Investigation of serotonergic receptors and transporter genes in vulnerability to depression, anxiety and neuroticism
A human population study

A thesis submitted to the University of Manchester for the degree of doctor of philosophy in the Faculty of Medical and Human Sciences

2010

Krisztina Mekli

School of Community Based Medicine
and School of Cancer and Enabling Science

Faculty of Medical and Human Sciences

TABLE OF CONTENTS

i.) List of tables……..7
ii.) List of figures……....8
iii.) List of abbreviations……..9
iv.) List of appendices……...11
v.) Abstract……12
vi.) Declaration……..14
vii.) Copyright statement……..15
viii.) Acknowledgement……..16
ix.) Alternative format……...17

1. Introduction 20
1.1 Psychiatric phenotypes 20
1.1.1 Depression 20
1.1.1.1 Description of depression 20
1.1.1.2 Epidemiology of depression 23
1.1.1.3 The course of depression 24
1.1.2 Anxiety 25
1.1.2.1 Description of anxiety 25
1.1.2.2 Epidemiology of anxiety disorders 27
1.1.2.3 The course of anxiety 27
1.1.3 Personality traits 28
1.1.4 Correlation between neuroticism, depression and anxiety 30
1.2. Biochemistry of the serotonergic pathway 32
1.2.1 Distribution of serotonergic cells in the brain 33
1.2.2 Metabolism and catabolism of serotonin 36
1.2.3 Serotonin receptors 39
1.2.3.1 5-HT1 receptor family 39
1.2.3.2 5-HT2 receptor family 41
1.2.3.3 5-HT3 receptor family 43
1.2.3.4 5-HT4 receptor family 44
1.2.3.5 5-HT5 receptor family 44
1.2.3.6 5-HT6 receptor family 45
1.2.3.7 5-HT7 receptor family 45
1.2.4 5-HT neurochemistry and depression; peripheral and post-mortem brain measures 48
1.2.4.1 Monoamine and catecholamine metabolites in the cerebrospinal fluid (CSF) of psychiatric patients 48
1.2.4.2 Post-mortem brain studies of serotonin and its metabolites in suicide and depression 48
1.2.4.3 Post mortem studies of serotonin receptors 49
1.2.4.4 Imipramine binding site studies 49
1.2.5 5-HT neurochemistry and depression; molecular imaging with Positron Emission Tomography 51
1.2.5.1 Receptor binding studies in depression 51
1.2.5.2 Receptor binding studies in anxiety disorders 52
1.3 Genetics of the serotonergic system 53

1.3.1 Genetics of the serotonin transporter (SERT) 53
1.3.1.1 SLC6A4 animal models 53
1.3.1.2 SLC6A4 genetic variations in humans 55
1.3.2 Genetics of 5-HT1A receptor 58
1.3.2.1 Animal models 58
1.3.2.2 HTR1A genetic variants in humans 59
1.3.3 Genetics of the 5-HT1B receptor 61
1.3.3.1 Animal models 61
1.3.3.2 HTR1B genetic variants in humans 62
1.3.4. Genetics of 5-HT1D, E and F receptors 63
1.3.4.1 Animal models 63
1.3.4.2 HTR1D, E and F variants in humans 63
1.3.5 Genetics of the 5-HT2A receptors 63
1.3.5.1 Animal models 63
1.3.5.2 HTR2A genetic variants in humans 64
1.3.6 Genetics of 5-HT2B receptors 66
1.3.6.1 Animal models 66
1.3.6.2 HTR2B genetic variants in humans 66
1.3.7 Genetics of 5-HT2C receptors 67
1.3.7.1 Animal models 67
1.3.7.2 HTR2C genetic variants in humans 68
1.3.8 Genetics of 5-HTR3 receptors 70
1.3.8.1 Animal models 70
1.3.8.2 HTR3 genetic variants in humans 71
1.3.9 Genetics of 5-HT4 receptors 72
1.3.9.1 Animal models 72
1.3.9.2 HTR4 genetic variants in humans 72
1.3.10 Genetics of 5-HT5 receptors 73
1.3.10.1 Animal models 73
1.3.10.2 HTR5 genetic variants in humans 73
1.3.11 Genetics of 5-HT6 receptors 73
1.3.11.1 Animal models 73
1.3.11.2 HTR6 genetic variants in humans 74
1.3.12 Genetics of 5-HT7 receptors 74
1.3.12.1 Animal models 74
1.3.12.2 HTR7 genetic variants in humans 74
1.4. Genome-wide association studies 75
1.5 Role of the environment 78
2. Aims of the study 82
2.1 Aim 1 82
2.2 Aim 2 82
2.3 Aim 3 82
2.4 Aim 4 83
2.5 Aim 5 83
3. Methods 84
3.1 New Mood cohort 84
3.1.1 Participants 84
3.1.2 Phenotypes 86
3.1.2.1 Mood state 86

3.1.2.2 Personality 87
3.1.2.3 Recent negative life events 87
3.1.2.4 Childhood adversity 87
3.1.2.5 Face emotion recognition task 88
3.1.3 SNP selection 88
3.1.4 DNA sample collection, extraction and genotyping 88
3.1.4.1. Extraction 89
3.1.4.2 Genotyping by Sequenom 91
3.1.4.2.1 Amplification 91
3.1.4.2.2 PCR products 93
3.1.4.2.3 SAP treatment 93
3.1.4.2.4 The iPLEX reaction 94
3.1.4.2.5 Clear Resin 96
3.1.4.2.6 Dispensing to SpectroCHIP Bioarrays 96
3.1.4.2.7 MALDI-TOF Analysis 96
3.1.4.3 Specifications: 97
3.1.4.4 Determination of the 5HTTLPR genotype 98
3.1.4.5 Determination of the 5-HTTLPR short/long A/G genotype (Triallelic method) 98
3.1.4.6 Determination of the rs25531 genotype (TaqMan) 99
3.1.4.7 Genotyping success rate 100
3.2 Dyne Steel cohort 101
3.2.1 Participants 101
3.2.2 Phenotypes 102
3.2.2.1 Mood state 102
3.2.2.2 Personality 103
3.2.3 DNA sample collection, extraction and genotyping 105
3.3 Statistical analysis 105
Paper 1 107
4. Study 1 1
Abstract 2
Introduction 3
Experimental procedures 6
Results 10
Discussion 11
References 17
Figures 27
Manuscript 1 101
5. Study 2 1
Abstract 2
Introduction 2
Methods 5
Results 9
Discussion 19
Figures 24
References 27
Manuscript 2 102
6. Study 3 1
Abstract 2

Introduction....2
Methods....8
Results....10
Discussion....18
References....22
7. General Discussion....103
8. Conclusions....117
9. References....118
Mamounas LA and Molliver ME (1988). Evidence for dual serotonergic projections to neocortex: axons from the dorsal and median raphe nuclei are differentially vulnerable to the neurotoxin p-chloroamphetamine (PCA). Experimental Neurology, 102:23-36....158
10. Appendix....187
10.1 Imaging studies on depression and anxiety....187
10.1.1 5-HT neurochemistry and depression; imaging with Magnetic Resonance Imaging....187
10.1.1.1 Functional changes in the amygdala in depression and anxiety disorders....188
10.1.1.2 Morphological changes in the amygdala in depression and anxiety disorders....189
10.1.1.3 Functional changes in the hippocampus in depression and anxiety disorders....189
10.1.1.4 Morphological changes in the hippocampus in depression and anxiety disorders....190
10.1.1.5 Functional changes in the basal ganglia in depression....190
10.1.1.6 Morphological changes in the basal ganglia in depression....191
10.1.1.7 Functional changes in cortical regions in depression and anxiety disorders....191
10.1.1.8 Morphological changes in the cortical regions in depression and anxiety disorders....193
10.2 Genetics of the serotonergic system....200
10.3 Questionnaires used in this study....203
10.3.1 Beck Depression Inventory....203
10.3.2 Eynseck Personality Questionnaire....205
10.3.3 Cornell Medical Index....207
10.3.3.1 Personality....207
10.3.3.2 CMI Cardiovascular system....209
10.3.3.3 CMI Fatigue....209
10.3.3.4 CMI Sleep disorders and Addiction....209
10.3.4 Yesavage Geriatric Depression Inventory....210
10.3.5 Big Five Inventory (BFI-44)....210
10.3.6 Brief Symptom Inventory....211
10.3.7 Life Events Questionnaire....212
10.3.8 Childhood Trauma Questionnaire....213
10.4 Primers and probes used in the New Mood study....213
10.4.1 Primers....213
10.4.2 Probes....215
10.4.3 Other primers and probes....215
10.5 List of Illumina SNPs of Dyne Steel cohort....216

10.5.1 HTR2A gene 216
10.5.2 SLC6A4 gene 217
10.5.3 HTR6 gene 217
10.5.4 HTR1D gene 217
10.5.5 HTR2B gene 217
10.5.6 HTR1F gene 217
10.5.7 HTR3 gene, C,D, E subunits 218
10.5 8 HTR4 gene 218
10.5 9 HTR1E gene 219
10.5.10 HTR5A gene 220
10.5.11 HTR7 gene 220
10.5.12 HTR3 gene, AB subunits 220
10.6. Supplementary material for Study 1 222
10.7 Manuscripts in preparation –Further results 225
10.8 Published version of Study 1 227
10.9 Supplementary materials for Study 2 239

Final word count: 83,432

i. List of tables

Table 1. Human serotonin receptors 47
Table 2. Demographic data of New Mood participants 86
Table 3. Parameters of extraction 90
Table 4. Components of the PCR reaction 92
Table 5. PCR cycles 93
Table 6. Components of the SAP treatment reaction 94
Table 7. Incubation of the SAP treatment reaction 94
Table 8. Mass differences between the iPLEX ddNTPs 95
Table 9. Components of the iPLEX reaction 95
Table 10. Cycles of the iPLEX reaction 96
Table 11. Genotyping success of SNPs in the New Mood Manchester and Budapest cohorts. 100
Table 12. Demographic data of Dyne Steel participants (Manchester and Newcastle together) 102
Table 13. Summary table of phenotypic measures used in the study 104

ii. List of figures

Figure 1. Relationship between depression, anxiety and neuroticism.....31
Figure 2. Location of serotonergic body cells in the rat central nervous system. 35
Figure 3. Location of serotonergic body cells in the human central nervous system .35
Figure 4. Serotonin metabolism. 38
Figure 5. The editing of human HTR2C mRNA.....69
Figure 6. PCR amplification reaction.....92
Figure 7. The PCR product 93
Figure 8. The iPLEX reaction 95
Figure 9. Fisher's bell curve. 104
Figure 10. Function of the serotonin transporter molecules in the synaptic cleft.....110
Figure 11. Schematic figure of the role of serotonergic system in the development of depression. 113

iii. List of abbreviations
5-HIAA: 5-hydroxyindole acetic acid
5-HT: 5-hydroxytiptamine (serotonin)
5-HTTLPR: 5-hydroxytryptamin transporter gene-linked polymorphic region
AADC: aromatic L-amino acid decarboxylase
AD: antidepressant
ACTH: adrenocorticotropic hormone
ATD: acute Tryptophan depletion
BFI-44: big five inventory
BP: binding potential
BSI: brief symptom inventory
CC2D1A: coiled coil C2 domain 1A
Chr: chromosome
CSF: cerebrospinal fluid
CNS: central nervous sytem
CRH: corticotropin releasing hormone
dACC: dorsal anterior cingulate cortex
ddNTP: di-dezoxy nucleotide triphosphate
DAT: dopamine transporter
DLPFC: dorsolateral prefrontal cortex
DSM-IV: diagnostic and statistical manual of mental disorders
DNA: dezoxyribonucleic acid
dNTP: dezoxy nucleotide triphosphate
EC: Enzyme Comission number
EPQ: Eysenck personality questionnaire
FDG: [C^{15}] flourodeoxyglucose
FDR: false discovery rate
fMRI: functional magnetic resonance imaging
Freud: Five-prime repressor element under dual repression
GABA: γ-amino butyric acid
GAD: generalised anxiety disorder
GWAS: genome-wide association studies
HA: harm avoidance
HC: healthy controls
Hes5: Hairy and enhancer of split 5 gene
HT: haplotype tagging
HPA: hypothalamic-pituitary-adrenal axis
HWE: Hardy-Weinberg equilibrium
ICD: international classification of diseases
KO: knock out
LD: linkage disequilibrium
LSD: lysergic acid diethylamide
LTE: life threatening experiences
L-Trp: L-Tryptophan
MAO-A: monoamino oxydase A gene
MDD: major depressive disorder
MRI: magnetic resonance imaging
NEO-PI-R: revised neuroticism-extroversion-openness personality inventory
NET: norepinephrine transporter

NS: novelty seeking
NUDR: nuclear Deaf-1 related protein
OCD: obsessive-compulsive disorder
OFC: orbitofrontal cortex
PCR: polymerase chain reaction
PD: panic disorder
Receptor genes
- *HTR1A*: 5-hydroxytryptamine receptor 1A
- *HTR1B*: 5-hydroxytryptamine receptor 1B
- *HTR1D*: 5-hydroxytryptamine receptor 1D
- *HTR1E*: 5-hydroxytryptamine receptor 1E
- *HTR1F*: 5-hydroxytryptamine receptor 1F
- *HTR2A*: 5-hydroxytryptamine receptor 2A
- *HTR2B*: 5-hydroxytryptamine receptor 2B
- *HTR2C*: 5-hydroxytryptamine receptor 2C
- *HTR3A*: 5-hydroxytryptamine receptor 3A
- *HTR3B*: 5-hydroxytryptamine receptor 3B
- *HTR3C*: 5-hydroxytryptamine receptor 3C
- *HTR3D*: 5-hydroxytryptamine receptor 3D
- *HTR3E*: 5-hydroxytryptamine receptor 3E
- *HTR4*: 5-hydroxytryptamine receptor 4
- *HTR5*: 5-hydroxytryptamine receptor 5
- *HTR6*: 5-hydroxytryptamine receptor 6
- *HTR7*: 5-hydroxytryptamine receptor 7

RD: reward dependence
RNA: ribonucleic acid
SAD: social anxiety disorder
SAP: Shrimp Alkaline Phosphatase
SCID: structured clinical interview for DSM-IV
SEM: standard error of the mean
SERT: serotonin transporter
SLC6A4: 5-hydroxytryptamine transporter gene
SNAP25: synaptic associated protein 25kDa gene
SNP: single nucleotide polymorphism
SSRI: selective serotonin reuptake inhibitor
TCI: temperament and character inventory
TPH2: typtophan hydroxylase 2 gene
UTR: untranslated region
VLPFC: ventrolateral prefrontal cortex
VNTR: variable number of tandem repeats
WHO: World Health Organization

iv. List of appendices
10.1 Imaging studies on depression and anxiety
10.1.1 5-HT neurochemistry and depression; imaging with Magnetic Resonance Imaging
10.1.1.1 Functional changes in the amygdala in depression and anxiety disorders
10.1.1.2 Morphological changes in the amygdala in depression and anxiety disorders
10.1.1.3 Functional changes in the hippocampus in depression and anxiety disorders
10.1.1.4 Morphological changes in the hippocampus in depression and anxiety disorders
10.1.1.5 Functional changes in the basal ganglia in depression
10.1.1.6 Morphological changes in the basal ganglia in depression
10.1.1.7 Functional changes in the cortical regions in depression and anxiety disorders
10.1.1.8 Morphological changes in the cortical regions in depression and anxiety disorders
Table 10.1 Receptor binding studies in depression
10.2 Genetics of the serotonergic system
Table 10.2. Genome position of genes investigated in this study
10.3 Questionnaires used in this study
10.3.1 Beck Depression Inventory
10.3.2 Eynseck Personality Questionnaire
10.3.3 Cordell Medical Index
10.3.4 Yesavage Geriatric Depression Inventory
10.3.5 Big Five Inventory (BFI-44)
10.3.6 Brief Symptom Inventory
10.3.7 Life Events Questionnaire
10.3.8 Childhood Trauma Questionnaire
10.4 Primers and probesused in the New Mood study
10.4.1 Primers
10.4.2 Probes
10.4.3 Other primers and probes
10.5 List of Illumina SNPs of Dyne Steel cohort
10.5.1 *HTR2A* gene
10.5.2 *SLC6A4* gene
10.5.3 *HTR6* gene
10.5.4 *HTR1D* gene
10.5.5 *HTR2B* gene
10.5.6 *HTR1F* gene
10.5.7 *HTR3* gene, C,D, E subunits
10.5 8 *HTR4* gene
10.5 9 *HTR1E* gene
10.5.10 *HTR5A* gene
10.5.11 *HTR7* gene
10.5.12 *HTR3* gene, AB subunits
10.6.1 Supplementary material for Study 1
10.7 Manuscript in preparation Study 3, Manuscript 2
10.8 Published version of Study 1
10.9 Supplementary materials for Study 2

v. Abstract

University of Manchester
Krisztina Mekli
Doctor of Philosophy

Investigation of serotonergic receptors and transporter genes in vulnerability to depression, anxiety and neuroticism. A human population study.

22 December 2010

Background

Depression and related phenotypes, such as anxiety and neuroticism are thought to have a common genetic background. The malfunction of the serotonergic system is likely to play an important role in the etiology of these phenotypes, with compelling evidence coming from animal and human studies. However, recent studies indicate that other factors should be investigated, such as environmental stress.

Aim

To investigate the role of the serotonergic gene variants (*HTR1A-7* and *SLC6A4*) in depression, anxiety and neuroticism, in interaction with each other and other factors, such as stressful events.

Method

Two large independent Caucasian cohorts, haplotype tagging single nucleotide polymorphisms (htSNPs) and existing genome-wide genotype data were used. Phenotypes were assessed by detailed questionnaires about psychiatric phenotypes (depression, anxiety and neuroticism) as well as background information, such as physical health. Environmental stress factors were investigated in one cohort by self-reported life event (recent and childhood) questionnaires. Healthy participants from this cohort took part in a computerised task to measure the effect of a functional polymorphism in the *HTR1A* gene on threat-related emotional information processing.

Results

My study confirmed the importance of stressful life events in depression and anxiety modulated via the 5-HT1A and 5-HT1B autoreceptor, but not via the *SLC6A4*. I found evidence for epistatic interaction between *HTR2A* and *SLC6A4* genes and between different subunits of the *HTR3* gene which may contribute towards the depressive phenotype. Finally, certain alleles of SNPs in other serotonergic receptors (5-HT4 and 5-HT6) were also associated with depression, anxiety and neuroticism however, this association was weak. On the other hand, my study did not provide evidence for the interaction between the serotonin transporter 5-HTTLPR polymorphism and stressful life events which is widely reported in the literature.

Conclusion

This study provides further support for the serotonergic hypothesis of depression and confirms the role of the environment in the aetiology of depression. The results show evidence of possible epistatic interaction between the *SLC6A4* and *HTR2A*

genes. These results highlight the complex interactions between the members of the serotonergic pathway as well as the role of the environment on the individual.

vi. Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

vii. Copyright statement

viii. Acknowledgement
First of all I am most grateful for my supervisors, Professor JF William Deakin, Dr Gabriella Juhasz and Dr Antony Payton. I learnt everything from them and they helped me in every possible way. I cannot imagine better supervisors and it has been a privilege to work with them. Thank you for everything.
I am also thankful for Professor Gyorgy Bagdy for the possibility of this PhD studentship. He and his team of young, enthusiastic scientists in Budapest, Hungary kindly let me use some of the New Mood Budapest cohort's data, which I believe contributed to the better quality of this thesis.
Miss Hazel Platt taught me the laboratory methods, with enormous patience and accuracy.
Colleagues at the Neuroscience and Psychiatry Unit and Centre for Integrated Genomic Medical Research were also ready to help, whenever I needed. I learnt a lot from Dr Diana Chase, Professor Ian Anderson, Ms Francine Jury, Ms Emma Davis.
Many people I have mentioned, I consider as friends, as well. I hope they feel the same way.
I am also grateful for the thousands of participants for filling in endless questionnaires and giving DNA samples, both in Hungary and in the UK.
Last, but not least I am grateful for my Parents back in Hungary, for believing in me and especially for making me take English courses at the age of 10.

ix. Alternative format

This thesis has been submitted as an alternative format thesis. This way I was able to include my published paper and other manuscripts.

“When I lie waking all alone,
Recounting what I have ill done,
My thoughts on me then tyrannise,
Feare and sorrow me surprise,
Whether I tarry still or goe,
Me thinkes the time moves very sloe,
All my griefes to this are jolly,
Naught so sad as Melancholy.”

Robert Burton, 1621

"The 20th century saw the coming together of biology and chemistry, this century is seeing the coming together of biology and psychiatry".

James D. Watson, 2007

1. Introduction

1.1 Psychiatric phenotypes

1.1.1 Depression

1.1.1.1 Description of depression

Depression describes both a transient mood state experienced by virtually all individuals at some time in their life as well as a clinical or behavioural syndrome, usually called Major Depressive Disorder (MDD). MDD is a medical condition that includes abnormalities of affect and mood, neurovegetative functions (such as appetite and sleep disturbances), cognition (such as inappropriate guilt and feelings of worthlessness), and psychomotor activity (such as agitation or retardation). It is one of the oldest, well-recognized medical disorders, having been clearly described in medical texts dating back to ancient Greece.

MDD can occur with a history of episodes of mania known as Bipolar Disorder (previously termed Manic-Depressive Illness) or without a history of mania known as Unipolar Depressive Disorder.

Current diagnostic criteria for MDD according to the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) (American Psychiatric Association, 1994) represent a clinical and historical consensus about the important symptoms and signs of depressive illness.

DSM-IV criteria for major depressive episode:

Five (or more) of the following symptoms have been present during the same two-week period and represent a change from the previous functioning; at least one of the symptoms is either (a) Depressed mood or (b) loss of interest or pleasure

(1) depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g. feels sad or empty) or observation made by others (e.g. appears tearful)
(2) markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly everyday (as indicated by either subjective account or observations made by others)
(3) significant weight loss when not dieting or weight gain (e.g. a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day
(4) insomnia or hypersomnia nearly every day
(5) psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)
(6) fatigue or loss of energy nearly every day
(7) feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)
(8) diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as objected by others)
(9) recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.

Another classification is the International Classification of Diseases (ICD-10, World Health Organization 1992)

ICD-10 criteria for depressive episode:

A.
Depressed mood
Loss of interest and enjoyment
Reduced energy and decreased activity

B.
Reduced concentration
Reduced self-esteem and confidence
Ideas of guilt and unworthiness
Pessimistic thoughts
Ideas of self-harm
Disturbed sleep
Diminished appetite

Mild depressive episode: at least 2 of A and at least 2 of B.
Moderate depressive episode: at least 2 of A and at least 3 of B.
Severe depressive episode: all 3 of A and at least 4 of B.

Patients often display other features as well, such as:

- Poor memory: patients often complain about impairments in the retrieval and recognition of recently learned material. This may be so severe that the clinical presentation resembles that of dementia.
- Other psychiatric features may also occur, such as depersonalization, obsessive symptoms and panic attacks.
- Physical symptoms are very common in depression, especially constipation, fatigue and aching discomfort anywhere in the body.
- Psychomotor changes are also common, including agitation (which is a state of restlessness that is experienced by the patient as restless activity), anxiety (which is particularly common in less severe depression) and irritability (which is the tendency to respond with undue annoyance to minor demands and frustrations).

It can be seen that, affected individuals display a wide variety of clinical symptoms and signs. Furthermore, current diagnostic conventions are, to some degree, arbitrary. Debate continues as to whether MDD is best conceptualized as a disease or as the extreme of a continuum of increasingly disturbed affective

regulation (Plomin *et al*. 2009). As a continuous trait, depressive symptoms can be measured with self-reported questionnaires such as the Zung Self-Rating Depression Scale (Zung 1965), the Beck Depression Inventory (Beck *et al*. 1961) and the Brief Symptom Inventory (Derogatis 1993), or interviewer rated questionnaires (e.g. Montgomery Asberg Depression Rating Scale, Montgomery and Asberg 1979).

1.1.1.2 Epidemiology of depression

Large-scale epidemiological studies, such as the National Comorbidity Survey in the USA, estimated the lifetime prevalence of MDD at 17% (DSM-III) (Blazer *et al*. 1994). The same survey found that nearly 5% of the population met criteria for MDD in the last 30 days. As has long been suspected, MDD is probably the most common of psychiatric disorders. In a prospective cohort study (in which people were assessed at regular intervals to minimise the problem of recall bias) by the age of 26, 37% of the participants had met criteria for a major depressive episode (Jaffee *et al.* 2003).

Psychiatric epidemiology has identified a list of putative risk factors for MDD; however it is difficult to discriminate association from causation. Four MDD risk factors stand out in the consistency of their association with MDD and the level of evidence suggesting that at least some of the association is indeed causal: gender, stressful life events, adverse childhood experiences and certain personality traits (Kendler *et al.* 2002, Kendler *et al*. 2006a).

- Across many studies, women have been shown to be at consistently greater risk for MDD than men. In the National Comorbidity Survey the lifetime prevalence of MDD in the US population was estimated to be 21.3% in women and 12.7% in men (Blazer *et al.,* 1994).
- A wide range of environmental adversities such as job loss, marital difficulties, major health problems, and loss of close personal relationships are associated with a substantial increase in risk for the onset of MDD (Kessler, 1997).
- A range of difficulties in childhood including physical and sexual abuse, poor parent-child relationships, and parental discord and divorce have been shown to increase the risk for MDD later in life (Arnow 2004, Weich *et al.* 2009).

- Certain personality traits appear to predispose to MDD, in particular "neuroticism". (Kendler *et al.* 2004, Kendler *et al.* 2006b). (Neuroticism is discussed in more detail below in section 1.1.3).

A range of other risk factors has been proposed for MDD, although in general the evidence for the existence of a causal association is weaker. These include low social class, urban residence, separated or divorced marital status, low levels of social support, and younger age (Muntaer *et al*. 2004, Fava and Kendler 2000).

A World Health Organization (WHO) report (Murray and Lopez 1996) predicted that depression would be the second most common condition with greatest disease burden worldwide by 2020, measured in Disability-Adjusted Life Years, which express years of life lost to premature death and years lived with a disability of specified severity and duration.

1.1.1.3 The course of depression

MDD is not a disorder exclusively limited to adult populations. A substantial proportion of patients experience their first episodes of MDD during childhood and adolescence. In such occurrences of early-onset these individuals typically continue to suffer from episodes of MDD during adulthood as well. For most people, MDD is a life-long episodic disorder with multiple recurrences (averaging one episode in every 5-year period), with approximately 20%–25% of major depressive disorder patients experiencing a chronic, unremitting course (Mueller and Leon 1996). The chronic recurrent course of MDD is a major clinical issue, often requiring long-term prophylactic treatment with an enormous economic expense (Anderson *et al.* 2008, Fostick *et al*. 2010).

1.1.2 Anxiety

1.1.2.1 Description of anxiety

Anxiety can be a personality trait as well as a disorder. The symptoms of anxiety are found in many disorders; meanwhile in anxiety disorders it is the most severe and prominent symptom. Anxiety is experienced very commonly and it may have beneficial effects in ordinary behaviour. At low a level of anxiety, behaviour may not be purposefully directed and performance is low. There is a middle range of anxiety where optimal performance has been reached. Above a certain level, a further increase in anxiety interferes with performance and therefore can be regarded as pathological anxiety. It is obviously very difficult, if not impossible to draw an exact dividing line between normal and pathological anxiety.

It is however possible to measure anxiety as a trait which reflects a stable tendency to respond in the anticipation of threatening situations. Several questionnaires measure trait anxiety, for example the Brief Symptom Inventory (Derogatis 1993), the Cordell Medical Index (Brodman *et al.* 1956), the Spielberger's State-Trait Anxiety Inventory (Spielberger 1970) and the Beck Anxiety Inventory (Beck *et al.* 1961).

Anxiety disorders are heterogeneous; both ICD-10 and DSM-IV divide anxiety disorders into different classes according to the evoking factors and the manifestation of anxiety symptoms. Among many other classes in both systems the three main categories are (1) Phobic anxiety disorders, (2) Panic disorder (PD), and (3) Generalised Anxiety Disorder (GAD).

Short description of the three main anxiety disorders:

1. Phobic anxiety disorders.

Anxiety symptoms occur only in particular circumstances. These circumstances may be few or many, but even so there are situations in which no anxiety is experienced.

a. Agoraphobia

Agoraphobic patients are anxious when they are away from home, in crowds or in situations that they cannot leave easily.

b. Social phobia

In this disorder inappropriate anxiety is experienced in situations in which the person is observed and could be criticized. Some patients become anxious in a wide range of social situations, while other patients are anxious only in specific situations, such as public speaking.

c. d. Specific phobias

In DSM-IV five types of specific phobia are recognized concerned with:

- Animals
- Aspects of the natural environment (for example thunder)
- Blood, injection and injury
- Situations (for example crowded places)
- Other provoking agents (for example the dentist's chair)

2. Panic disorder (PD)

The main feature of this disorder is the occurrence of sudden attacks of anxiety (panic attacks) in which physical symptoms predominate (e.g. palpitations, shortness of breath, nausea, dizziness, and paresthesias) and are accompanied by fear of serious consequences such as a heart attack or dying.

3. Generalized anxiety disorder (GAD)

Symptoms:

- Worry and apprehension which are difficult to control. The worries are widespread and not focused on a specific issue.
- Psychological arousal (e.g. difficulty concentrating, irritability).
- Autonomic arousal (e.g. muscle tension, sleep disturbances).
- Other features. These include tiredness, depressive symptoms obsessional symptoms and depersonalization; however these symptoms are never the most prominent features.

Anxiety and depressive symptoms often occur together. The correlation is greatest when the symptoms are mild (0.5) with about 0.20-0.30 correlation when symptoms are more severe (Gelder *et al*. 2001). MDD and anxiety disorders are also frequently co-morbid. Furthermore, anxiety disorders can cause similar levels of disabilities as MDD (Hettema 2008).

1.1.2.2 Epidemiology of anxiety disorders

The lifetime prevalence of any anxiety disorders in the general population is about 28.8% (for generalised anxiety disorder: 5.7%, for panic disorder: 4.7%). Projected lifetime risk by the age of 75 is 31.5% for any anxiety disorder, 8.3% for generalised anxiety and 6.0% for panic disorder (Kessler *et al.* 2005). The lifetime prevalence of the different anxiety disorders vary from 1.7% (agoraphobia in men) to 8.9% (GAD in women). In general these disorders affect twice as many women as men, which is a similar ratio to that seen for depression. In addition, other MDD risk factors such as childhood maltreatment, stressful life events and socioeconomic factors also increase the prevalence of anxiety disorders (Hettema 2008) suggesting common pathophysiological mechanisms.

1.1.2.3 The course of anxiety

As well as the characteristic differences, the time course of anxiety symptoms is also different in the various disorders:

- In phobic anxiety disorders anxiety only arises in particular circumstances.
- In panic disorders anxiety also is present occasionally but its occurrence is unrelated to any particular circumstances.
- In generalized anxiety disorders anxiety is continuous, with fluctuations in its intensity.

Despite these differences, the nature of anxiety disorders is chronic and disabling thus requiring both acute and preventive therapies (McIntosh *et al.* 2004). Interestingly, follow-up studies show that anxiety disorders tend to develop into depression or co-morbid anxiety-depression but pure depression rarely develops into pure anxiety (Hettema 2008).

1.1.3 Personality traits

Personality traits can be defined as habitual patterns of behaviour, thought and emotion. Traits are relatively stable over time; differ among individuals and influence behaviour. The statistical technique of factor analysis has demonstrated that particular clusters of specific traits reliably correlate together; therefore the human personality can be described with a very limited number of domains, called factors. These factors are continuous, can be distinguished from temporary states, can describe individual differences and are intended to be uncorrelated.

By employing factor analysis Eysenck found two basic dimensions of personality, Neuroticism, characterized by high levels of emotional arousal, and Extroversion, characterized by being outgoing, talkative and high on positive affect. Further research demonstrated the need for a third category of temperament and later another scale was included in the Eysenck Personality Queationnaire (EPQ) (Eysenck and Eysenck 1964) called Psychoticism, which is a measure of hostility.

Although Eysenck's personality factors seem to be universal across cultures, languages and people, further models have been developed to describe human personality. The most widely used model has five factors. The five factor personality model can be measured by self-administered questionnaires, such as the Big Five Inventory (John *et al.* 1991) or the Revised Neuroticism-Extroversion-Openness Personality Inventory (NEO-PI-R; Costa and McCrae 1992).

The five factors of the Five Factor Personality Model:

1. ***Openness to experience (sometimes called intellect):***

   is a general appreciation for art, emotion, adventure, unusual ideas, imagination, curiosity and variety of experience. People who are open to experience are intellectually curious, appreciative of art, creative and aware of their feelings

2. ***Conscientiousness:***

   is a tendency to show self-discipline, act dutifully and aim for achievement. This trait shows a preference for planned, rather than spontaneous behaviour

3. ***Extroversion:***

   is characterized by positive emotions and tendency to seek out stimulation and the company of others. Extroverts enjoy being with people, tend to be enthusiastic, action-oriented and are often perceived as full of energy

4. ***Agreeableness:***

   is a tendency to be compassionate and cooperative rather than suspicious and antagonistic toward others. Agreeable individuals are generally considerate, friendly, generous, helpful and willing to compromise their interests for others

5. ***Neuroticism:***

   is the tendency to experience negative emotions, such as anger, anxiety or depression. It is sometimes referred to as emotional instability. Those people who score high in neuroticism are emotionally reactive and vulnerable to stress. They are more likely to interpret ordinary situations as threatening, and minor frustrations as hopelessly difficult. Neuroticism is also a risk factor for internalizing mental disorders such as phobia, depression, panic disorder and other anxiety disorders

Another personality hypothesis uses three personality factors: novelty seeking (NS), harm avoidance (HA) and reward dependence (RD) that can be measured by the Temperament and Character Inventory (TCI) (Cloninger *et al.* 1994) or by the Three-dimensional Personality Questionnaire (TPQ) (Cloninger *et al.* 1991). Harm avoidance shows close resemblance to neuroticism as it is defined as a tendency to overreact to signals of aversive stimuli and as a result inhibit or stop behaviour.

1.1.4 Correlation between neuroticism, depression and anxiety

There have been attempts to estimate the genetic correlation between depression, anxiety and neuroticism. These studies used monozygotic and dizygotic twin pairs as well as families to establish the genetic correlations between these psychiatric phenotypes.

A study using 20692 members of same-sex twin pairs concluded that correlation between neuroticism and liability to major depression is 0.25.This was largely the result of shared genetic risk factors, with a genetic correlation of 0.46-0.47 in women and men, respectively (Kendler *et al.* 2006b). According to another report the genetic correlation between MDD and neuroticism was as high as 0.60 (Hettema *et al.* 2006). In an independent longitudinal epidemiologic study inhibited personality characters (such as shy, fearful and easily upset) at age 3 years predicted the likeliness of major depression diagnosis at age 21 years (Caspi *et al*. 1996).

Environmental factors have also been shown to be important with one study showing that level of stress exposure was directly related to an increase in the risk of depression (Kendler *et al.* 2004). This study also demonstrated that neuroticism moderated the pathogenic effects of the stress exposure, as individuals with low levels of neuroticism were less sensitive to the depressogenic effects of adversity. In addition, it has been reported that individuals with higher neuroticism are more likely to be prone to the occurrence of multiple depressive episodes (Duggan *et al*. 1995). However, this study was not able to distinguish whether this elevation in neuroticism was the cause or the result of a depressive episode. Neuroticism shows an even greater phenotypic correlation to generalized anxiety than to depression with one study finding a correlation of 0.80 (Hettema *et al*. 2004). The genetic correlation between neuroticism and generalized anxiety disorder was 0.77; meanwhile between neuroticism and different phobias it varied between 0.58 and 0.82 (Hettema *et al.* 2006).

In conclusion, the high phenotypic and genotypic correlations between depression, neuroticism and anxiety disorders suggest shared biological pathways which are likely to predispose an individual's response to stress and stress tolerance (Figure 1).

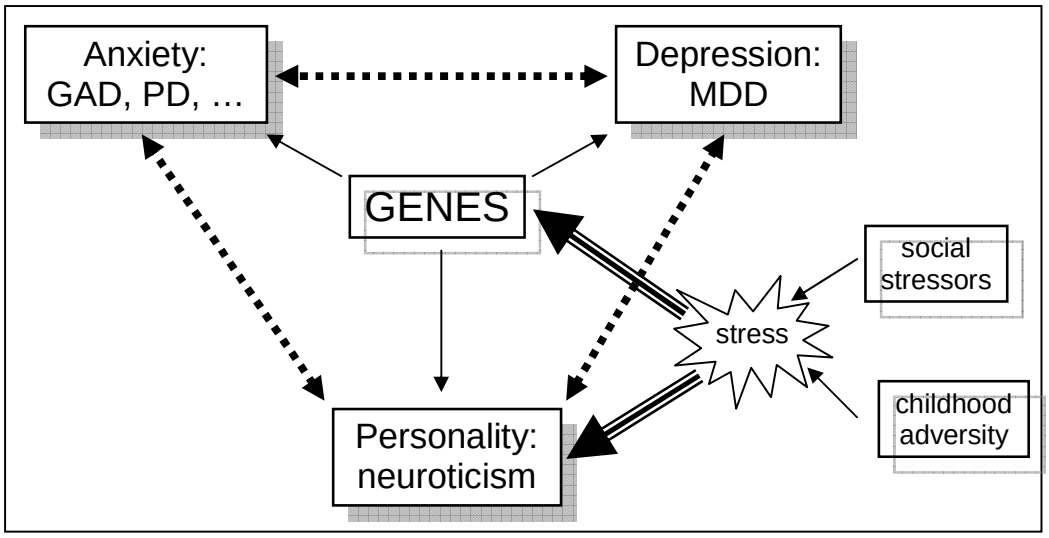


Figure 1. Relationship between depression, anxiety and neuroticism
Arrows indicate the complex interactions between genes and the environment which in some cases may lead to psychiatric phenotypes. These phenotypes are in high correlation with each other, possibly because they share the same susceptibility genes.

1.2. Biochemistry of the serotonergic pathway

It has been known since the mid-19th century that after blood clots, a substance in the resulting serum constricts vascular smooth muscles and increases vascular tone. Around the turn of the 20th century, platelets were identified as the source of this substance. In 1948 Rapport, Green and Page isolated and identified the serum vasoconstrictor factor as 5-hydroxytryptamine (5-HT) (Rapport *et al.* 1948). As this serum tonic factor was released into sera from platelets during the clotting of blood it was named 'serotonin'. Independently, Esparmer characterized a substance found in large amounts in the enterochromaffin cells of the gastrointestinal tract, which also constricted smooth muscle, and called it '*enteramine*'. In 1952 Esparmer and Areso reported (in Siegel *et al.* 2006) that serotonin and enteramine were the same substance. In 1953 Twarog and Page detected serotonin in brain extracts (Twarog and Page, 1953). Thus, serotonin was localized to three key systems in the body: platelets, gastrointestinal tract and brain. As a hydrophilic substance, 5-HT does not pass the lipophilic blood-brain barrier. Therefore, its discovery in brain indicated that it was synthesized in the brain. About the same time the observation was made by Woolley and Shaw (Woolley and Shaw 1954) that (+)lysergic acid diethylamide (LSD) produced marked disturbances in thought and vision and had distinct structural similarities to 5-HT.

A further accidental discovery was that an antihypertensive drug called *reserpine*, which was extracted from the plant *Rauwolfia serpentina*, caused symptoms resembling those of depression (Pletscher *et al.* 1956). The discovery that reserpine depletes both the central and the peripheral vesicular stores of 5-HT added impetus to the idea that 5-HT might have an important role in the causation of mental illness.

It has been known for 30 years that many of the tricyclic antidepressants such as imipramine and amitriptyline are potent inhibitors of both 5-HT and norepinephrine reuptake (Siegel *et al.* 2006). The selective serotonin reuptake inhibitors (SSRIs) have a high affinity for the serotonin transporter (SERT) (a protein responsible for the reuptake of serotonin from the synaptic cleft), and therefore prevent 5-HT reuptake. SSRIs are now the most widely used agents in the treatment of depression and are

also used to treat other neuropsychiatric disorders, such as anxiety and panic disorder (PD) which are often associated with the depressed phenotype.

In-vivo studies in patients using the technique of acute tryptophan depletion (ATD) have made a compelling case that SSRIs work in the short-term through their ability to enhance the synaptic availability of 5-HT (Ruhe *et al*. 2007). L-Tryptophan (L-Trp) is the precursor molecule of serotonin synthesis (see details in Metabolism and catabolism of serotonin, 1.2.2.). Participants drink a mixture of amino-acids that does not include tryptophan. The liver responds by synthesising protein and this uses up circulating tryptophan since it is not present in the drink. Circulating levels decrease to about 20% of normal after 4-5 hours and rapidly return to normal after a meal. In addition, increased concentrations of large neutral amino acids compete with diminished levels of tryptophan for active transport into the brain. ATD has been shown to trigger a transient relapse of depression in patients who have recently recovered and are still taking SSRIs. ATD has little effect in people with no history of depression or who have been well for more than year (Ruhe *et al.* 2007). These results suggest that the depletion reflects a biological vulnerability of the 5-HT system in remitted patients, however there is no direct correlation of 5-HT levels in the brain and mood.

1.2.1 Distribution of serotonergic cells in the brain

Serotonin-containing neuronal cell bodies are restricted to discrete clusters of cells located along the midline of the brainstem. However, their axons innervate nearly every area of the central nervous system (CNS).

Dahlstrom and Fuxe (Dahlstom and Fuxe 1964) described nine groups of serotonin containing cell bodies in the rat brain which they designated B1-B9, and which correspond for the most part with the raphe nuclei. Subsequent animal and *post-mortem* human anatomical techniques (Azmitia and Gannon 1986, Geyer *et al.* 1976, Steinbusch and Nieuwenhuys 1981, Anderson and Felten 1982) have allowed an accurate characterization of the serotonergic innervations of forebrain areas. The largest group of serotonergic cells is group B7, which are continuous with a smaller group of serotonergic cells, B6. Groups B6 and B7 are often considered together and form the dorsal raphe nucleus, with B6 being its caudal extension. Group B8

corresponds to the median raphe nucleus. Group B9 (part of the ventrolateral tegmentum of the pons and midbrain) forms a lateral extension of the midbrain raphe. Ascending serotonergic projections innervating the cerebral cortex and other regions of the forebrain arise primarily from the dorsal raphe, median raphe and the B9 group. The median raphe projects heavily to the hippocampus, septum and hypothalamus, whereas the striatum is innervated predominantly by the dorsal raphe. The dorsal and median raphe nuclei send overlapping neuronal projections to the neocortex. Moreover, raphe neurons send collateral axons to areas of brain that are related in function, such as the amygdala and hippocampus, or substantia nigra and caudate putamen (Molliver, 1987).

The other raphe nuclei, B1-B4 are more caudally situated (mid-pons to caudal medulla) and contain a smaller number of serotonergic cells. These cell body groups give rise to serotonergic axons that project within the brainstem and the spinal cord. The spinal cord receives a strong serotonergic innervation through three principal descending pathways. Afferent connections to the raphe nuclei include connections between the dorsal and median raphe nuclei, B9, B1 and B3. The raphe nuclei also receive input from other cell body groups in the brainstem such as the substantia nigra and ventral tegmentum area (dopamine), superior vestibular nucleus (acetylcholine), locus ceruleus (noradrenaline), nucleus prepositus hypoglossi and nucleus of the solitary tract (adrenaline). Other afferents include neurons from the hypothalamus, cortex and limbic forebrain structures such as the amygdala. The location of serotonergic cell body groups in the rat and human central nervous system can be seen in Figures 2 and 3.

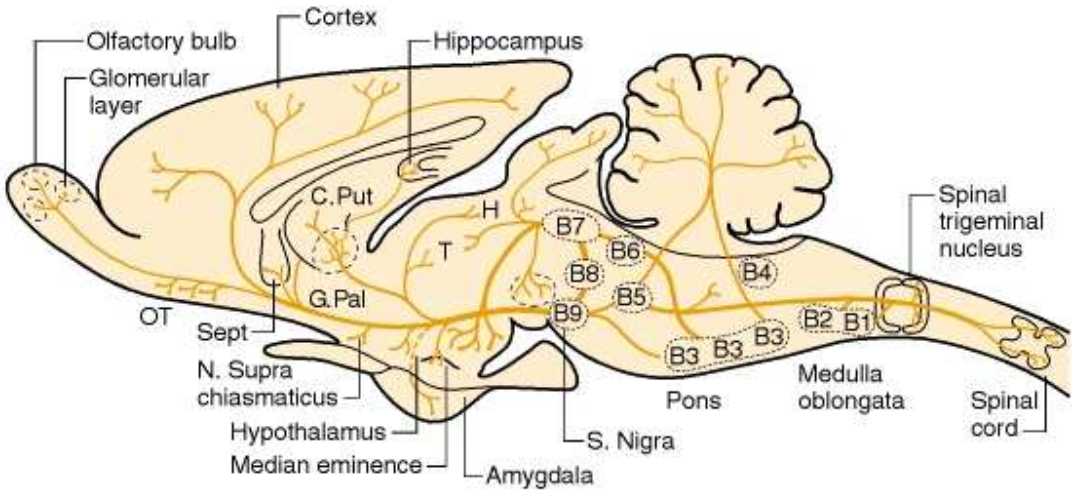


Figure 2. Location of serotonergic body cells in the rat central nervous system. OT: olfactory tuberculum, sept: septum, C. Put: nucleus caudate-putamen, G. Pal: globus pallidus, T: thalamus, H: Habenula (Siegel *et al.* 2006).

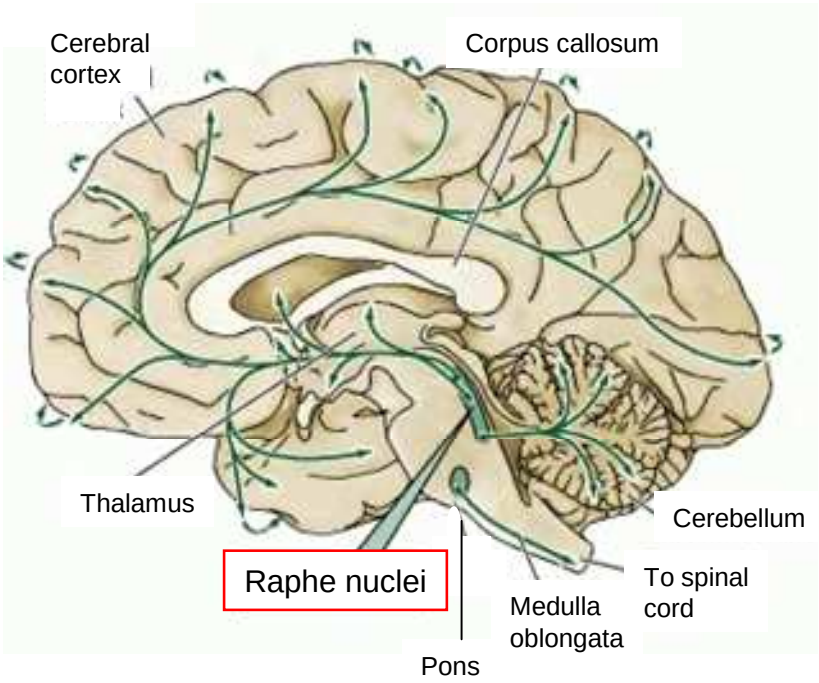


Figure 3. Location of serotonergic body cells in the human central nervous system (Purves *et al.* 2001).

Serotonergic axon terminals appear to exhibit morphological differences related to the raphe nucleus of origin. Serotonergic axons from the median raphe nucleus (type M) look relatively coarse with large spherical varicosities. These axons also appear to be more vulnerable to neurotoxic amphetamine derivatives (Siegel *et al.* 2006). In contrast, axons from the dorsal raphe (type D) are very fine and typically have small pleomorphic varicosoties. Type D axons appear to be more resistant to the neurotoxic effects of amphetamine (Mamounas and Molliver, 1988, Siegel *et al*. 2006).

1.2.2 Metabolism and catabolism of serotonin

The metabolic machinery for synthesis and degradation of serotonin is present at the nerve ending. The amino acid L-Trp is the precursor for the synthesis of 5-HT, whose primary source is dietary proteins. L-Trp is unique among amino acids in the blood, in that it is bound to plasma albumin. As a result, only approximately 5% is left free and available for transport into the CNS. Free L-Trp is transported into the brain across the brain-blood barrier by an active transport system, which is not specific to L-Trp. Instead, L-Trp competes for uptake with large neutral amino acids (Leucine, Isoleucine, Tyrosine, Valine, Methionine and Phenylalanine). Therefore, the availability of free L-Trp and its concentration relative to large neutral amino acids are limiting factors for the synthesis for 5-HT. The first stage of serotonin synthesis is the conversion of L-Trp to 5-hydroxytryptophan by the enzyme L-tryptophan-5-monooxygenase (Enzyme Commission number: EC.1.14.16.4.) (this enzyme is also referred to as Trp-hydroxylase). L-tryptophan-5-monooxygenase is synthesized in serotonergic cell bodies of the raphe nuclei and is found only in cells that synthesize 5-HT. Because this enzyme has low activity (the K_M value, which is equal to the substrate concentration at which the reaction rate is half its maximal value is 30-60µmol/l for L-Trp) and is not normally saturated with its substrate, the hydroxylation is the rate limiting step of the 5-HT synthesis. The next stage in the synthesis is the conversion of 5-hydroxytryptophan to 5-HT by removing a carboxyl group. This is carried out by an enzyme called aromatic L-amino acid decarboxylase (AADC, Enzyme Commission number: EC.4.1.1.28.). AADC is present not only in serotonergic neurons but also in catecholaminergic neurons, where it converts 3,4-

dyhydroxy-phenylalanine (DOPA) to dopamine. The K_M for L-Trp of this enzyme is 10µmol/l, indicating higher activity than that of Trp-hydroxylase.

The synthesized 5-HT is stored in vesicles. Storage is carried out by a vesicular transporter. The vesicular transporter uses the electrochemical gradient generated by a vesicular H^+ATP-ase to drive transport, such that cytoplasmic 5-HT is exchanged for a luminar H^+. The release of 5-HT from the vesicles into the synaptic cleft occurs by exocytosis, i.e. the membrane of the vesicle fuses with the plasma membrane of the nerve cell and the entire content of the vesicle is discharged into the cleft.

The synaptic effect of 5-HT is terminated by the binding of 5-HT to SERT. SERT is a member of a family of ion coupled cotransporters that rely on the electrochemical gradient for Na^+ to provide the energy for the transport of substrates against a concentration gradient. SERT's closest relatives in this family are the norepinephrine transporter (NET) and dopamine transporter (DAT) with over 50% sequence homology. The SERT protein contains 630 amino acids, which form 12 transmembrane domains, with both the amino- and carboxy-termini being intracellular. The largest (second) extracellular loop contains glycosylation sites which appear necessary for optimal stability of the transporter in the membrane but not for 5-HT transport or ligand binding (Blakely *et al.* 1994) The current accepted model of transport has one Na^+, one Cl^- and one protonated 5-HT^+ binding to the transporter extracellularly to form a quarternary complex that subsequently undergoes a conformational change to release the neurotransmitter and the ions into the cytoplasm. In the cytoplasm, K^+ associates with the SERT to promote the reorientation of the unloaded carrier for another transport cycle.

Within the nerve cell, the main catabolic pathway of 5-HT is oxidative deamination and dehydrogenation to 5-hydroxyindole acetic acid (5-HIAA). In the first step monoamine oxidase (MAO, Enzyme Commission number: EC.1.4.3.4.) converts 5-HT to 5-hydroxy-indol-acetaldehyde. In humans there are two isoenzymes of MAO, referred to as type A and type B. These isoenzymes are integral proteins of outer mitochondrial membranes in neurons and in glia. The amino acid sequences of type A and type B MAOs show approximately 70% homology. The role of MAO-B in serotonergic neurons is to prevent the cells from accumulating various natural substrates (tyramine, dopamine, adrenaline and noradrenaline) that could interfere

with the storage, release and uptake of 5-HT. Meanwhile MAO-A performs an oxygenizing reaction. In the next step 5-hydroxy-indol-acetaldehyde is dehydrogenated to 5-HIAA by an NAD^+-dependent aldehyde dehydrogenase. 5-hydroxy-indol-acetaldehyde cannot normally be detected in the brain, suggesting this step occurs very rapidly. Alternatively, 5-hydroxy-indol-acetaldehyde can be reduced by aldehyde-reductase to the alcohol 5-hydroxy-tryptophol, which can be detected in the urine and in the liver. The serotonin metabolism flow-chart can be seen in Figure 4.

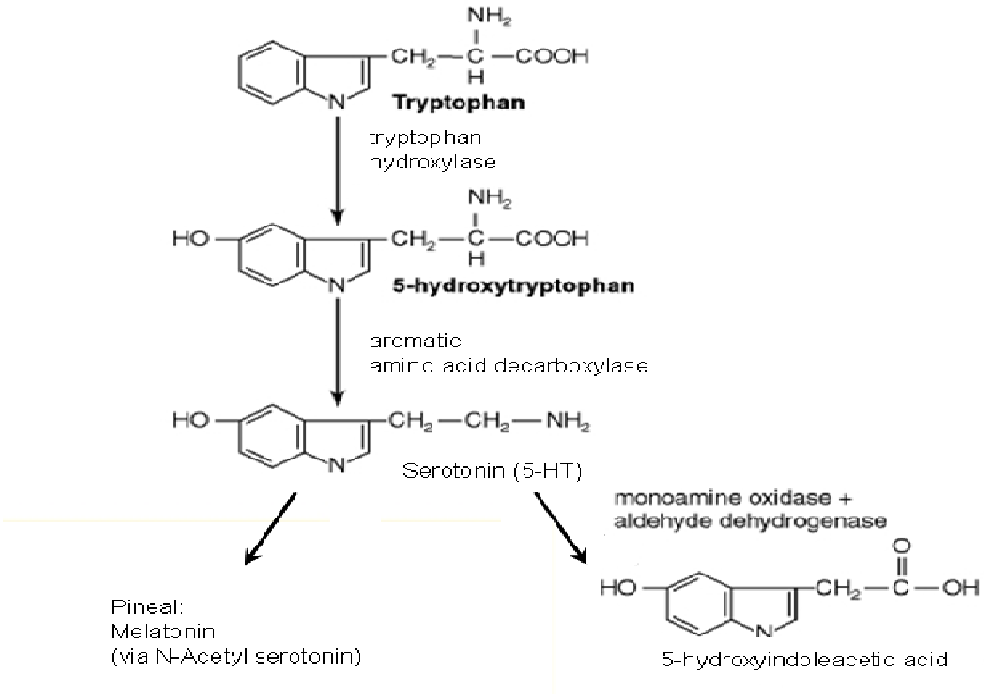


Figure 4. Serotonin metabolism. Serotonin is synthetised from amino acid L-tryptophan and the main metabolite is 5-hydroxyindole acetic acid, however in the pineal gland it is metabolised to melatonin (Modified from Siegel *et al.* 2006).

1.2.3 Serotonin receptors

In the synaptic cleft 5-HT encounters various receptors which mediate its effect. Serotonin receptors were originally classified based on pharmacological data and were termed 5-HT1, 5-HT2, 5-HT3 and 5-HT4 according to their binding profiles and secondary messenger coupling. Pharmacological and selective ligand binding studies (Barnes and Sharp 1999, Hannon and Hoyer 2008) have since identified at least 14 molecular subtypes that are divided into 7 families (5-HT1-7). The diversity of these receptors suggests an evolutionary requirement for the fine tuning of serotonergic signalling in distinct brain regions that are involved in the modulation of feeding (Jacobs *et al.* 1990), cognition (Sirviö *et al.* 1994), pain perception (Harvey *et al*. 1975), sexual behaviour (Gessa and Tagliamonte 1974), sleep (Jacobs *et al.* 1990), body temperature (Nagai 1992) and mood (Siegel *et al*. 2006). Outside the brain recent studies indicate serotonin's role in platelet aggregation (Berger *et al.* 2009) and bone remodelling (Kawai and Rosen 2010).

1.2.3.1 5-HT1 receptor family

The 5-HT1 receptor family is comprised of at least 5 subtypes classified as 5-HTR1A, 5-HTR1B (formerly 5-HT1Dβ), 5-HTR1D (formerly 5-HT1Dα), 5-HTR1E and 5-HTR1F. All of these receptors are G-protein coupled receptors with seven transmembrane domains that are generally negatively linked to adenylyl cyclase. They are encoded by intronless genes with each receptor being comprised of between 365 and 422 amino acids. The family shares 40% protein sequence homology (Barnes and Sharp 1999, Siegel *et al.* 2006).

- 5-HT1A receptor

  The gene encoding the 5-HT1A receptor (*HTR1A*) was the first 5-HT receptor to be fully sequenced (Lanfumey and Hamon 2004). This receptor is present in high density in cortical structures, in the hippocampus, and in the amygdala. In these areas, 5-HT1A receptors are located postsynaptically and are coupled to the inhibition of adenylyl cyclase and involved in the opening of potassium channels, which result in neuronal hyperpolarization. The receptors are also present in high

density on serotonergic cell body areas, particularly in the dorsal and median raphe nuclei. Here they function as somatodendritic autoreceptors and are involved in negative feedback modulation of serotonergic neuronal activity. In the dorsal raphe nucleus 5-HT1A receptors are coupled to opening potassium channels but do not appear to be coupled to the inhibition of adenylyl cyclase (Barnes and Sharp 2002, Siegel *et al.* 2006, Filip and Bader 2009).

Stimulation of 5-HT1A receptors facilitates acetylcholine and noradrenaline release in the brain, cortisol level in blood and decreases serotonin and glutamate release in the brain (Filip and Bader 2009).

- 5-HT1B receptor

In the human brain these receptors are highly expressed in the globus pallidus and the substantia nigra, with lower densities located in the hippocampus, amygdala, the hypothalamus and the frontal cortex. (Drago *et al*. 2010, Sari 2004). HTR1B is predominantly expressed as a presynaptic protein where it acts as an autoreceptor to inhibit neuronal firing. However, it also acts as a heteroreceptor on different neurons where it modulates the release of other neurotransmitters such as acetylcholine in the hippocampus and γ-amino-butyric acid (GABA) in the prefrontal cortex. It also serves as heteroreceptor for glutamate in the cingulate cortex (Barnes and Sharp, 1999). Postsynaptic HT1B receptors are also located on cerebral arteries (Siegel *et al.* 2006).

- 5-HT1D, 5-HT1E and 5-HT1F receptors

5HTR1D, 1E and 1F genes are paralogs of HTR1B in that they are related by duplication, although at some point in evolution they have acquired alternative functions. All three receptors are found in both the CNS and blood vessels. 5-HT1D receptors have a similar distribution to 5-HT1B, although 5-HT1D is much less abundant in comparison (Varnas *et al*. 2001). 5-HT1D, 1E and 1F receptors are also present in the spinal cord (Filip and Bader 2009). 5-HT1D receptors are either autoreceptors on the terminals of 5-HT neurons, where they inhibit serotonin release, or heteroreceptors on other nerve-terminals, where they control the release of GABA, acetylcholine and glutamate. Activation of these receptors inhibits 5-HT, glutamate, GABA and acetylcholine in the brain, and

reduces cortisol levels in the blood (Filip and Bader 2009). 5-HT1E receptors are mainly located in the amygdala, hippocampus, cortex (especially in the frontal and entorhinal cortices), caudate putamen and claustrum (Lanfumey and Hamon 2004). The function of this receptor remains unknown, although they may act as heteroreceptors, as 5-HT lesions do not alter the 5-HT1E receptor binding (Filip and Bader 2009). 5-HT1F receptors are found in the cortex, dorsal raphe nucleus, hippocampus and hypothalamus. Their function also remains unknown, but based on localization it has been suggested that they function as autoreceptors (Filip and Bader 2009).

1.2.3.2 5-HT2 receptor family

The three receptor subtypes in the 5-HT2 family (5-HT2A, 2B and 2C) share 46-50% sequence homology. They couple to Gq/11 and the phosphoinositol hydrolysis signal transduction system to stimulate the inositol 1,4,5- triphosphate accumulation and intracellular release (Hannon and Hoyer 2002, Siegel *et al.* 2006).

- 5-HT2A receptor

The HT2A receptor is coupled to the phospholipase C signaling cascade and increases intracellular Ca^{2+}-ion concentration. This receptor also closes K^{+} channels (Hannon and Hoyer 2002).

These receptors are found postsynaptically to serotonergic neurons and are particularly concentrated in the frontal cortex. In the medial prefrontal cortex (mPFC) they are located on GABAergic interneurons, as well as on pyramidal projection neurons, which are known to be glutamatergic, and which modulate mPFC activity. In addition, 5-HT2A receptors increase the stress-induced release of dopamine in the mPFC. Exposure to chronic, unpredictable stress and isolation has been reported to increase 5-HT2A receptor binding in the mPFC and in the amygdala (Holmes *et al.* 2008). An interesting feature of this receptor is that it shows desensitization and downregulation in response to serotonin depletion.

5-HT2A receptors are also found in high densities in parts of the limbic system, such as the amygdala and hippocampus and in the basal ganglia. In the

amygdala, they are present on the GABAergic interneurons, which probably accounts for the inhibitory actions of the 5-HT2A receptor in this region.

- 5-HT2B receptor

The expression of 5-HT2B receptor is restricted to certain brain areas, such as the cerebellum, septum, hypothalamus and amygdala. Autoradiographic studies suggest that 5-HT2B is a heteroreceptor (Filip and Bader 2009). Stimulation of these receptors in the rodent brain has been reported to yield changes in motor behaviour, food intake and pain perception and also provoked anxiolytic effects in rat social interactions (Filip and Bader 2009). This receptor is also present in a number of blood vessels (such as pulmonary arteries) (Hannon and Hoyer 2008) and has a vasodilatory role (Siegel *et al.* 2006).

- 5-HT2C receptor

These receptors are present in high density in the choroid plexus where they probably regulate the composition and volume of the cerebrospinal fluid (CSF). The 5-HT2C receptors are also found throughout the brain, in the cerebral cortex, hippocampus, amygdala and areas associated with motor behaviour such as the substantia nigra and globus pallidus (Hannon and Hoyer 2002, Siegel *et al.* 2006). 5-HT2C receptors are localized to GABA, glutamate and dopamine neurons, where they act as somatodendritic heteroreceptors. Activation of these receptors leads to hyperpolarisation in several brain areas and inhibits the release of dopamine and noradrenaline (Filip and Bader 2009).

An interesting feature of this receptor is that its transcribed mRNA undergoes post-transcriptional A-to-I RNA editing by specific enzymes. During this process specific adenosines are converted to inosines by adenosine deaminase enzymes that act on RNA. Since inosine is read as guanosine by translation machinery, A-to-I RNA editing leads to amino acid substitution (Iwamoto *et al*. 2009). To date, 14 5-HT2C receptor isoforms have been reported with different distributions in the brain (Iwamoto *et al*. 2009, Siegel *et al.* 2006). These isoforms may have different functional and regulatory properties, and thus provide a novel mechanism for the regulation of 5-HT synaptic signalling and plasticity.

1.2.3.3 5-HT3 receptor family

The 5-HT3 receptor family is a cation-selective ligand-gated ion channel (Na^+, Ca^{2+} influx, K^+ efflux) (Hannon and Hoyer 2002, Siegel *et al*. 2006).

The excess of splice variants and isoforms of this receptor is comparable to that of 5-HT2C and 5-HTR4 receptors. There exists at least five 5-HT3 genes (termed A, B, C, D and E), and their protein products are referred to as A, B, C, D and E subunits, respectively. Including the canonical receptor forms, there are four isoforms of the 5-HT3A, three of 5-HT3B, six of the 5-HT3C and three of 5-HT3E receptors that have been described. A hypothetical unprocessed variant may also exist for the 5-HT3D receptor, which has not yet been detected. Within the same region of chromosome 3, where HTR3C, D and E genes are located, maps a fourth putative gene (F). The transcript of this possible gene has not been detected so far (Walstab *et al.* 2010).

The 5-HT3 receptor functions as pentamers of several subunits. A homomer of 3A subunits is functional. Meanwhile the other subunits are not able to assemble into functional homomers but form part of the heteropentamer together with the A subunit. The reason for this may be that only the A subunit possesses the ability to integrate into the cell membrane and B, C, D and E subunits lack a Trp residue which is crucial for ligand binding (Walstab *et al*. 2010). The properties of the 5-HT3A homomer and 5-HT3AB heteromer (probably B-B-A-B-A) have been studied extensively. 5-HT3AB receptors have higher single channel conductance, lower Ca^{2+} permeability and lower 5-HT potency, compared to homomeric 5-HT3A receptors (Walstab *et al.* 2010).

The 5-HT3A and B subunits are found in the hippocampus, amygdala and caudate nucleus. 5-HT3A is also abundant in the cerebral cortex (Siegel *et al.* 2006, Filip and Bader 2009). 5-HT3 receptors are heteroreceptors, localized on GABA, glutamate and acetylcholine neurons. Their activation results in an increase in 5-HT, dopamine and GABA release and a decrease in acetylcholine release. Functional effects of these receptors are induction of motor behaviour, dysfunction of cognition, pain, nausea and vomiting. These receptors have also been shown to regulate emotional behaviour with their stimulation causing increased anxiety (Filip and Bader 2009).

1.2.3.4 5-HT4 receptor family

5-HT4, 5-HT6 and 5-HT7 receptors couple to adenylyl cyclase, increasing cAMP formation via the G_s family of G proteins. They show 35% overall protein sequence homology (Hannon and Hoyer 2002, Siegel *et al*. 2006).

Like the 5-HT3 receptor, the 5-HT4 receptor also exists in multiple isoforms, due to post-translational modification of the C-terminus. These isoforms (termed 5-$HT4_{a\text{-}h}$ and 5-$HT4_{hb}$) possess similar pharmacology (Filip and Bader 2009). Only 5-$HT4_{a\text{-}c}$ isoforms have been found in the brain where they localise in the substantia nigra, dorsal and ventral striatum, prefrontal cortex and hippocampus. This receptor is located postsynaptically to serotonergic neurons and modulates the direct release of several neurotransmitters including acetylcholine, dopamine and GABA. The receptor is also responsible for the indirect release of 5-HT (Siegel *et al.* 2006). Activation of this receptor in the brain induces neuronal excitability and slows repolarisation. In monkeys and rats 5-HT4 receptor activation has been shown to enhance cognitive performance (Siegel *et al*. 2006).

In the periphery 5-HT4 is expressed in the human atrium and may be responsible for serotonin responsiveness (Blondel *et al.* 1997). This receptor is also in the gastrointestinal tract where it evokes secretions and the peristaltic reflex (Siegel *et al*. 2006).

1.2.3.5 5-HT5 receptor family

The 5-HT5 receptor family consists of two subunits termed 5-HT5A and 5-HT5B that are located on two different chromosomes (Chromosome 7 and 2). Their sequences show approximately 80% homology, whereas their homology with other 5-HT receptors is very low (Siegel *et al.* 2006). Their preferential coupling has not been fully established yet but there is some evidence to indicate that it may be $G_{i/o}$ or G_s (Hannon and Hoyer 2008).

In rodents, 5-HT5A receptors are predominantly expressed by astrocytes in the hippocampus, hypothalamus, olfactory bulb, cerebral cortex, thalamus and striatum (Filip and Bader 2009). 5-HT5B expression has been detected in the hippocampus and dorsal raphe in rats and humans (Filip and Bader 2009). It has been proposed

that 5-HT5 receptors can act as either heteroreceptors on GABA neurons or autoreceptors in the frontal cortex of mice (Filip and Bader 2009). 5-HT5A and 5-HT4 receptors have been shown to exert a negative feedback control over the dorsal raphe nucleus via direct autoreceptor-mediated (5-HT5A) and indirect mPFC-mediated (5-HT4) mechanisms (Holmes *et al.* 2008).

1.2.3.6 5-HT6 receptor family

The 5-HT6 receptor exists as two splice variants with no known pharmacological differences between them (Filip and Bader 2009). This receptor is expressed in the striatum, amygdala, hippocampus, cortex and olfactory tubercle. It is localized postsynaptically to serotonergic neurons on acetylcholine, GABA and glutamate neurons. Receptor stimulation yields an increased 5-HT, dopamine and GABA release and a decreased release of acetylcholine (Filip and Bader 2009). During embryonic development this receptor may have a role in the 5-HT concentration-dependent interneuron migration in the cortex (Riccio *et al.* 2009).
Its importance in psychiatric research is unique since it has a high affinity for a wide variety of antipsychotic and antidepressant drugs (Siegel *et al*. 2006).

1.2.3.7 5-HT7 receptor family

5-HT7 receptors have three splice variants (5-HT7A, 5-HT7B and 5-HT7D) that differ in the C-terminus. There are no known pharmacological or signal transduction differences between them (Filip and Bader 2009).

This receptor is expressed in high density in the thalamus, hippocampus, cerebral cortex, suprachiasmatic nucleus and amygdala. It is found in lower density in the hypothalamus and the dorsal raphe nucleus. They are located on the membranes of GABA and glutamate neurons (Filip and Bader 2009). 5-HT7 receptors may be the modulators of 5-HT induced regulation of the circadian rhythm regulation in the suprachiasmatic nuclei which is the site of the mammalian circadian clock (Lovenberg *et al.* 1993). These receptors are also involved in pain perception, cognition, thermoregulation and sleep patterns (Hannon and Hoyer 2008, Siegel *et al*. 2006). This receptor may play a role in psychiatric phenotypes based on the observation that

several antipsychotics and some antidepressants display high affinity for this receptor (Siegel *et al*. 2006).

Table1 summarizes the main properties of serotonin receptors.

Table 1. Human serotonin receptors

Receptor	Distribution	Effector mechanism	Coupled
5-HT1A	Hippocampus, amygdala, septum, entorhinal cortex, raphe nuclei	Inhibition of adenylyl cyclase; opening of K^+ chanels	$G_{i/o}$
5-HT1B	Basal ganglia (particularly substantia nigra, globus pallidus, caudate putamen), frontal cortex, raphe nuclei	Inhibition of adenylyl cyclase	$G_{i/o}$
5-HT1D	Globus pallidus, substantia nigra, caudate putamen	Inhibition of adenylyl cyclase	$G_{i/o}$
5-HT1E	Cortical areas, caudate, putamen, amygdala.	Inhibition of adenylyl cyclase	$G_{i/o}$
5-HT1F	Dorsal raphe nucleus, cerebral cortex, striatum, hippocampus, thalamus, hypothalamus.	Inhibition of adenylyl cyclase	$G_{i/o}$
5-HT2A	Claustrum, cerebral cortex, olfactory tubercle, striatum, nucleus accumbens	Stimulation of phospolipase C; closing of K^+ channels	G_q
5-HT2B	Cerebellum, septum, hypothalamus, amygdala	Stimulation of phospolipase C	G_q
5-HT2C	Choroid plexus, globus pallidus, cerebral cortex, hypothalamus, septum, substantia nigra, spinal cord	Stimulation of phospolipase C	G_q
5-HT3	Hippocampus, entorhinal cortex, amygdala, caudate nucleus, cerebral cortex, dorsal vagal complex of the brainstem also in gastrointestinal tissues (colon, intestine and stomach)	Ligand-gated ion channel Na^+, Ca^{2+} influx, K^+ efflux	
5-HT4	Hippocampus, striatum, substantia nigra, dorsal and ventral striatum, prefrontal cortex, olfactory tubercle	Stimulation of adenylyl cyclase	G_s
5-HT5A	Hippocampus, hypothalamus, olfactory bulb, cerebral cortex, thalamus, striatum, pons	Inhibition of adenylyl cyclase	$G_{i/o}$ / G_s
5-HT5B	Hippocampus, habenula, dorsal raphe nucleus	Pseudogene?	
5-HT6	Striatum, amygdala, hippocampus, cortex, olfactory tubercle	Stimulation of adenylyl cyclase	G_s
5-HT7	Cerebral cortex, septum, thalamus, hypothalamus, amygdala, hippocampus	Stimulation of adenylyl cyclase	G_s

1.2.4 5-HT neurochemistry and depression; peripheral and post-mortem brain measures

1.2.4.1 Monoamine and catecholamine metabolites in the cerebrospinal fluid (CSF) of psychiatric patients

Many studies in the 1960s and 70s measured metabolites in the cerebrospinal fluid as an indirect assessment of brain chemistry in depression. Ashcroft and his colleagues (1966) reported that 5-hydroxyindoles (5-hydroxytryptamin, 5-hydroxyindolacetaldehyde, and 5-hydroxyindole-acetic acid) in the cerebrospinal fluids were lowered in depressed patients (Ashcroft *et al.* 1966). Unfortunately, further studies on indole concentrations in the CSF have failed to provide a clear result, with some studies finding reduced concentrations of the 5-hydroxyindole-acetic acid (its concentration is thought to reflect turnover of 5-HT and perhaps activity within serotonergic neurons) in depressives, while others have failed to detect a change (Brown and Linnoila 1990). One possible explanation for these contradictory findings is that 5-HIAA and other metabolites do not reflect central amine changes, but instead are a measure of the neuronal activity of the spinal 5-HT system.

1.2.4.2 Post-mortem brain studies of serotonin and its metabolites in suicide and depression

Examination of brain tissue removed post-mortem from patients provided direct measures of serotonin in the brain, and was first carried out by Shaw *et al.* (1967).A statistically significant decrease in 5-HT concentration was found in the hind-brain region of 11 previously depressed cases who committed suicide, compared to a control group of 17 individuals who had died from natural causes. This finding was replicated by Pare and colleagues (Pare *et al.* 1969) but not by another study (Bourne *et al*. 1968). The latter study also investigated the concentration of 5-HIAA and found it decreased in the hind-brains of suicide completers. Further studies investigating 5-HT and 5-HIAA concentrations in more brain regions did not find significant

differences between suicide cases and non-suicidal cases (Lloyd et al. 1974, Beskow et al. 1976, Cochran et al 1976). It is noteworthy that in these studies several other factors affected the measurements, including the handling of the tissue between the time of death and freezing and the type of death.

1.2.4.3 Post mortem studies of serotonin receptors

Developments of the original hypotheses for the aetiology of depressive disorders and the mode of action of antidepressant treatments have suggested that events at the neurotransmitter receptor level are important. Investigations of the effects of antidepressant treatment on the brains of laboratory animals have demonstrated changes in the density of 5-HT_1 and 5-HT_2 receptors. Fuxe and colleagues (Fuxe *et al.* 1981) reported a decrease in the 5-HT_1 receptors with long-term zimelidine (a 5-HT uptake inhibitor) treatment and Peroutka and Snyder (Peroutka and Snyder 1980) demonstrated a decrease in the density of 5-HT_2 receptors. This latter finding has been confirmed by many (Blackshear and Sanders-Bush 1982, Goodwin et al. 1984) though not all (Green *et al.* 1983) attempts at replication. These observations suggested hypotheses involving alterations in serotonergic receptor densities (Curzon 1982). In human studies, Stanley and Mann (1983) reported a 45% increase in the density of 5-HT_2 receptors, as assessed by ^{3}H-spiroperidol binding in frontal cortex tissue from 11 suicide victims compared to 11 control subjects thus providing further evidence that receptor density is important.

1.2.4.4 Imipramine binding site studies

The search for a peripheral marker of the central 5-HT system has focused much of its attention on blood platelets as they also contain 5-HT. Coppen *et al.* (1978) and Meltzer *et al.* (1981) have both found the uptake of 5-HT into blood platelets from depressed patients to be reduced (Coppen and Wood 1978, Meltzer et al. 1984) and have suggested that this may reflect a defect in the brain. Sites to which the antidepressant drug imipramine binds have been identified on both platelets and neurons (Langer *et al.* 1981) and seem to be closely related to the uptake sites for 5-HT (Sette *et al.* 1981, Raisman *et al.* 1982). A reduction in the number of these sites

has been found on platelets from depressed patients (Briley *et al.* 1980, Paul *et al.* 1981). Investigation of post-mortem brain tissue from suicide victims, using ^{3}H-imipramine as the ligand, has so far been inconclusive with findings for both an increase (Meyerson *et al*. 1982) and a decrease (Stanley *et al.* 1984) in the number of binding sites. Assessment methods varied between these studies making a comparison of results difficult. Perry and colleagues (Perry *et al.* 1983) examined ^{3}H-imipramine binding in samples of hippocampus and occipital cortex from a group of depressed patients who died of natural causes. They found a marked decrease in the number of imipramine binding sites in both brain regions in the depressed group compared to the control group. Treatment of laboratory animals with antidepressant drugs has been reported to reduce imipramine binding (Kinnier *et al.* 1980) Using learned helplessness (an animal model of depression), Sherman and Petty (1984) were able to demonstrate a reduction in imipramine binding in frontal cortical areas, but not in the septal or hippocampal areas of 'helpless' rats.

In conclusion, despite a number of early studies supporting the hypothesis that disturbance of serotonergic metabolism contributed towards MDD, later studies failed to find an association. Serotonin receptor (HT1A and HT2) and imipramine binding studies were a little more consistent, but were not free from contradictory results. This may be due to many confounder factors such as technology limitations for measuring, delays of processing post-mortem samples, uncertain diagnoses and the confounding effects of drug treatment. For more accurate results methods were needed which made it possible to measure the components of the serotonergic system *in vivo*. Such methods are Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET). In the next section I will summarise the findings of imaging studies focusing on those brain regions where the serotonergic system may contribute to the pathology of anxiety and depression.

1.2.5 5-HT neurochemistry and depression; molecular imaging with Positron Emission Tomography

1.2.5.1 Receptor binding studies in depression

The literature is fairly consistent on the findings that the 5-HT1A receptor binding potential (BP) is decreased in depressed in the amygdala, hippocampus and cortical regions (Sargent *et al.* 2000, Bhagwagar *et al*. 2004, Meltzer *et al.* 2004, Hirvonen *et al*. 2008, Drevets *et al.* 2007) and in epileptic depressed patients (Theodore *et al*. 2007). This lowered BP does not seem to be correlated with the severity of symptoms (Hirvonen *et al.* 2008, Drevets *et al.* 2007) and cannot be modified with Tryptophan depletion or antidepressant treatment (Meltzer *et al.* 2004), which may indicate a trait abnormality. However, the literature is not entirely in agreement as elevated 5-HT1A receptor binding in depressed patients' cortical regions, hippocampus and amygdala (Parsey *et al.* 2006b) and no differences (Mickey *et al.* 2008) have been reported.

Animal studies are generally in agreement with the findings of human studies. Subordinate monkeys who showed behavioural signs of depression after exposure to social defeat, had reduced 5-HT1A receptor binding in the raphe nuclei, amygdala, hippocampus and anterior cingulate cortex (Shively *et al.* 2006). A decrease in postsynaptic 5-HT1A receptor binding and mRNA expression is also observed in the hippocampus and the cortex of rats exposed to repeated or chronic stress (Mendelson *et al*. 1991, McKittrick *et al.* 1995, Lopez *et al.* 1998).

The binding potential of SERT in depression has also been thoroughly investigated. As with 5-HT1A the BP tends to be decreased and the areas of brain affected are predominantly the amygdala and midbrain (Miller *et al.* 2008, Parsey *et al.* 2006a). In the cingulate cortex studies have either reported an increase in SERT BP (Reivich *et al.* 2004, Cannon *et al.* 2007) no difference in BP (Meyer *et al.* 2004) and a decrease in BP (Miller *et al*. 2008). In the cerebral cortex a study reported increased BP in depressed patients with Parkinson disease (Boileau *et al.* 2008) whilst another group found no difference (Bhagwagar *et al.* 2007).

BP of 5-HT2A receptors in depression has also been investigated. The radioligand altanserin, which is a compound with a high specificity for 5-HT2A

receptors, has been used to demonstrate reduced BP in the hippocampus of depressed, medication free MDD patients (Mintun *et al*. 2004, Sheline *et al.* 2004). In these studies the cortical regions where 5-HT2A receptor is abundant showed no difference in BP between healthy controls and depressed patients, except for (Bhagwagar *et al.* 2006) which found increased BP in cortical regions of medication free recovered depressed patients but it was not correlated with the clinical features of depression (such as lifetime duration of illness or number of depressive episodes). In another study the increase of BP in the cortical regions was 21-29% higher in depressed patients compared to controls (Meyer *et al.* 2003). In contrast a 20-26% decrease in BP in the cortical regions of depressed individuals (Messa *et al.* 2003) has also been reported.

1.2.5.2 Receptor binding studies in anxiety disorders

Reduced 5-HT1A receptor binding has been observed in PD patients with various brain regions (raphe, amygdala, orbitofrontal cortex (OFC), anterior and posterior cortices) being implicated (Nash *et al.* 2008, Neumeister *et al.* 2004a). In the latter study, PD patients with comorbid MDD did not show different binding when compared to patients with MDD.

5-HT1A receptor BP has been correlated with anxiety in the cingulate cortex and orbitofrontal cortex but not in the amygdala, hippocampus and raphe in depressed, medication free patients (Sullivan *et al.* 2005). As lowered 5-HT1A BP seems to be relatively consistent in the depression and anxiety literature, a result of a study reporting decreased receptor binding after 12 weeks of treatment with the antidepressant escitalopram may seem surprising (Spindelegger *et al*. 2009). However, this may reflect that the 5-HT receptors in the CNS form a complex interaction and measuring the actions of only one of them is unlikely to unravel the aetiology of the illness.

Similar inconsistencies have been found for the 5-HT2A receptor in cortical regions and in the limbic system where the BPs have either been decreased or unaltered (Nikolaus *et al.* 2009). A study of PD showed 29% less SERT BP in cortical regions which returned to normal levels in the remitted state (Nikolaus *et al.* 2009).

In conclusion, there is some evidence of reduced 5-HT1A receptor and SERT BP in the amygdala, hippocampus and cortical regions in depression, which is supported by animal studies. The lowered 5-HT1A receptor BP seems to be present in anxiety disorders too. Results on HT2A receptor are conflicting both in depression and anxiety.

A summary of binding potential studies on different serotonergic receptors and functional and morphological changes in depression and anxiety can be seen in Appendix 10.1 (Imaging studies on depression and anxiety).

1.3 Genetics of the serotonergic system

The field of behavioural genetics has been extensively explored over the past 20 years and the high number of contrasting findings is testament to its complexity. In the following chapter I will briefly review the most important genetic studies regarding the role of the serotonergic receptors and the transporter molecule in psychiatric phenotypes, focusing on depression and anxiety.

1.3.1 Genetics of the serotonin transporter (SERT)

1.3.1.1 SLC6A4 animal models

Mice with absent or reduced serotonin transporter (Sert) activity have been generated by targeted disruption of the *Slc6a4* gene (Bengel *et al.* 1998). Gene knockout (KO) results in a failure to re-accumulate released serotonin and as a consequence there is an increase in basal extracellular levels of serotonin in the striatum, cortex (Mathews *et al.* 2004) and substantia nigra (Fabre *et al.* 2000) and also a 40–60% decreased serotonin brain–tissue concentration (Bengel *et al.* 1998, Fox *et al.* 2008, Kim *et al.* 2005). The deficient recycling of serotonin in *Slc6a4* knock-out (KO) mice causes serotonin synthesis and turnover to increase across brain regions (Fox *et al.* 2008, Kim *et al.* 2005). These KO mice also exhibit a shift in receptor binding with reduced 5-HT1A receptor binding in the raphe (Bouali *et al*. 2003, Fabre *et al.* 2000, Gobbi *et al*. 2001, Li *et al.* 1999, *Li et al.* 2000, Li *et al*. 2004), decreased 5-HT2A receptor

binding in the striatum and cortex, an increase in 5-HT2A binding in the hypothalamus (Li *et al.* 2003, Rioux *et al.* 1999) and an increase in 5-HT2C receptor binding in the amygdala and choroid plexus (Li *et al.* 2003).

These neurochemical changes cause increased anxiety-like behaviours in both male and female KO mice (Holmes *et al.* 2003a, Holmes *et al.* 2003b, Lira *et al.* 2003, Zhao *et al*. 2006, Kalueff *et al*. 2007). For example, when exposed to predator odour, Sert KO mice display an enhanced anxiety-like phenotype (Adamec *et al.* 2006, Carola *et al.* 2008). In terms of behavioural responses to stress, repeated exposure to stressful experiences causes these mice to exhibit an increased 'depression related' phenotype on the forced swim test, tail suspension test and passive avoidance task (Ansorge *et al*. 2004, Holmes *et al.* 2002a,Holmes *et al.* 2002b, Lira *et al.* 2003). These animals are also found to be less aggressive than control animals in the intruder test (Holmes *et al.* 2002a).

Interestingly, *Slc6a4* knockout mice have been found to show elevated levels of rapid eye movement (or paradoxical) sleep; an abnormality that is also found in human depression (Alexandre *et al*. 2006, Wisor *et al*. 2003). Finally, *Slc6a4* knockout mice are insensitive to the antidepressant-like effects of drugs that target Sert, such as fluoxetine, but not other monoamine transporters, such as desipramine (Holmes *et al.* 2002b).

Mice heterozygous for the *Slc6a4* KO phenotype have fewer specific Sert binding sites, decreased serotonin clearance, around three-fold elevated extracellular serotonin concentrations in striatum and cortex and reduced serotonin uptake (Bengel *et al.* 1998, Mathews *et al.* 2004, Montanez *et al.* 2003, Perez and Andrews 2005). In addition, they have unchanged tissue serotonin concentrations in the brain and periphery and have unchanged brain serotonin synthesis and turnover (Bengel *et al.* 1998, Kim *et al.* 2005, Mathews *et al.* 2004). Thus the loss of one *Slc6a4* allele leads to a decrease in many transporter functions, but this single copy of a *Slc6a4* allele is adequate to maintain overall tissue serotonin homeostasis.

As a possible explanation for the increased anxiety in the KO and Sert-decreased mice, it was found that while these animals have lower baseline adrenal and pituitary serotonin concentrations, they respond to mild stress with greater than normal level of adrenocorticotropic hormone (ACTH) (1.5 increase in the *Slc6a4* +/+ genotype, 3.5 fold in the +/- genotype and 4.5 fold in the KO animals) and oxytocin

release (Li *et al.* 2000, Li *et al.* 1999, Li *et al.* 2003, Murphy *et al*. 2001). These differences may explain one of serotonin's many roles, namely to restrain adrenomedullary activation in response to stress.

In contrast to the KO results, transgenic two to three-fold overexpression of the *Slc6a4* gene yields marked increase in SERT binding sites, reduced extracellular fluid concentration of serotonin and reduced anxiety-like behaviours (Jennings *et al.* 2006). This finding appears to indicate a direct, two-way relationship between serotonin availability and anxiety-related behaviours (Jennings *et al.* 2006). (Reviews on KO animals: Murphy *et al.* 2008, Holmes 2008**,** Kalueff *et al.* 2010, Murphy and Lesch 2008)

1.3.1.2 SLC6A4 genetic variations in humans

The mouse *Slc6a4* model finds strong parallels in the human literature. Most extensively studied variants are the 5-HTTLPR which is a common 44 base pair (bp) insertion/deletion polymorphism (short allele abbreviated to s and long allele abbreviated to l) in the promoter region of human serotonin transporter (SERT) gene (*SLC6A4*), the rs25531 (within the 5-HTTLPR) and rs25532, and the intron 2 variable number tandem repeat (VNTR) (9, 10 or 12 copies, termed STin2.9, STin2.10 and STin2.12, respectively), as well as the less common I425V, I425L, P339L and G56A variants in *SLC6A4* coding regions. The short variant of the 5-HTTLPR (14 repeats) (Nakamura *et al.* 2000) as well as the single nucleotide polymorphism (SNP) rs25531 and rs25532 variants (and most likely the shorter STin2.9 and STin2.10, 9 and 10 repeat alleles, respectively) plus the P339L SNP may be responsible for as much as 50–80% lower SERT expression (Murphy *et al.* 2008, Sakai *et al*. 2002). In contrast, the higher-expressing *SLC6A4* alleles (such as the 16 repeats long allele of the 5-HTTLPR) can result in a 5-fold increase in serotonin uptake capacity (Hu *et al.* 2006, Prasad *et al.* 2005, Wendland *et al.* 2007, Wendland *et al.* 2008b). Thus, combinations of the frequent *SLC6A4* variants, and possibly the less common variants such as P339L (Prasad *et al.* 2005), may act together to confer from five to twenty-fold differences in SERT expression and function levels (Murphy *et al.* 2008).

The human 5-HTTLPR homozygous s/s genotype plus *SLC6A4* P339L genotype closely resembles the heterozygote *Slc6a4* KO mice in regard to reduced

levels of SERT expression, decreased number of SERT binding sites and reduced 5-HT uptake rate (Murphy *et al.* 2008). Anxiety and depression-related personality traits (such as neuroticism and harm avoidance) and affective disorders including MDD (Lesch *et al.* 1996, Munafo *et al.* 2008, Sen *et al.* 2004, Lopez-Leon *et al*. 2008) have been associated with *SLC6A4* polymorphisms, particularly the 5HTTLPR s/s genotype. Other associations with *SLC6A4* have been reported for alcohol and other drug dependencies (Lesch 2005), as well as sleep and thermoregulatory disorders (Rausch *et al.* 2003, Wisor *et al.* 2003).

How the loss of 5-HT reuptake results in depression remains unknown but one hypothesis suggests that loss of *SLC6A4* gene function results in the failure of cortical systems to exert sufficient inhibitory control over the amygdala during stressful situations. Increased amygdala activity then reduces the individual's capacity to cope with stress, which results in anxiety and depression. In this model, serotonin acts as a moderator on amygdala activity. In humans, Hariri and colleagues first demonstrated in a relatively small sample (n=14) that exposure to threatening faces, a stimulus that reliably engages the amygdala in humans, produces a relatively greater amygdala response in s allele carriers than long (l) allele carriers, as measured by fMRI (Hariri *et al.* 2002). During the task, s allele carriers exhibited nearly five-fold greater amygdala activity than l/l homozygotes, a difference that accounted for approximately 20% of the total variance in the amygdala response to fearful and angry faces. This amygdala hyperactivity in s allele carriers to emotionally provocative stimuli in comparison with emotionally neutral stimuli has been corroborated by further studies in five independent cohorts, as well as panic disorder patients and social phobics (Hariri and Holmes 2006).

The 5-HTTLPR has been studied extensively in antidepressant response. Results appear to indicate that the l/l genotype is associated with better and earlier response than the s/s genotype to SSRI antidepressant treatment (such as citalopram, fluoxetine and paroxetine) (Schlosser and Kasper 2009). However, the literature is not entirely consistent. The heterozygous genotype has been shown to be associated with both better (Smeraldi *et al.* 1998, Kato *et al.* 2005) and poorer response (Yu *et al.* 2002) and the s/s genotype has been associated with better response (Kim et al. 2000) whilst in several studies no association has been found at all (Schlosser and Kasper 2009).

Finally, structural MRI studies found that the l/l genotype was associated with smaller hippocampal volumes in patients with MDD. This was also observed in geriatric depressed patients with late onset depression. However, another study was unable to show any difference in hippocampus/amygdala volume differences between the genotype groups (Frodl *et al.* 2008).

The VNTR promoter polymorphism is not the only potentially interesting variant in the human *SLC6A4* gene. An intron2 VNTR polymorphism in *SLC6A4* has also been associated with depression and suicide, although the findings have been inconsistent (Anguelova *et al.* 2003a, Anguelova *et al*. 2003b). This VNTR is an intronic 17 bp repeat, followed by an AP1 motif, a putative binding site for a transcription factor comprising the heterodimer c-fos/c-jun, and may play a role in the regulation of SERT expression (Lesch *et al.* 1994). In functional studies the 12 copy variant was shown to be the stronger enhancer when compared to the 10 copy variant (D'Souza and Craig 2006). This intronic variant as well as a rare gain-of-function mutation (I425V) (Kilic *et al.* 2003), has recently been implicated in other psychiatric conditions with major stress components, including autism and obsessive compulsive disorder (Ozaki *et al.* 2003, Sutcliffe *et al.* 2005, Delorme *et al.* 2005).

Other coding SNPs are also present in the *SLC6A4* gene. T4A and G56A (rs6355) in exon2, E215K in exon3, I425V (rs28914832) in exon9, K605N (rs6352) in exon12, P621S in exon13 seem to increase serotonin transport in transfected cells; meanwhile P33L seems to decrease transport (Murphy *et al.* 2008).

Despite SERT's important role in the synapse, the association between depression, anxiety and the *SLC6A4* genetic variations is somewhat inconclusive (Anguelova *et al.* 2003a, Lasky-Su *et al.* 2005, Gratacos *et al.* 2008). The reasons for this include the use of different diagnostic measures and population stratification where the frequencies of susceptibility alleles may be significantly different between populations and therefore give different results (Moskvina *et al.* 2010). Another factor that may result in between report discrepancies is that the different receptors (not only the serotonergic ones) act together and influence each-others function. An example of this is that in the *Slc6a4* KO mice the 5-ht1a receptors are downregulated in the raphe and 5-ht2a receptor binding is decreased in the cortex and increased in the hypothalamus. This may indicate that one receptor may compensate in response to a change in another receptor. This compensation may also occur between different

neurotransmitter systems. In *Slc6a4* KO mice the dopamine transporter (DAT) is capable of transporting the excess serotonin in the substantia nigra (Zhou *et al*. 2002). Also, the *SLC6A4* gene itself is known to have alternative promoters (in exon1) (Philibert *et al.* 2007) involved in alternative splicing (Ozsarac *et al.* 2002, Bradley and Blakely 1997) and the 3' untranslated region's variability (Vallender *et al.* 2008) can yield different mRNAs from the same gene with different stability. Finally, as an epigenetic effect, methylation also should be taken into account, as the *SLC6A4* gene has at least one methylation CpG island in its promoter (Philibert *et al.* 2007).

In conclusion, it is very likely that the serotonergic system and particularly the genotypic variants of the SERT molecule have a role in the aetiology of depression. However, being such an important molecule in the body, the level of serotonin is very tightly regulated. Therefore when searching for the biological explanations of depression and anxiety one may have to investigate additional factors.

1.3.2 Genetics of 5-HT1A receptor

1.3.2.1 Animal models

As a significantl increase in anxiety behaviour is present in both the homozygous and heterozygous *Ht1a* KO mice, it seems that unlike the serotonin transporter, the mouse requires both copies of the gene to maintain the wild-type phenotype. Indeed, both anxiety-related responses and harm avoidance have been shown to increase and exploratory activity decrease in KO homo- and heterozygous animals (Zhuang *et al.* 1999 and as reviewed in Akimova *et al.* 2009).

Using a tissue specific and conditional inducible gene KO (so called "rescue" mouse line) researchers showed that increased anxiety only appears when the receptor is not present in the raphe nuclei at a critical period of the early postnatal development, whereas KO of the gene in adulthood does not affect anxiety (Gross *et al.* 2002, Zhuang *et al.* 1999). During this critical early postnatal period the serotonergic system is still under heavy development and the Ht1a receptor may be a critical mediator of at least some of the developmental effects of serotonin. Observations, such as increased 5-HT turnover in the hippocampus of rat pups which were separated from their mothers, compared to undisturbed pups (Mitchell *et al.*

1990) and elevated anxiety-like behaviour in the offspring of mothers who lick and groom their pups less frequently (Caldji *et al.* 1998) suggest that early stress in life may cause disturbances in the serotonergic system with long lasting consequences. The rescue mouse models also showed that rescuing the expression of the Ht1a receptor in the hippocampus and the cortex in the early postnatal period is enough to rescue the behavioural phenotype of the KO mice (Gross *et al.* 2002). Overexpression of Ht1a receptors has been reported to decreas anxiety-related responses and harm avoidance as well as result in increased aggression and exploratory activity (Akimova *et al*. 2009).

Finally, it is worth mentioning that *Slc6a4* KO mice have reduced Ht1a receptor binding in the raphe, hypothalamus and amygdala (Bouali *et al.* 2003, Fabre *et al*. 2000, Gobbi *et al.* 2001, Li *et al.* 1999, Li *et al.* 2000, Li *et al.* 2004) indicating a complex interplay within the serotonergic system.

1.3.2.2 HTR1A genetic variants in humans

An SNP (rs6295, -C1019G) exists in an imperfect 26 bp palindromic region of the promoter of the gene (Wu and Comings 1999). This palindrome sequence harbours a transcription factor-binding site for two transcription factors: deformed epidermal autoregulatory Nuclear DEAF-1 Related protein (NUDR; which shares a 46% amino acid similarity with the Drosophila Deaf-1) and Hairy and enhancer of split 5 (Hes5). NUDR shows differential activity at somatodendritic and postsynaptic 5-HT1A receptors. In the raphe, NUDR has a presynaptic inhibitory action, whilst postsynaptically it acts as an enhancer of transcription in nonserotonergic neurons in the amygdala and the hypothalamus and the interneuronal cells of the cortex and hippocampus (Czesak *et al.* 2006). In a functional study it has been demonstrated that the rs6295 mutant G allele disrupts the NUDR/Hes5 binding site (Lemonde *et al.* 2003). In the raphe, the G allele up-regulates autoreceptor expression, decreasing the firing rate of these cells, and reducing serotonergic neurotransmission in projection areas (Lemonde *et al.* 2003). Consistent with this hypothesis, the G/G genotype has been associated with an increase in raphe 5-HT1A autoreceptor expression (Savitz *et al.* 2009). Meanwhile, postsynaptically the G allele results in lowered expression of the 5-HT1A receptor, as an abrogation of enhancer function

(Czesak *et al*. 2006, Savitz *et al.* 2009). This hypothesis was confirmed in a post-mortem study, which found both decreased 5-HT1A receptor and Deaf-1 protein numbers in the PFC of depressed female suicide victims (Szewczyk *et al.* 2009). The G allele also prevents the binding of Hes5 which represses transcription at both somatodendritic and postsynaptic 5-HT1A receptor expressing neurons (Czesak *et al.* 2006, Savitz *et al.* 2009). The initial finding of this polymorphism in depression and suicide (Lemonde *et al.* 2003) was replicated in numerous studies (as reviewed by Drago *et al.* 2008) and with other psychiatric phenotypes such as anxiety (Akimova *et al.* 2009 and neuroticism (Strobel *et al.* 2003) and with antidepressant treatment response (Le Francois *et al.* 2008). However, attempts at replication were not always successful (Drago *et al.* 2008, Gratacos *et al.* 2008). Another serotonin-related transcription factor, Five-prime repressor element under dual repression or CC2D1A, coiled-coil C2 domain 1A (Freud-1), may be another factor connecting the 5-HT1A receptor and stress. Freud-1 is a transcriptional repressor factor for the 5-HT1A receptor (Ou *et al*. 2003), and in male rats exposed to constant stress the mRNA and protein levels of this repressor have been demonstrated to decrease in the PFC, together with equally enhanced levels of 5-HT1A receptor mRNA (Iya *et al.* 2009). The correspondent repressor of 5-HT1A on non-serotonergic neurons is Freud-2 (CC2D1B) (Hadjighassem *et al.* 2009). It is a very attractive speculation that these repressors may malfunction in psychiatric phenotypes.

Finally, fMRI studies have reported that G allele carriers showed hyperactivity of the amygdala in response to emotional stimuli and that this was negatively associated with amygdala volume in depressed patients with borderline personality disorder (Savitz *et al*. 2009).

Several other SNPs in the *HTR1A* gene exist with possible involvement in depression, anxiety and other psychiatric illnesses (as reviewed in Drago *et al.* 2008). Among them is a non-synonymous Arg→Leu change (rs1800044) in the coding region that has been associated with impairment in signal transduction (Bruess *et al*. 2005).

In conclusion, the role of HTR1A in anxiety and depression has received much support for some well characterized functional genetic variants. However, the gene itself is very unlikely to be the only factor influencing the aetiology of depression and related behavioural traits.

1.3.3 Genetics of the 5-HT1B receptor

1.3.3.1 Animal models

Together with the 5-HT1A receptor, 5-HT1B also shows reduced receptor binding in *Slc6a4* KO homozygous and heterozygous mice models (Fabre *et al.* 2000). On the other hand, *Htr1b* KO animals show opposite phenotypes to those of *Htr1a* KO mice, namely decreased anxiety and increased exploratory behaviour in standard tests (Zhuang *et al.* 1999). These mice also show increased aggressiveness and reduced impulse control (Zhuang *et al.* 1999, Holmes 2008, Saudou *et al.* 1994) which may be important clues for suicide in human depression if it were to be interpreted as self-directed aggression. These animals are also prone to increased alcohol and cocaine consumption (Crabbe *et al*. 1996, Moret and Briley 2000, Rocha *et al.* 1997).

In rat models virus-induced overexpression of *Htr1b* in the dorsal raphe nuclei (DRN) with exposure to a stressor has been shown to increase the animals' anxiety-like behaviour 24 h later; meanwhile, in the absence of stress it increased exploratory behaviour (Clark *et al.* 2002). This suggests that the impact of overexpression of *Htr1b* in the DRN depends on the context, i.e. presence or absence of stressor provokes different types of behavioural response. An intracellular molecule, called p11 (or S100A10) also may play an important role in depression and anxiety. This molecule, which localizes 5-Ht1b receptor to the cell surface, was found to be downregulated in mouse brain after antidepressant treatment and was also downregulated in the post-mortem brains of human depressive suicide victims (Svenningsson *et al.* 2006). *P11* KO mice models showed increased depression-related behaviour, lower 5-Ht1b receptor binding and increased level of 5-HT turnover (Svenningsson *et al.* 2006). In contrast, the same study showed that overexpression of p11 gene yielded the opposite phenotype and increased functional 5-Ht1b receptors in the substantia nigra.

Htr1b animal models further corroborate the finding on the importance of stress factors in the development of depression and demonstrate important interactions between various genes.

1.3.3.2 HTR1B genetic variants in humans

The *HTR1B* gene harbours eight mutations in the coding region, one of which (rs6296; G861C) is featured extensively in the literature. Its role seems to be most significant in substance abuse/dependence (Huang *et al.* 2003, Fehr *et al.* 2000, Drago *et al.* 2010) and antisocial alcoholism (Lappalainen et al. 1998) but it also seems to be involved in depression (Huang *et al.* 2003, Drago *et al*. 2010 Review) and anxiety related disorders, such as obsessive-compulsive disorder (OCD) (Drago *et al.* 2010). The literature is not in agreement with regards to which allele confers susceptibility (C or G) and some studies have found no association (Drago *et al.* 2010, Sanders *et al.* 2002, Gratacos *et al.* 2008). In a post-mortem study the G allele was associated with a 20% reduction in receptor number in the PFC, but was not associated with depression or alcoholism (Huan *et al.* 1999). Another SNP (rs6298; C129T) was investigated in the same study, with the C allele responsible for a lower expression of 5-HT1B receptor although no association has been reported for any of the phenotypes mentioned above (Huan *et al.* 1999, Drago *et al*. 2010). Both of these variations are synonymous and therefore cause no change in the amino acid residue. Therefore, an explanation for the influence on receptor density may be that these two SNPs are in linkage disequilibrium with another functional polymorphism(s) nearby. A study investigating this possibility has found that a haplotype of HTR1B, containing the G allele of rs11568817 (T-261G) and the A allele of rs130058 (A-161T) surrounded a deletion in between (-182INS-181) and this yielded a 2.3-fold enhanced transcriptional activity. The SNPs rs11568817 and rs130058 were found in LD with rs6296 whilst rs6296 and rs6298 were in perfect LD with each other (Duan *et al.* 2003). In the same study it has been found that the rs11568817 G allele generates a new AP2 transcription factor binding site, meanwhile the T allele of rs130058 disrupts a site for c-Jun (central component of AP1 complexes) binding (Duan *et al.* 2003). On the other hand, within the 3'UTR region another polymorphism's (rs13212041) G allele disrupts a binding site for a microRNA (miR-96), which is a repressor element and which has been reported to increase expression in a reporter system (Jensen *et al.* 2009). In the same study, individuals carrying the A allele (capable of binding the silencer miR-96) were more likely to be involved in aggressive actions (Jensen *et al.* 2009) as hypothesised by KO animal models.

The role of this receptor is established in aggressivity and substance abuse with possible involvement in depression, possibly due to variations in the 5' and 3' regulatory regions. However, inconsistency within the literature indicates either false positive results or that other factors may be involved.

1.3.4. Genetics of 5-HT1D, E and F receptors

1.3.4.1 Animal models

There are no animal models existing for these receptors to date.

1.3.4.2 HTR1D, E and F variants in humans

Although the role of these receptors is not well known, genetic polymorphisms in both *HTR1D* and *HTR1E* have been associated with attention deficit hyperactivity disorder (Amin *et al.* 2009,Lasky-Su *et al.* 2008). A post-mortem study (Goswami *et al.* 2010) showed that mRNA concentrations of the 5-HT1D receptor and the transcription factor NUDR (See HTR1B receptor) were increased in dorsal raphe neurons of female MDD subjects compared to female controls. No differences were found between male patients and controls. No genetic association of the 5-HT1D receptor to depression is known but it may have a role in substance abuse and OCD (Gratacos *et al*. 2008).

As for the 5-HT1F receptor, no association with chronic fatigue syndrome (often present in MDD (Smith *et al*. 2008)) and a nominal significance in eating and bipolar disorder have been reported (Gratacos *et al.* 2008).

1.3.5 Genetics of the 5-HT2A receptors

1.3.5.1 Animal models

In homozygous *Slc6a4* KO animal models 5-HT2A receptors are decreased in the cortex but increased in the hypothalamus (Murphy and Lesch 2008).

Gene knock-out in mice yields a decrease in anxiety-like behaviour in various tests, without affecting depression-related behaviour in the forced swim test (Weisstaub *et al*. 2006). Rescuing the expression in these KO animals in the cortex was enough to normalize the anxiety-like behaviour, showing the importance of cortical 5-Ht2a receptors in stress related behaviours (Weisstaub *et al*. 2006).

Recently, a study investigated the hypothesis that increases in *Slc6a4* gene expression may influence the abundance and function of 5-Ht2a receptors, using a transgenic mouse model, which overexpresses the transporter 2-3 fold (Jennings *et al.* 2008). Using the 5-Ht2a receptor agonist 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) in these mice they found increased expression of *Arc* (activity-regulated cytoskeleton-associated protein, Lynford *et al.* 1995) and *c-fos* (an immediate early gene) genes. This is evidence of increased 5-Ht2a receptor function because the expression of these genes is mediated via the 5-Ht2a receptor, as previous studies have shown in rat models (Pei *et al*. 2000, Scruggs *et al.* 2000). The distribution of *Htr2a* mRNA and the receptor binding sites of 5-Ht2a did not differ in the brains of 5-HT overexpressing and wild type mice (Jennings *et al.* 2008).

Finally, an *in vitro* study has showed increased levels and stability of *Htr2a* mRNA levels in rat pituitary cells (P11 cells) exposed to 5-HT (Wohlpart *et al.* 1998).

1.3.5.2 HTR2A genetic variants in humans

On the National Center for Biotechnology Information (NCBI) Reference Assembly website (http://www.ncbi.nlm.nih.gov/projects/SNP/) there are 6 SNPs listed within the *HTR2A* gene which cause amino acid residue change. One of them is rs6314 in exon3 which yield in a His→Tyr change in the protein. In a study the 452Tyr residue was associated with a smaller peak amplitude in Ca^{2+}-mobilisation after stimulation with 5-HT, and also exhibited a different time course of response, compared to the 452His residue, whilst there was no observed difference between the two variants in 5-HT binding and the levels of G-protein coupled to the receptor (Ozaki *et al.* 1997). The A allele of the rs6311 polymorphism in the 5' region has been show to significantly enhance promoter activity when compared against the G allele (Parsons et al. 2004). A possible explanation for this finding is that the A allele creates a consensus binding site for Th1/E47 which is a transcription factor implicated in the

development of the nervous system (Smith *et al.* 2008). This increased promoter activity does not necessarily manifest in increased mRNA level (Spurlock *et al*. 1998) indicating the presence of perhaps additional promoter polymorphisims or balancing mechanisms.

The role of these polymorphisms along with others without known function, such as rs6313 (Bray *et al.* 2004) have been investigated in the literature of affective disorders. In MDD no association has been found for any of these SNPs (Anguelova *et al*. 2003a, Norton and Owen 2005, Gratacos *et al*. 2008). However, the SNP rs6311 has been reported to be associated with anxiety by two independent studies of obsessive-compulsive disorder (OCD) (Enoch *et al.* 1998, Walitza *et al.* 2002).

Recent investigations raised the interesting possibility of an interaction between the 5-HT2A receptor and SERT. Pharmacological studies found that efficacy of SSRI antidepressant medications were significantly associated with polymorphisms in the *HTR2A* gene (Wilkie *et al*. 2009, McMahon *et al.* 2006, Choi *et al.* 2005, Peters *et al.* 2004), whilst polymorphisms within the *SLC6A4* gene were not associated (Wilkie *et al.* 2009, McMahon *et al.* 2006, Peters *et al.* 2009, Kraft *et al*. 2007). Recently, in a genome-wide association study (GWAS) similar results were found. Ten previously MDD- or antidepressant-related SNPs of the HTR2A gene were associated either with response or remission to citalopram SSRI treatment (7 of them with both) but none of the SNPs in the *SLC6A4* gene (Garriock *et al.* 2010).

The possible functional relationship between the 5-HT2A receptor and SERT (mentioned among the animal models) may be apparent in humans, too. One study showed that *HTR2A* SNPs (rs733412, rs7997012, rs977003) were associated with SERT binding potential (which is proportional with SERT density) in the thalamus and insula, and association with another two SNPs (rs985933 and rs594242) was observed only in the thalamus (Laje *et al.* 2010). Notably, two of these SNPs (rs7997012 and rs799003) have been shown to be associated with SSRI treatment response in previous studies (McMahon *et al.* 2006, Garriock *et al.* 2010). This receptor seems to have a role in chronic fatigue syndrome, often present in depressed patients, as three SNPs (rs6311, rs6313 and rs1923884) showed significant association to this syndrome in a recent study (Smith *et al.* 2008).

Finally, like the *SLC6A4*, the *HTR2A* gene also has some very interesting features including a possible four transcription initiation sites (Shih *et al.* 1996, Zhu *et*

al. 1995). In addition, one study observed significant negative heterosis with rs6313 on neuroticism scores (Comings and MacMurray 2000). Negative heterosis occurs when heterozygous subjects show a significantly lesser effect for a quantitative or dichotomous trait than homozygous subjects. Another study suggested gender differences in the allele distribution of the rs6311 polymorphism in OCD, with female OCD patients possessing a higher A allele frequency compared to male OCD patients (Enoch *et al.* 2001). Gender differences have also been noticed in receptor binding, with men demonstrating significantly more receptor binding capacity than women, especially in the frontal and cingulate cortices (Biver *et al.* 1996) and expression of *HTR2A* (together with *HTR2C*) being generally higher in men than in women (Sugden *et al*. 2009).

1.3.6 Genetics of 5-HT2B receptors

1.3.6.1 Animal models

In mice inactivation of this gene led to embryonic and neonatal death caused by heart defects, accompanied by reduction in the levels of a tyrosine kinase receptor, ERBB2 (Nebigil *et al.* 2000). This result suggests that 5-HT2B receptors use tyrosine kinase receptor ERBB2 signaling pathway for cardiac differentiation.

1.3.6.2 HTR2B genetic variants in humans

This receptor has been rarely investigated in psychiatric phenotypes. One study which investigated various psychiatric phenotypes such as major depression, panic disorder, OCD, and substance abuse found no evidence of association (Gratacos *et al.* 2008). However, in another study that investigated (Lin *et al.* 2004) two missense variations in the coding region (Arg6Gly and Glu42Gly), observed that the Gly/Gly variant showed a trend toward drug abuse. In the same study and a third variant (a synonymous Gln11Gln) was significantly associated with drug abuse. Furthermore, the 247 bp allele of a microsatellite marker (termed D2S427, a tetranucleotide repeat) which is 216 kb upstream of the *HTR2B* gene has also been demonstrated to be

significantly associated with drug abuse (Lin *et al.* 2004). Finally, a group investigating the role of serotonergic genes in chronic fatigue syndrome which often accompanies depression, found no evidence for this gene's role in the syndrome (Smith *et al.* 2008).

1.3.7 Genetics of 5-HT2C receptors

1.3.7.1 Animal models

In *Slc6a4* KO homozygous mice 5-Ht2c binding sites are increased in the amygdala and the choroid plexus (Murphy *et al.* 2008).

Mice lacking 5-Ht2c receptors suffer from obesity by abnormal feeding behaviour, are prone to spontaneous death by convulsions (Telcott *et al.* 1995) and dysregulation in anxiety-like behaviours (Heisler *et al.* 2007).

This receptor is unique among the serotonin receptors, in that its mRNA undergoes A-to-I type RNA editing. During this process two adenosine-deaminase enzymes acting on the mRNA convert adenosine to inosine on the pre-mRNA. These inosine residues are then recognized as guanosines by the translation machinery, and depending on the number of editing sites, yield several different mRNAs and proteins. There are 11 potential transcription combinations, generating 7 protein isoforms in rats due to four editing sites (A, B, C and D). The editing occurs in the second intracellular loop of the receptor, which is crucial for the association to G-proteins. Therefore, it is proposed that the different variants have different receptor activities (Burns *et al.* 1997). Without major changes in the different receptor isoforms' ligand binding activity, the fully edited isoform (termed VSV) exhibits markedly reduced receptor-G protein coupling, meaning less efficient coupling to the intracellular signaling machinery. The distribution of 5-Ht2c isoforms has been shown to be different across brain regions (Burns *et al.* 1997). For example a partially edited isoform termed VNV is present in 36% of the hippocampus and only 6% in the choroid plexus. On the other hand, a common variant in the choroid plexus (INV, 38%) is not present in the hippocampus (Burns *et al*. 1997). Later, with a discovery of

an additional minor editing site (termed E) the number of possible mRNA variants rose to 32 and the number of proteins to 24 (Niswender *et al*. 1998).

In a rat model of depression (termed learned helplessness model; LH), mRNA editing efficiency at site E is increased (Iwamoto et al. 2005). This study also showed that administration of the SSRI fluoxetine decreased mRNA editing in three sites (A, B and E) and imipramine administration decreased at site E, and that the animals underwent a significant reversal of the LH condition (Iwamoto *et al.* 2005).

mRNA editing of the 5-Ht2C receptor is present in mice too, which have five editing sites (Niswender *et al.* 1998, Du *et al*. 2006). Whilst measuring the mRNAs in four different developmental stages of the mouse brain, a study (Wahlstedt *et al*. 2009) found that A-to-I editing is low in the early embryogenesis, with the non-edited version INI being the most common (39% throughout the brain), and that this steadily increased during development. At later postnatal stages only 7% percent of the transcripts are non-edited (Wahlstedt *et al.* 2009). This indicates that mRNA editing has an important role during brain maturation.

1.3.7.2 HTR2C genetic variants in humans

Many edited mRNA variants has been reported in humans as well as animals. With five editing sites (termed A, B, C, D and E) the possible *HTR2C* mRNA varieties are 32 with 24 protein isoforms. In the human brain the full editing (change in all possible amino acids) is more prevalent (45%) than in the rat brain (11%) (Fitzgerald *et al.*1999). Unlike in rats, human mRNA editing changes the receptor's ligand binding affinity. Finally, mRNA editing reduces both affinity of ligand binding and receptor-G protein binding in a graded fashion, with the unedited isoform (INI) having the least apparent reduction and the fully edited VGV isoform having the greatest reduction in binding (Fitzgerald *et al.* 1999, Niswender *et al.* 1999). Figure 5 summarises the amino acid residues of mRNA editing in human HTR2C.

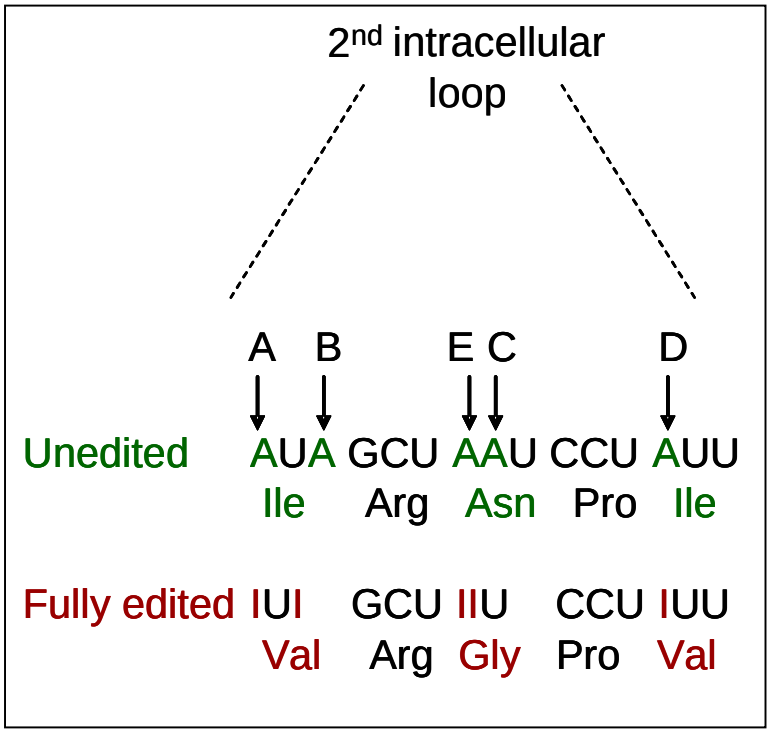


Figure 5. The editing of human HTR2C mRNA
(Adapted from Iwamoto *et al.* 2009)

Studies using post-mortem brains attempted to link the mRNA editing variants to affective disorders like depression. Unfortunately, these studies reported mixed results. In a study (Gurevich *et al*. 2002) comparing mRNA variants in the dPFC of healthy controls and depressed suicide victims it was found that while non-edited mRNA was commonly found in controls, it was very rare in depressed suicides. Also, in the brains of suicide victims increased E and C side editing, and decreased D side editing was observed (Gurevich *et al*. 2002). Another study also found increased editing in depressives but in the D side in the PFC (Iwamoto and Kato 2003). A third study did not find any difference between controls, depressives and schizophrenics and reported only a small increase in A editing side in suicides, regardless of the diagnosis (Niswender *et al.* 2001).

According to the NCBI Reference Assembly website (build 132) there are over 2500 SNPs in the human *HTR2C* gene. Among the 10 missense variations is rs6318 G/C, which causes a Cys→Ser amino acid change in position 23 (exon4), disrupting a disulfide (S-S) bridge in the protein (Drago and Serretti 2008). The Ser23 allele was shown to be more active than the Cys23 allele (Okada *et al.* 2004). Associations between this polymorphism and depression, drug efficacy and side effect studies

have yielded inconclusive results (Frisch *et al.* 1999, Lerer *et al.* 2001, Gratacos *et al.* 2008, Drago and Serretti 2008). The SNP rs6318 has been investigated in other psychiatric phenotypes, and produced more consistent results in eating and bipolar disorders (Drago and Serretti 2008) but once again not all studies found an association (Gratacos *et al.* 2008). A polymorphism in the 5' UTR region (rs3813929; -759C/T), where the C allele is associated with a reduction in transcriptional activity, has been reported to significantly influence antidepressant drug induced weight gain, but not all studies could replicate this finding (Drago and Serretti 2008). Two microsatellite repeated sequences in position -175 (GT, number of repats=12-14-15-16-17-18) and a CT repeat (number of repeats=4 or 5) divided by a 45 bp spacer were also investigated in MDD in females, with no association and with no difference in expression rate (Meyer *et al*. 2002).

Finally, a recent mutation screening study (Bundo *et al.* 2010) using post-mortem brains of 58 psychiatric patients sequenced all exons, exon-intron boundaries and the promoter region and identified eight polymorphisms (among them were rs6318 and rs3813929). However, none of them was associated with altered mRNA expression or mRNA editing efficacy, nor specific to patients (Bundo *et al.* 2010), suggesting that either the study did not have sufficient power to detect association, that the HTR2C influence was dependent upon an non-investigated interaction with additional gene or environmental influences or that the *HTR2C* gene does not influence the behavioural phenotype.

1.3.8 Genetics of 5-HTR3 receptors

1.3.8.1 Animal models

In homozygous *Sert* KO mice this receptor's binding and mRNA level are increased in various brain regions, especially in the mPFC (50%) (Holmes et al. 2008).

Hht3a KO mice show enhanced amygdala-mediated fear conditioning with male mice exhibiting reduced anxiety-related behaviours (Bhatnagar *et al*. 2004). Post-developmental transgenic overexpression of the 5-Ht3 receptor results in a reduction of voluntary ethanol self-administration (Holmes *et al.* 2008).

1.3.8.2 HTR3 genetic variants in humans

The NCBI Reference Assembly lists 7 missense variations in the *HTR3A* gene. Two functional variants with unknown population frequencies are A33T and M257I in the 5-HT3A receptor which are both associated with a reduced level of cell surface expression. The T allele of another SNP in the 5' UTR region of the gene, (rs1062613; C178T) was associated with a significant increase in expression as measured using a luciferase report system (Niesler *et al.* 2001). Another study has reported that the T allele of this SNP was associated with lower harm avoidance in females (Melke *et al*. 2003). The C/C genotype has also been shown to significantly increase harm avoidance and neuroticism (Mizuta *et al.* 2008). An fMRI study indicated that the C/C genotype was associated with increased levels of neuronal activation in the right amygdala and the PFC during a face recognition task (Iidaka *et al.* 2005) and participants showed a faster reaction time compared with those possessing the C/T genotype. Two other SNPs (rs1150226 and rs1176713) showed no association to depression in an Estonian study (Koks *et al.* 2006). Finally, rs1062613 may have a role in antidepressant response in anxiety symptoms (Kato *et al*. 2006).

The *HTR3B* gene harbours 6 missense polymorphisms (NCBI Reference Assembly). One of them is rs1176744 (A386C) which is thought to be functional. The A→C transversion causes a Tyr→Ser change with functional consequences. The mutant receptor's deactivation and desensitization kinetics are slower, due to a 7-fold increased mean open time (Krzywkowski *et al.* 2008). This mutation does not affect the receptor's surface expression or 5-HT binding (Krzywkowski *et al.* 2008). This finding was confirmed in another study which found that the mutant receptor had significantly increased eliciting concentration of 5-HT (a measure of 5-HT response) without cell surface expression alteration (Walstab *et al*. 2008). Interestingly, in a haplotype analysis a haplotype block (containing rs1176744) was associated with depression in females. Tyr/Tyr variant carrying individuals were found in significantly higher frequency in the depressed group (Yamada *et al.* 2006), indicating a Ser variant's protective role against depression.

Other functional mutations V183I (rs17116138) and S156R (rs72466469) (Walstab *et al.* 2008) and a deletion termed -100_-102AAG (rs3831455) (Meineke *et al.* 2008) have also been identified but their role in depression and anxiety has not yet been extensively investigated. However, one study observed significant improvement in somatic anxiety in response to paroxetine treatment (Kato *et al.* 2006).

The *HTR3C*, *HTR3D* and *HTR3E* genes also harbour polymorphisms, but these have not been investigated in depression and anxiety (Walstab *et al.* 2010).

1.3.9 Genetics of 5-HT4 receptors

1.3.9.1 Animal models

5-Ht4 KO mice show normal feeding and motor behaviours under baseline conditions, but stress-induced hypophagia (reduced food intake) and novelty-induced exploratory activity were decreased in these animals (Compan *et al*. 2004).

1.3.9.2 HTR4 genetic variants in humans

This receptor has not been investigated extensively in psychiatric phenotypes, but it may play a role in affective disorders. In a Japanese sample, haplotype analysis showed association between bipolar disorder (but not to depression) and two polymorphisms surrounding exon d, which encodes the divergent C-terminals of the receptor (Ohtsuki *et al.* 2002). This association was confirmed in an independent sample in the same study. However, other polymorphisms in exon1 to intron4 were not associated with any psychiatric phenotypes (Ohtsuki *et al.* 2002). An earlier study did not find an association between this receptor and various psychiatric phenotypes, whilst recent literature provides evidence of this receptor's role in depression (Lewis *et al.* 2010) and in antidepressant response (Uher *et al.* 2010). No SNP in this region showed association to chronic fatigue syndrome in another study (Smith *et al.* 2008).

1.3.10 Genetics of 5-HT5 receptors

1.3.10.1 Animal models

The *Htr5a* KO mice showed increased exploratory behaviour, but not apparent changes in anxiety-like behaviours (Grailhe *et al.* 1999).

1.3.10.2 HTR5 genetic variants in humans

During a full screening of the *HTR5A* gene four variants were found: two in the 5' UTR region (-19G/C, rs79028878 and -18C/T, rs74873317) and two in the coding region (12A/T, rs6320 and 789C/T, rs6319, none of them causing an amino acid change) (Shimron-Abarbanell *et al*. 1997). These latter synonymous mutations together with a 43T/C transition (rs79630720) showed no association in alcoholic patients (Iwata *et al.* 1998). The -19G/C however, showed association to psychiatric phenotypes, including depression in another study, where the G allele had a protective effect (Birkett *et al.* 2000). In the same study 12A/T also showed association to depression, although an attempt at replication by an independent study did not find an association (Arias *et al.* 2001). *HTR5A* SNPs showed no association to chronic fatigue syndrome (Smith *et al.* 2008) or psychiatric phenotypes (Gratacos *et al.* 2008) in the two aforementioned studies.

HTR5B is considered as a pseudogene, due to the presence of a stop codon within the gene which would result in the expression of a short, probably non-functional protein (Rees *et al.* 1994).

1.3.11 Genetics of 5-HT6 receptors

1.3.11.1 Animal models

5-Ht6 KO mice appeared to perform normally in a wide-variety of cognition and anxiety tests (Bonasera *et al.* 2006) but showed reduced response to the ataxic and sedative effects of ethanol, without differences in ethanol-induced hypothermia, compared to the wild-type.

In rats, continuous intraventricular infusion of 5-HT6 receptor antisense oligonucleotides yielded increased anxiety-like behaviours (Otano *et al.* 1999).

1.3.11.2 HTR6 genetic variants in humans

The *HTR6* SNP rs1805054 showed a positive heterosis effect in response to antidepressant treatment in depressive patients in a Korean study (Lee *et al.* 2005). However a case-control study using five haplotype-tagging SNPs (including rs1805054) and meta-analysis of four studies of rs1805054 found no evidence of the association between any of these polymorphisms and MDD (Fukuo *et al.* 2010). The 5-HT6 receptor showed no association to chronic fatigue syndrome (Smith *et al.* 2008) or psychiatric phenotypes (Gratacos *et al.* 2008) in the two aforementioned studies.

1.3.12 Genetics of 5-HT7 receptors

1.3.12.1 Animal models

5-Ht7 KO mice showed no difference in psychotic and anxiety states compared to wild-type animals (Guscott *et al.* 2005). In the forced swim test they showed a significant decrease in immobility (Guscott *et al.* 2005). Similar findings were reported in a tail suspension test (Hedlund *et al.* 2005). (The forced swim and tail suspension tests are highly predictive for antidepressant drug activity.) KO animals also showed indications of disturbed circadian rhythm (Guscott *et al.* 2005) where they spent less time in and had less frequent episodes of rapid eye movement (REM) sleep, which is also consistent with the antidepressant phenotype (Hedlund *et al.* 2005). KO animals also showed evidence that 5-HT induced hypothermic response may be modulated by this receptor (Hedlund *et al.* 2003).

1.3.12.2 HTR7 genetic variants in humans

Despite the implications from animal and pharmacological studies the role of this receptor has been investigated in only in a few studies which found no association

with fatigue (Smith *et al.* 2008) or depression although an association with panic disorder has been reported (Gratacos *et al.* 2008).

Table 10.2 in the Appendix shows the positions of serotonergic receptor genes and the SLC6A4 gene in the human genome.

1.4. Genome-wide association studies

In genome-wide association studies (GWAS) a large number of genetic polymorphisms across the genome are examined for association with phenotypes. The GWAS are conducted on chips that can characterize over a million SNPs and copy-number variants (CNV). These common variants (minor allele frequency >5%) cover around 90% of the human genome for those of European or East Asian ancestry (Craddock *et al.* 2008, Psychiatric GWAS Consortium Coordinating Committee 2009b).

To date 9 GWAS on MDD have been conducted, with a total of 12926 case and 9618 control subjects (Psychiatric GWAS Consortium Coordinating Committee 2009a, Psychiatric GWAS Consortium Coordinating Committee 2009b). The lessons from GWAS on depression are as follows. GWAS often fails to detect genome-wide significant evidence of association to depression (Shi *et al.* 2010, Shyn *et al.* 2009 , Muglia *et al.* 2010) and they occasionally fail to confirm previously reported associations, such as *HTR1A*, *HTR2A*, *HTR2C* and *HTR3B* (Bosker *et al.* 2010). A reason for this is that depression is thought to be genetically complex involving many small effect genes, with possible gene-gene and gene-environment interactions, and the criteria for genome-wide significance is very stringent with SNPs needing to reach 10^{-8} to be considered significantly associated. On the other hand, these studies often come up with previously unidentified loci, such as 18q22.1 (rs17077540) (Shi *et al*. 2010), 19q12 (rs12462886) (Shyn *et al.* 2009), 3p21.1 (rs2251219) (McMahon *et al.* 2010) and *Corf20* (Bosker *et al*. 2010). These findings, if they are not false positives are intriguing because they draw attention to the gene desert regions and help us, researchers to understand the organization of the genome. On a nominal significance level, (not on genome-wide significance level) GWAS corroborate previous findings,

such as *HTR2A* gene's association to antidepressant treatment (Uher *et al.* 2009) and to major depression (Muglia *et al.* 2010), together with *HTR3B* (Bosker *et al.* 2010). In a study by McMahon and colleagues the HTR2A SNP rs7997012 was associated with antidepressant response (McMahon *et al.* 2006). An association was also observed by an independent study although with the opposite direction of effect (Horstmann *et al*. 2010). Neuroticism has also been investigated in GWAS. These studies investigated healthy individuals where neuroticism was measured as a continuous trait. Because neuroticism has a high correlation with depression and is thought to share genetic determinants (Hettema *et al.* 2006), the results of GWAS are often similar to those of depression studies. For example, neuroticism GWAS has failed to confirm previously implicated genes from candidate gene studies including serotonergic receptors and SLC6A4 (van den Oord *et al*. 2008, Shifman *et al.* 2008, Terracciano *et al.* 2010). However, new genes have been identified including *MAMDC1*, a gene involved in regulating neuronal migration and axonal guidance (van den Oord *et al.* 2008) and phosphodiestarease 4D cAMP specific (*PDE4D*) gene (Shifman *et al.* 2008). Also, they have found significant associations in gene desert regions (rs6047641, rs1159275 and rs7329003) (Terracciano *et al.* 2010). The most interesting finding was a confirmation of a previously suspected gene's involvement in depression, *SNAP25*. This is a 25kDa synaptosomal associated protein with role in neurotransmitter release, axonal growth and synaptic plasticity. SNAP25 protein levels have been previously shown to be decreased in bipolar disorder and schizophrenia (Honer *et al.* 2002) and increased in depression (Fatemi *et al.* 2001). In a Dutch study a strong correlation was found between intelligence and three SNPs within the *SNAP25* gene in two independent cohorts (Gosso *et al*. 2006), and in a Spanish cohort nominal association was observed between this gene and anxiety and schizophrenia (Gratacos *et al.* 2008).

Finally, as these studies often use more than one independent cohort they are not always able to replicate the findings (van den Oord *et al.* 2008, Shifman *et al.* 2008, Terracciano *et al.* 2010).

GWAS are very useful tools but do have some limitations.

- Some regions of the genome are not well covered and low-frequency alleles (minor allele-frequency is less than 1%) are generally not interrogated in

current designs. Therefore, GWAS are not particularly suitable to detect rare variants of small effects.

- As GWAS produce a lot of significant results it may be tempting to focus on the strongest associations, meanwhile it has been shown that the true risk loci in suitable sample sizes may fall within the top few hundred or even thousand hits (Craddock *et al*. 2008).
- Initial GWAS analyses often assumed a simple additive genetic model. In reality it is more likely, that behind different phenotypes (especially psychiatric ones) there are a very large number of contributing loci each with very small effects, more complex single locus effect (such as dominance or heterosis), interactions (within gene, gene x gene, gene x environment) and other factors, such as epigenetics and RNA editing (Craddock *et al.* 2008).
- In psychiatric genetics the careful evaluation of phenotypes is crucial. The current official classification systems (Diagnostic and Statistical Manual of Mental Disorders and International Classification of Diseases) developed valid entities; but there are overlaps between the categories and heterogeneity within them. Among the possible solutions is to use endophenotypes which are heritable sub-clinical traits, associated with the expression of an illness (Hasler *et al.* 2004, Leboyer *et al.* 1998, Gottesman and Gould 2003). In major depression endophenotypes may be psychiatric, such as depressed mood (mood bias towards negative emotions) and anhedonia (impaired reward function) or biological such as REM sleep variations (Gottesman and Gould 2003). This approach assumes that effect sizes of genetic loci contributing to endophenotypes are larger than those contributing to disease susceptibility, hence increasing the chance that genetic linkage and association tests will detect them. While endophenotypes measures may afford greater reliability, a meta-analysis has found no evidence of this larger effect size therefore it is unlikely that endophenotypes demonstrate simple genetic aetiology (Flint and Munafo 2007). In mood disorder GWAS for more accurate diagnosis it is also advantageous to use continuous variables instead of binary ones to measure the disease.
- Finally, despite the continuously decreasing price this technique is still expensive.

1.5 Role of the environment

This ‘pure genetics’ approach is not able to answer two problems:

- Not every individual shows the signs of the psychiatric illness, even if they carry those alleles which are proven to modulate the gene’s function. This problem is mirrored very well in the inconclusive results of genetic association studies.
- These models do not take into consideration that the observed phenotype is a product of the genotype and the environment. Individuals do not exist independently from their environment, but they are in constant interaction with it. Even metabolic illnesses can be modified from the environment; a good example is phenylketonuria.

Since the infamous Harlow experiment (Harlow and Harlow 1962) it has been known that early social deprivation, such as separation from the mother, has a long-lasting and irreversible effect on the behaviour of monkeys. This experiment has been repeated with other mammalian species (including mice and rats) with the same result: animals exposed to stressful conditions during development manifest short- and long-term cognitive dysfunction and abnormal behaviour (Neigh et al. 2009).

This phenomenon exists in humans, too. Epidemiologic studies indicate that children exposed to early adverse experiences are at increased risk for depression, anxiety disorders or both (Heim and Nemeroff 2001). One such study (Chapman *et al.* 2004) showed that not only childhood emotional abuse increased the lifetime MDD with the odds ratio of 2.7 in women and 2.5 in men, but also concluded that this association was dose-dependent. In other words, multiple forms of abuse during childhood have particularly deleterious consequences on adult mental health. In some cases, childhood adverse experiences caused neurobiological changes, such as reduced hippocampal (Heim *et al.* 2008) or corpus callosum (Teicher *et al.* 2004) volumes. However, it was not always observed (Lenze *et al.* 2008).

These studies showed that adverse events contribute to the increased risk of depression and anxiety disorders. Taken together with the estimated heritability of depression, which is 30-50% (Levinson 2006) it was obvious that the individual’s

sensitivity to stressful life events is moderated by genes as well. As for which genes, the serotonergic genes, especially the transporter gene, were plausible candidates. The serotonin pathway is thought to play an important role in the hypothalamic-pituitary-adrenal (HPA) axis stress response. The amygdala triggers the response via this axis. In the amygdala the basolateral nuclei process the incoming sensory information and relay it to neurons in the central nucleus and when the central nucleus becomes active, the stress response commences. 5-HT inhibits the excitability of neurons in the basolateral nucleus of the amygdala, preventing the unnecessary stress response. In animal studies, stressed animals showed stress hormone and serotonergic abnormalities (Coplan *et al.* 2000, Rosenblum *et al.* 1994). Non-primate, human, and primate studies have found that adult influences, particularly maternal input, are critical to modulate the development of the CNS serotonin system. In the absence of adult influence, the development of serotonin functioning is impaired (Higley and Linnoila 1997). Also, the adverse consequences of early maternal separation were reversed by treatment with paroxetine SSRI antidepressants in an animal study (Huot *et al.* 2001).

The landmark study of Caspi and colleagues suggested a link between nature and nurture in depression (Caspi *et al.* 2003). In their study no direct association between the *SLC6A4* gene and depression was observed, but in young adults the effect of stressful life events of the past five years on depression symptoms was significantly stronger among individuals carrying the less active s allele than among l/l homozygotes. This study also recorded childhood maltreatment occurred during the first decade of life and concluded that childhood maltreatment predicted adult depression only among individuals carrying an s allele but not among l/l homozygotes. Many later studies found association between childhood adversity and lifetime negative events and depression too (Caspi *et al.* 2010).

In the rhesus macaque (*Macaca mulatta*) there is an analogous 21 bp length variant rh5-HTTLPR, located in the same region as the human 5-HTTLPR polymorphism (Lesch *et al*. 1997). In a study (Barr *et al.* 2003) an interaction was found between rearing condition and rh5-HTTLPR interaction for both social play and aggression. While there was no behavioural difference between genotypes in the mother-reared group, the peer-reared monkeys who possessed the rh5-HTTLPR s/l genotype of the rh5-HTTLPR were engaged less in social play and were more

aggressive, compared to the l/l genotype monkeys. The CSF 5-HIAA concentrations were significantly influenced by the rh5-HTTLPR genotype in the peer-reared, but not in parent-reared monkeys in another study (Bennett et al. 2002).

A known functional polymorphism in the *HTR1A* gene (rs6295) was also investigated, with regard to its role in MDD and negative environmental effects. In a Chinese study it was reported, that the G/G genotype had a modifying effect on the 5-HTTLPR polymorphism and MDD in interaction with negative life events, especially in the younger age group (Zhang *et al.* 2009). In a recent study the association between the rs6295 polymorphism and depression or anxiety in interaction with childhood or recent life events were not confirmed (Chipman *et al.* 2010).

The gene x environment interaction is a recent hot topic in the literature. There is no doubt, that not every attempt to replicate the original Caspi-finding has been successful (Caspi *et al*. 2010), some did not find association, and some did but in the opposite direction, like the aforementioned Chinese study (Zhang *et al.* 2009). These studies often use different methodologies to determine phenotypes and the individual genes affecting behaviour have small effect sizes. Furthermore, it appears that the effect of the stressor depends on its context (Kalin *et al.* 2008). Therefore, several researchers adopt the theory that gene x environment interaction is an important part of the genetic research and the results are not merely 'findings by chance', due to publication biases (Munafo and Flint 2009) or to the life events themselves (Risch *et al.* 2009).

As a conclusion, it appears from the gene x environment studies, that stressful life events (recent or past) affect those individuals and cause depression and anxiety disorders in those who have the less effective transporter variant (s/s genotype), meaning more synaptic 5-HT. This seems to be in line with the animal study of the elevated 5-HIAA in CSF of maternally neglected rhesus monkeys. It cannot be linked directly to the HPA axis modulated stress response, as the 5-HT is thought to inhibit the excitability of the basolateral amygdala nuclei (Cheng *et al*. 1998), therefore preventing the stress response. Furthermore, SSRI antidepressant drugs which successfully treat depression and anxiety disorders (panic disorder, for example) also increase the amount of serotonin in the synaptic cleft. Perhaps, the way out of this contradiction is the observation of poor correlation between genotype of the 5-HTTLPR and availability of serotonin transporter in the adult brain (Nordquist and

Oreland, 2010) which indicates highly complex not yet understood levels between genotypes and phenotypes.

2. Aims of the study

2.1 Aim 1

Based on the literature of association studies (Drago *et al.* 2008, Anguelova *et al.* 2003a, Savitz *et al.* 2009, Filip and Bader 2009, Sari 2004) and animal models (Murphy and Lesch. 2008, Gross *et al.* 2002, Zhuang *et al.* 1999) I sought to confirm the possible role of *HT1A* and *HT1B* autoreceptors in depression and anxiety in the New Mood Manchester population. As environmental stress factors are thought to play a role in depression and anxiety (Sullivan *et al.* 2000, Kessler 1997), I hypothesised that childhood adversity, recent life events or both contribute towards the development of these phenotypes.

fMRI studies have reported that *HTR1A* rs6295 G allele carriers showed hyperactivity of the amygdala in response to emotional stimuli (Savitz *et al*. 2009). In this study 101 healthy participants took part in a computerised face-recognition task which engages the amygdala. I hypothesised that *HTR1A* rs6295 G allele carriers react to negative emotions (fear and sadness) quicker than C allele carriers.

2.2 Aim 2

There is strong evidence in the literature that the *SLC6A4* (especially the 5-HTTLPR polymorphism) and the *HTR2A* genes are involved in depression and anxiety (Reviewed in Murphy *et al.* 2008, Norton and Owen 2005). I wished to investigate whether these genes have any role in these psychiatric phenotypes in the New Mood cohort. The Caspi study (Caspi *et al*. 2003) showed genetic interaction with environmental stress factors. I sought to attempt to replicate this finding in the New Mood cohort by including recent life events or childhood maltreatment into the association analysis.

2.3 Aim 3

In the literature there is evidence of association between *HTR2A* polymorphisms and response to SSRI treatment (Uher *et al*. 2009, McMahon *et al*. 2006, Choi *et al.* 2005). On the other hand, animal models indicate that genetic variation that alters the

SERT expression is linked to changes in the 5-HT2A receptor function (Jennings *et al.* 2008). Therefore, I wished to investigate the possible interaction between the *SLC6A4* and *HTR2A* genes.

2.4 Aim 4

While the serotonin transporter (*SLC6A4*) and serotonin receptor (*HTR1A, HTR1B* and *HTR2A*) genes have received much attention in depression research, other serotonergic receptors, such as 5-HT1D, 5-HT1E, 5-HT1F, 5-HT2B, 5-HT3A, 5-HT3B, 5-HT4, HT5A, 5-HT6 and 5-HT7 are lesser featured in the literature (Shimron-Abarbanell *et al*. 1997, Birkett *et al.* 2000, Lee *et al.* 2005). I wished to investigate the involvement of these receptors in depression and associated phenotypes (anxiety and neuroticism) using existing Genome Wide Association study data from the Dyne Steel cohort. Based on the literature I hypothesised that among these receptors 5-HT4 (Lewis *et al*. 2010, Uher et al. 2010), 5-HT6 (Riccio *et al*. 2009) and 5-HT7 (Uher *et al.* 2010, Lewis *et al.* 2010) may play a role in depression and in related phenotypes.

2.5 Aim 5

Association studies reported the possible involvement of the *HTR2C* gene in depression (Drago and Serretti 2008). I wished to investigate the association between this gene and depression in the New Mood cohort.

3. Methods

3.1 New Mood cohort

3.1.1 Participants

Manchester

This study was part of the EU funded New Mood (New Molecules for Mood Disorders) research program. The participants (aged between 18-60 years) were recruited from Greater Manchester, UK through general practices, and a web-site (http://www.newmood.co.uk).

Flow-chart of the recruitment and DNA collection process in Manchester (phase Level 1):

4500 information/questionnaire packs were sent to GPs in Greater Manchester Area (Heaton Mersey Medical Practice and Cheadle Medical Practice)

↓

Approximately 30% responded, of which approximately 60% agreed to participate

200 information/questionnaire packs were sent to people enquiring through the New Mood website (launched in August 2005)

↓

Over 65% responded

↓

Altogether, over 2000 participants returned the completed New Mood questionnaire (39% through general practices and 61% through the New Mood website). Of these, 92% were white, 68% were women (which reflects the gender ratio of people with depression), 48% had a self-reported history of depression (28% in the last year), 40% had had antidepressant treatment in the past, 35% had a family history of depression, and 84% were willing to give DNA.

↓

Participants who returned the signed consent form to provide DNA (n=1679) were then sent a genetic sampling kit.

↓

Of these subjects, 1520 returned the kit (35% from general practices and 65% from NewMood website). Women (78 vs 71% of men) and those who had a history of depression (55 vs 42% of those who reported no depression in the past) were more likely to send a DNA sample.

↓

In the study there were 1387 participants included who were Caucasian origin and had a complete New Mood questionnaire. Participants who reported manic or hypomanic episodes, psychotic symptoms or obsessive-compulsive disorder were not excluded.

Demographic data of this population can be seen in Table 2.
In the second phase (Level2) a subgroup of Level1 participants supplemented by new recruits took part in face-to-face interviews and computerized tasks (n=257).
This study was approved by the local ethics committees and was carried out in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Budapest

The recruitment was conducted by colleagues in Budapest as described elsewhere (Lazary *et al.* 2008). Participants were recruited from:

- The practices of general practitioners,
- Adult students participating in a long-distance learning program,
- Community-based population.

As the recruitment continued in Budapest in my study I used the phenotypic and genotypic data of 1053 participants instead of the 706 participants decribed by Lazary *et al.* 2008. The demographic data of this cohort can be seen in Table 2.

Table 2. Demographic data of New Mood participants

	Manchester	Budapest
Total number of volunteers included in the study	1387	1053
Women (%)	968 (70%)	733 (70%)
Men (%)	419 (30%)	320 (30%)
Age, mean (SEM) in years	33.97 (0.27)	31.2 (0.31)
Depression score (Brief Symptom Inventory, range 0-4); mean (SEM)	1.132 (0.03)	0.570 (0.024)
Anxiety score (Brief Symptom Inventory, range 0-4); mean (SEM)	1.015 (0.027)	0.689 (0.022)
Neuroticism score (Big Five Inventory, range 1-5); mean (SEM)	3.365 (0.027)	2.815 (0.026)

Abbreviation: SEM: standard error of mean.

3.1.2 Phenotypes

Hungarian participants completed the same questionnaires as the Manchester participants; validated Hungarian versions of the questionnaires were used.

3.1.2.1 Mood state

Depression and anxiety questions from the 53-item Brief Symptom Inventory (BSI) were used to measure mood state over the past week (Derogatis 1993). A continuous weighted dimension score (sum of item scores on the dimension divided by the number of items completed) was calculated as questions occasionally left unanswered by participants. The sum of Depression and Anxiety were used in my study.

The full list of questions in BSI can be found in the Appendix (10.3 Questionnaires).

3.1.2.2 Personality

To assess personality the 44-item Big Five Inventory (BFI-44) was used (John *et al.* 1991). For the analysis a continuous weighted dimension score was calculated for Neuroticism. The full list of questions used in BFI-44 can be found in the Appendix (10.3 Questionnaires).

Validation data of neuroticism and symptom scores were published previously (Juhasz *et al.* 2009). Briefly, depression and anxiety symptoms of 140 participants were also rated by independent trained investigators using the Montgomery-Asberg Depression Rating Scale (Montgomery and Asberg 1979) and the Clinical Anxiety Scale (Snaith *et al.* 1982). The results showed highly significant ($p<0.001$) correlations between the self-reported symptom scores and the independent ratings (Pearson's correlation: depression $R=0.79$; anxiety $R=0.80$) in the New Mood Manchester population. Also, 142 participants filled out both the NEO PI-R (Costa and McCrae 1992) and BFI-44 questionnaires for validation purposes and the results once again showed highly significant ($p<0.001$) correlations between the two instruments (Pearson's correlation: neuroticism $R=0.81$).

3.1.2.3 Recent negative life events

The Recent Negative Life Events questionnaire was based on the validated List of Life Threatening Experiences (LTE) (Brugha *et al.* 1985, Rijsdijk *et al.* 2001). The full list of questions used in LTE can be found in the Appendix (10.3 Questionnaires).

The sum of the life event items was used in my study.

3.1.2.4 Childhood adversity

Four questions derived from the Childhood Trauma Questionnaire (CTQ) (Bernstein *et al*. 1994) were used to determine Childhood Adversity related to emotional and physical abuse, and emotional and physical neglect. An additional question asked about parental loss during childhood. The sum of item scores was used in the analysis. Summary scores from the full version of CTQ significantly correlated with

scores derived from our short questionnaire (Pearson correlation: R=0.75, p<0.001, n=142).

The full list of five questions used in CTQ can be found in the Appendix (10.3 Questionnaires).

3.1.2.5 Face emotion recognition task

The detailed description of this task can be found in Paper 1 (The *HTR1A* and *HTR1B* receptor genes influence stress-related information processing.)

3.1.3 SNP selection

HaploView software (www.broad.mit.edu/personal/jcbarret/haploview/) was employed to identify haplotype tagging SNPs using the confidence interval method (Barrett *et al.* 2005, Gabriel *et al.* 2002). Specifications: confidence interval minima for strong LD, upper: 0.98, lower: 0.7, upper confidence interval maximum for strong recombination: 0.9, fraction of strong LD in informative comparisons at least: 0.95, markers excluded below 5% minor allele frequency, r^2=0.8, pairwise method.

The tagging was based on the CEPH population genotype data that were available at the International HapMap Project Phase I, June 2005 release (www.hapmap.org). In some cases, previously identified and possibly functional SNPs were also investigated (Duan *et al.* 2003, Lemonde *et al.* 2003, Ozaki *et al.* 1997).

3.1.4 DNA sample collection, extraction and genotyping

The same method was used for both the Manchester and the Budapest cohorts. After collection, the Budapest cohort's DNA samples were sent to Manchester for further processing. The Manchester and Budapest DNA were extracted and genotyped together. The genotyping results were sent back to Budapest.

The DNA collection and extraction is a modified version of Freeman *et al*. 2003 (Freeman *et al*. 2003).

3.1.4.1. Extraction

Sample collection buffer was made in the following concentration:
100mM NaCl;
10mM Tris HCl pH 8.0
10mM EDTA
0.2mg/ml Proteinase K enzyme
0.5% SDS
The sample collection buffer was kept at room temperature and in the dark.

2 ml of sample collecting buffer was aliquoted in 15 ml Falcon tubes. Each participant received one sample kit containing the buffer and a cytological brush (Cytobrush plus C0012, Durbin PLC) and one New Mood questionnaire booklet by post. Participants were instructed to use the cytological brush and brush inside their cheeks, gums and teeth vigorously for a minute in the morning before having lunch, drink or cleaning their teeth. Then they were asked to put the brush into the Falcon tube containing the buffer. The sample kit and the completed questionnaire were returned by pre-paid post.

The DNA extratction method:
The samples were centrifuged at 1000RPM for 1 minute which collected all the liquid at the base. Then the samples were incubated at 65^{o}C (water bath) overnight which activated the Proteinase-K enzyme. After a short centrifugation (to recover any condensation), the lids were removed and the 15 ml Falcon tubes (still containing the cytological brush) were placed upside down into larger (50ml) Falcon tubes (without lids). The double tubes were centrifuged at 2500RPM for 1 minute. The 15ml Falcon tubes were removed as well as the brushes. The supernatants were transferred to 1.5ml Eppendorf tubes (two Eppendorf tubes/sample) 50µl organic deproteinisation reagent solution (Autogen Bioclear Yeast Reagent 3) was added to each tube and was mixed vigorously. The tubes were incubated on ice for 10 min, to promote the deproteinisation reaction. The tubes were centrifuged at 13000RPM for 5 minutes, so the denatured debris and remaining organic mix compacted at the bottom of the tubes. The clear supernatants (containing the DNA) were transferred to fresh

Eppendorf tubes. The deproteinising step was repeated to yield as clean samples as possible (free from protein and other cell debris). The supernatants were transferred to 15ml Falcon tubes and 2ml 95% ethanol (room temperature) were added and mixed gently to precipitate the DNA. The samples were centrifuged at 4000RPM for 20 min, to collect the precipitated DNA. The ethanol was decanted and the tubes were left overnight to allow the pelleted DNA to dry. Next morning the dry DNA was resuspended in 100µl TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0). After resuspension, the ribonucleic acid concentration was measured with a spectrophotometer. The absorbance of the suspension was measured at 260 (absorbed by nucleic acids) and 280 nanometres (absorbed by Tryptophan and Tyrosine) (NanoDrop Technologies, USA). This technique cannot measure the DNA concentration directly. The DNA concentration is calculated by using the Lambert-Beer law:

$c=(A x \varepsilon)/b$

where
c= DNA concentration [ng/µl]
A=absorbance of the sample at 260 nm [AU]
ε=wavelength dependent extinction coefficient [ngxcm/µl]
b=path length [cm]

The purity of the sample was estimated from the 260/280 absorbance ratio. A sample purity of 1.8 was considered as pure.

The Manchester and Budapest population differed in the average DNA concentration (Table 3).

Table 3. Parameters of extraction

Samples	**Average DNA concentration (ng/µl)**	**Average purity (Absorbance ratio 260/280nm)**
from Budapest	104.922	2.1
from Manchester	328.016	2.0

The reasons for having less DNA in Budapest samples compared to Manchester samples may be due to the exposure of the sample buffer to colder temperature on the plane journey.

After measuring the DNA concentrations, samples were normalized to 20 ng/µl with TE buffer.

3.1.4.2 Genotyping by Sequenom

Primers and probes for the PCR reactions were designed by the software Primer3 version 0.4 (http://primer3.sourceforge.net)
Background of the genotyping method (Sequenom Inc. San Diego, USA)
This method's most important property is that all reactions for the iPLEX assay are terminated after a single base extension (SBE). The use of a single base extension is coupled with Matrix-Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF) mass spectrometry.

Steps involved in the iPLEX assay:

3.1.4.2.1 Amplification

In this step the amplification of a short region of DNA flanking the SNP of interest takes place. It is a standard PCR reaction performed in a Thermo-Fast® 384 PCR plate (ABgene Ltd, UK) using an MJ PTC-220 Dyad TM thermocycler (MJ research, San Francisco, Ca).

This reaction uses HotStar Taq polymerase enzyme, which is more accurate than ordinary polymerases. The accuracy of a polymerase reaction is highly dependent on the temperature. If the temperature is high enough, bonds will only proceed between corresponding base pairs, namely between A and T, G and C. At a lower temperature than the optimal melting temperature mismatches are more frequent. The false base pairing can cause problems when primers anneal to non-specific

regions and yield non-specific PCR products or in the extension step resulting in incorrect combinations of base pairs which may result in wrong genotype calls. Previously used Taq-enzymes may initiate PCR reactions before reaching the optimal temperature, while HotStar Taq requires heat activation that ensures the reaction takes place under the optimal temperature.

This amplification step can be seen on Figure 6 below, the SNP is coloured.

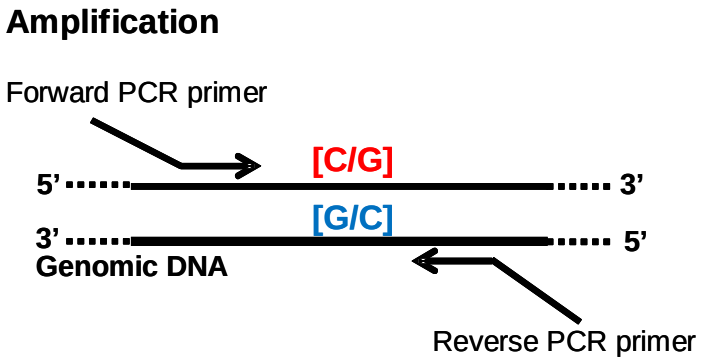


Figure 6. PCR amplification reaction

In Table 4 below the required concentrations of components of the PCR reaction can be seen as well as the reaction cycles.

Table 4. Components of the PCR reaction

Reagent	**Conc. in 5µl reaction volume**	**Volume (in 5µl reaction mix)**	**Volume of mastermix (420x)***
Nanopure H_2O	NA	2.850µl	1197µl
PCR Buffer with $MgCl_2$ (10x)	1.25x	0.625µl***	162.5µl
$MgCl_2$ (25mM)	1.625mM	0.325µl	136.5µl
DNTP mix (25mM)**	500µM	0.100µl	42µl
Primer mix (500nM each)	100nM	1.000µl	420µl
Hotstar Taq (5U/µl)	0.5U	0.100µl	42µl
TOTAL		5.000µl	2100µl
Genomic DNA (25-30ng)	25-30ng	Dried down	

*Volume includes 180µl overhang
**No more than 5 freeze thaw cycles
***The buffer concentration in the reaction is 1.25 rather than 1

5µl of PCR mastermix was added to each sample. The PCR cycles can be seen in Table 5.

Table 5. PCR cycles

Temp	Time	Aim	
94°C	15 mins	Activating HotStar Taq enzyme	
94°C	20 secs	Denature strands of template DNA	
56°C	30 secs	Primers bind to complementary DNA	35 cycles
72°C	1 min	HotStar Taq DNA polymerase extends strands from the primers using dNTPs	
72°C	3 mins	Final extending step	
10°C	forever		

3.1.4.2.2 PCR products

The amplified product is approximately 100 bp long. The exact lengths of the PCR products depend on its primers (Figure 7).

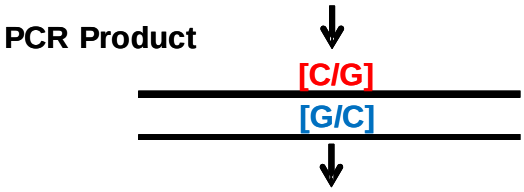


Figure 7. The PCR product

3.1.4.2.3 SAP treatment

In this step the Shrimp Alkaline Phosphatase (SAP) enzyme is used to dephosphorylate unincorporated dNTPs. It is essential; otherwise functional dNTPs can extend in the primer extension reactions causing contaminant peaks that greatly complicate data interpretation.

The compound of the SAP reaction mix is as follows (Table 6).

Table 6. Components of the SAP treatment reaction

Reagent	**Volume (in 2µl reaction mix)**	**Volume of mastermix (450x)***
Nanopure H_2O	1.530µl	688.5µl
10x SAP Buffer	0.170µl**	76.5µl
SAP enzyme (1U/µl)	0.300µl	135.0µl
TOTAL	2.000µl	900.0µl

*Volume includes 132µl overhang
**The buffer concentration in the reaction is 0.85 rather than 1

2µl of SAP mastermix was added to each 5 µl PCR reaction.

The SAP-treated PCR reaction was incubated as follows (Table 7).

Table 7. Incubation of the SAP treatment reaction

Temp	Time	Aim
37^{o}C	40 mins	Dephosphorylating reaction
85^{o}C	5 mins	Deactivating SAP enzyme
10^{o}C	15 mins	

3.1.4.2.4 The iPLEX reaction

In the next step, a PCR reaction takes place involving only one primer for each SNP.

These oligonucleotide primers (termed probes) are designed to cover the DNA directly upstream of the SNP of interest. Their length and therefore their molecular weight are also very important as multiple probes of different length can be multiplexed. The probes are then extended by a single dideoxynucleotide called a terminator nucleotide in a thermal cycled reaction.

A general method to select extension probes is to divide the probes into a low mass group and a high mass group. All probes in the high mass group are doubled in concentration with respect to the low mass group. This way, the peak heights are equilibrated. It is important to ensure that adequate signal-to-noise ratios and peak areas are generated from the high mass extension products.

The incorporated terminator nucleotides are mass modified dideoxy nucleotides (ddNTPs). This mass modification intensifies the natural molecular weight differences of DNA bases.

Due to the mass modified ddNTPs and the different molecular weight of the different extension primers, no two alleles for the iPLEX assay are within 15Da of each other. It is therefore possible to perform highly multiplexed genotyping of SNPs. Table 8 shows the mass differences of the mass modified ddNTPs.

Table 8. Mass differences between the iPLEX ddNTPs

Terminator	**A**	**C**	**G**	**T**
A	0	-24	16	55.9
C	24	0	40	79.9
G	-16	-40	0	39.9
T	-55.9	-79.9	-39.9	0

In the iPLEX step the single base extension takes place using the mass modified ddNTPs (Figure 8).

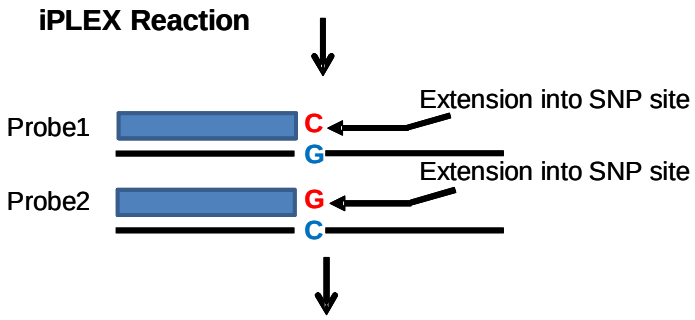


Figure 8. The iPLEX reaction

The combined items and the two-step 200 short cycles program can be seen in Tables 9 and 10 below.

Table 9. Components of the iPLEX reaction

Reagent	**Conc. in 9µl**	**Volume (in 2µl reaction mix)**	**Volume of mastermix (450x)****
Nanopure H_2O	NA	0.755µl	339.75µl
iPLEX Buffer (10x)	0.222x	0.200µl	90.00µl
IPLEX termination mix	1x	0.200µl	90.00µl
Probe mix (7µM: 14µM)*	0.625µM: 1.25µM	0.804µl	361.80µl
iPLEX enzyme	1x	0.041µl	18.45µl
TOTAL		2.000µl	900.00µl

*7µM and 14µM illustrate the doubled concentration of the high mass probes relative to the low mass probes. Low mass probes should be at 0.625µM and high mass probes at 1.25µM in the final 9µl reaction.
**Volume includes 132µl overhang

2µl iPLEX mastermix was used to each sample. The final reaction volume of each sample was 9µl.

The iPLEX reaction uses a two-step 200 short cycles program as shown in Table 10. It takes place using an MJ PTC-220 Dyad TM thermocycler (MJ research, San Francisco, Ca).

Table 10. Cycles of the iPLEX reaction

Temp	Time		
94°C	30 secs		
94°C	5 secs		
52°C	5 secs	5 cycles	40 cycles
80°C	5 secs		
72°C	3 secs		
10°C	forever		

3.1.4.2.5 Clear Resin

The iPLEX reaction has to undergo a desalting process to optimize mass spectrometric analysis. If samples are not properly desalted, sodium (22Da) and potassium (38Da) adducts are of concern since they can interfere with accurate heterozygote allele discrimination for A/C (24Da) and G/C (40Da).

3.1.4.2.6 Dispensing to SpectroCHIP Bioarrays

A nanodispenser (Sequenom Samsung Massarray Nanodispenser, Samsung Inc.) was used to dispense reaction products onto a 384-element SpectroCHIP (Sequenom Inc. San Diego, USA) bioarray, which serves as a solid matrix for the mass spectrometry.

3.1.4.2.7 MALDI-TOF Analysis

The instrument releases laser (λ=337nm) pulses onto the samples fixed on the chip producing charged ions which arrive at the detector through a vacuum area.

Spectra are acquired by measuring the time of flight. The data processing software automatically converts averaged time-of-flight to mass then to alleles.

3.1.4.3 Specifications:

All reactions were carried out according to the recommended method by Sequenom (Sequenom Inc. San Diego, USA).

Amplification step. Reagents used:

- Nanopure H_2O
- 10x PCR Buffer with $MgCl_2$ from Bioline, UK
- DNTPs were from Bioline, UK
- Primers were from Metabion, GmbH, Germany. (The list of primers can be found in Appendix 10.4.1)
- Hotstar Taq (5U/µl) from Bioline, UK

PCR reactions were performed in a Thermo-Fast® 384 PCR plate (ABgene Ltd, UK) using an MJ PTC-220 Dyad TM thermocycler (MJ research, San Francisco, Ca).

SAP treatment step. Reagents used:

- 10x SAP Buffer (iPLEX Gold, Sequenom Inc.)
- SAP enzyme (1U/µl) (iPLEX Gold, Sequenom Inc.)

SAP treatment reactions were performed in a Thermo-Fast® 384 PCR plate (ABgene Ltd, UK) using an MJ PTC-220 Dyad TM thermocycler (MJ research, San Francisco, Ca).

Primer extension step (iPLEX reaction). Reagents used:

- iPLEX Buffer (10x) (iPLEX Gold, Sequenom Inc.)
- IPLEX termination mix (iPLEX Gold, Sequenom Inc.)
- Probe mix (7µM: 14µM) were from Metabion, GmbH, Germany. (The list of probes can be found in Appendix 10.4.2)
- iPLEX enzyme (iPLEX Gold, Sequenom Inc.)

SAP treatment reactions were performed in a Thermo-Fast® 384 PCR plate (ABgene Ltd, UK) using an MJ PTC-220 Dyad TM thermocycler (MJ research, San Francisco, Ca).

Desalting reaction. Reagent used:

- Resin from Sequenom Inc. San Diego, USA

3.1.4.4 Determination of the 5HTTLPR genotype

Polymerase chain reactions (PCR) used a forward primer (5'- GCCAGCACCTAACCCCTAAT -3') labelled with 6-FAM (6-carboxyfluorescein) and a reverse primer (5'- GTAGGGTGCAAGGAGAATGC -3'), to give a product size of 366 bp and 323bp for the long and the short allele, respectively. Reactions were performed in a Thermo-Fast® 384 PCR plate (ABgene Ltd, UK) on a PTC-220 DyadTM thermocycler (Bio-rad, Hercules, CA). PCR amplification was performed in a final 10 µl volume containing 0.25 µM of each primer (Metabion GmbH, Germany), 1x NH4 buffer, 1.5 mM MgCl2, 0.1 mM of each dNTP, 0.2 units of Taq DNA polymerase (all from Bioline, UK) and 20 ng DNA. Cycling conditions were as follows: initial 15 min denaturation at 95°C, then 34 cycles of denaturation at 94°C for 20 s, annealing at 64°C for 30 s and extension at 72°C for 30 s; and a final extension for 10 min at 72°C. Polymerase chain reaction products were analyzed using an ABI3100 Genetic Analyzer and GeneScan analysis software (Applied Biosystems, Nieuwerkerk aan den IJssel, the Netherlands).

3.1.4.5 Determination of the 5-HTTLPR short/long A/G genotype (Triallelic method)

The 5-HTTLPR region underwent PCR amplification using the following primers. Forward: 5' ATGCCAGCACCTAACCCCTAATGT 3', reverse: 5' GGACCGCAAGGTGGGCGGGA 3'. Reactions were performed in Thermo-Fast® 384

PCR plate (ABgene Ltd. UK) on a PTC-220 Dyad TM thermocycler (MJ Research, San Francisco, CA). PCR amplification was performed in a final 20µl volume containing 0.25µM of each primer (Metabion GmbH, Germany),1xNH_4 buffer, 1.5mM MgCl2, 0.1mM of each dNTP, 0.4 units of Taq polymerase (all from Bioline, UK) and 20ng DNA. Cycling conditions were as follows: an initial 15 minute denaturation at 95^{o}C, then 34 cycles of denaturation at 95^{o}C for 20 seconds, annealing at 64^{o}C for 30 seconds and extension at 72^{o}C for 30 seconds, and a final extension for 10 minutes at 72^{o}C. The amplicons were 419 bp and 376 bp for the long and the short allele, respectively.

The *MspI* restriction digestion was performed in a final 20µl containing 1xNEB buffer2, 10 unit *MspI* endonuclease (both from New England Biolabs, Ipswich, MA, USA) and 10µl PCR product. The digestion was performed at 37^{o}C for 3 hours, followed by deactivation of the enzyme at 65^{o}C for 20 minutes. The digested products were ran on a 2% agarose gel with a 100bp DNA marker (Invitrogen Ltd, UK), and were visualised on a UV trans-illuminator with gel red stain (Cambridge Biosciences, UK). Long G alleles yield 152 bp fragments, long A alleles yield 326 bp fragments and short alleles yield 283 bp fragments when incubated with *MspI.* A second *MspI* site 93 bp from the 3' end of the amplicon provides a positive control for the restriction reactions yielding cut products of 326 or 283 bp for the long and short, alleles respectively.

3.1.4.6 Determination of the rs25531 genotype (TaqMan)

The TaqMan assay used a forward primer (5'- CCCTCGCGGCATCCC -3'), reverse primer (5'- ATGCTGGAAGGGCTGCA -3'), a reporter1 probe (5' CTGCACCCCCAGCAT 3') labelled with VIC® and a reporter2 probe (5' CTGCACCCCCGGCAT 3') labelled with FAM^{TM}. Reactions were performed in Thermo-Fast® 384 PCR plate (ABgene Ltd. UK) on a PTC-220 Dyad TM thermocycler (MJ Research, San Francisco, CA). The final volume was 5µl, containing 36µM of each primer, 8 µM of each reporter probe (Applied Biosystems, Foster City, CA, USA), 2.5µl 2xABI TaqMan Master Mix without UNG (Roche Molecular Systems Inc., Alameda, CA, USA) and 20ng DNA. The cycling conditions were as follows: initial enzyme activation at 95^{o}C for 10 min, then 40 cycles of

denaturation at 95^{o}C for 15 s and annealing/extension at 60^{o}C for 60 s. The reaction plate was covered with an optical lid and read on the LC480 Lightcycler (Roche Molecular Systems Inc., Alameda, CA, USA).

3.1.4.7 Genotyping success rate

Table 11 shows the genotyping success rates in the New Mood Manchester and Budapest cohorts. In the Manchester cohort the following SNPs were out of HWE: rs9316235 (HTR2A), rs2020932, rs2020933, rs2020934, rs25533 (SLC6A4) and therefore excluded from the analysis. In the Budapest cohort the success rate was lower and the SNPs included in Table 11 were in HWE.

Table 11. Genotyping success of SNPs in the New Mood Manchester and Budapest cohorts.

Chr	Gene and SNP	Cohort Manchester	Budapest
5	HTR1A_6295	96.97	
5	HTR1A_878567	99.50	
5	HTR1A_749098	96.25	
6	HTR1B_6296	93.01	
6	HTR1B_130058	91.35	
6	HTR1B_1156881	98.63	
13	HTR2A_6311	99.71	98.00
13	HTR2A_6310	92.86	61.00
13	HTR2A_985934	95.20	92.90
13	HTR2A_731779	92.10	93.90
13	HTR2A_1928040	90.60	82.90
13	HTR2A_2770296	97.30	95.40
13	HTR2A_2296972	93.50	92.60
13	HTR2A_9316232	95.70	94.20
13	HTR2A_6314	99.93	70.70
13	HTR2A_3125	92.43	57.26
17	5HTT_LPR	97.60	95.30
17	SLC6A4_25531	94.80	95.30
17	SLC6A4_2020934	99.92	70.00
17	SLC6A4_6354	99.71	70.37
17	SLC6A4_2020942	95.70	93.00
17	SLC6A4_140700	96.40	95.90
17	SLC6A4_3794808	95.20	94.60
17	SLC6A4_1042173	94.80	94.20

The average call rate in the Manchester cohort was 92.00%, in the Budapest cohort it was 89.05%.

3.2 Dyne Steel cohort

3.2.1 Participants

A detailed description of recruitment and phenotype measures for this cohort can be found in Rabbit *et al.* 2004.

Briefly, this study was funded by the U.K. Social Science Research Council in 1983 for measuring cognitive changes in increasing age. During the 20 years of the study, participants completed repeated cognitive tests as well as provided DNA samples. As part of collecting background data, the participants completed questionnaires related to their emotional well being, which included measures of depression, anxiety and personality traits, such as neuroticism.

Recruitment began in Newcastle-upon-Tyne during 1983/1984 with advertisements in local newspapers, radio and television. This attracted 2052 volunteers, 513 men aged from 49 to 86 years (mean= 65.2 years) and 1539 women aged from 46 to 92 years (mean=67.4 years). The recruitments continued until 1992 in the Newcastle-upon-Tyne area. From 1984 to 1986 similar advertisements recruited 2193 residents of Greater Manchester, 690 men aged from 45 to 93 years (mean=65.6 years) and 1503 women aged from 50 to 92 years (mean=64.4 years). The recruitment of new volunteers continued until 1994 in the Greater Manchester area.

Table 12 shows the demographic data of this cohort.

Table 12. Demographic data of Dyne Steel participants (Manchester and Newcastle together)

Total number of individuals	1563
Women (%)	1109 (71%)
Men (%)	454 (29%)
Age mean (SEM)	67.40 (1.45)
Depression score (Beck Depression Inventory, range: 0-63 (SEM)	6.834 (0.147)
Depression score (Yesavage Geriatric Depression Scale, range: 0-30) (SEM)	6.329 (0.134)
Depression score (Cordell Medical Index, range: 0-6) (SEM)	0.277 (0.018)
Anxiety score (Cordell Medical Index, range: 0-9) (SEM)	0.82 (0.033)
Neuroticism score (Eysenck Personality Questionnaire, range: 0-23) (SEM)	10.094 (0.15)

Abbreviation: SEM: standard error of mean.

3.2.2 Phenotypes

3.2.2.1 Mood state

I used the following phenotype data in my study:

Depression was assessed by

- Beck Depression Inventory, 21 items (Beck *et al*. 1961),
- Yesavage Geriatric Depression scale 30 items (Yesavage *et al*. 1983)
- Cordell Medical Inventory Depression Scale 6 items (Brodman *et al*. 1956). Anxiety was assessed by Cornell Medical Index Anxiety score 9 items (Brodman *et al.* 1956).

Beck Depression Inventory was completed in two waves:

- Manchester: Wave 1: 1985-1989 and 1991
  Wave 2: 2002
- Newcastle: Wave 1: 1983-1985, 1988-1990 and 1992
  Wave 2: 1986-1987

Yesavage Geriatric Depression Inventory was filled in

- Manchester: Wave 1: 1991 and 1996
  Wave 2: 1995 and 1999
  Wave 3: 1999 and 2003
  Wave 4: 2003
- Newcastle: Wave 1: 1991 and 1995
  Wave 2: 1995 and 2000

Wave 3: 2000

Cordell Medical Index was filled in

- Manchester: Wave 1: 1994-1996
  Wave 2: 1999
  Wave 3: 2001-2002
- Newcastle: Wave 1: 1994-1996
  Wave 2: 1999-2001

The original study was designed to measure cognition, therefore depression, anxiety and neuroticism were background information and participants often failed to fill in these questionnaires during their regular psychometric testing sessions. As a consequence despite the longitudinal design of the study data on psychiatric phenotypes were only collected once or twice during the study. To obtain the largest possible phenotypic data I compiled a data set, in which each individual has 3 scores for depression (measured on the three different inventories) and one score for anxiety. I used the first year of completion preferentially, to match this cohort's mean age as close as possible to that of the New Mood cohort. Gaps of missing data were filled in from the second year of completion, and then third, finally fourth if existed. This way I obtained a large data set, in which nearly every individual has three depression scores and one anxiety score. Even with this compilation the number of individuals with available data on psychiatric phenotypes and with DNA sample remained well below the 6000 individuals that were originally recruited on to the study. When using the data I adjusted the phenotype data according to the age of the completion year.

The full list of questions used in Beck Depression Inventory, Yesavage Geriatric Depression Inventory and Cordell Medical Index can be found in Appendix (10.3 Questionnaires).

3.2.2.2 Personality

Personality was measured by using the Eysenck Personality Questionnaire (23 items) (Eysenck and Eysenck 1964). This questionnaire was completed once during the 20 years:

- Manchester: between 1986-1990.

- Newcastle: between 1988-1991.

I used the neuroticism data in my study.

The full list of questions used in Eysenck Personality Questionnaire can be found in the Appendix (10.3 Questionnaires).

A summary table shows the phenotypic measures in the New Mood and Dyne Steel cohorts (Table 13).

Table 13. Summary table of phenotypic measures used in the study

Phenotype	**Assessed in the New Mood Manchester cohort by:**	**Assessed in the New Mood Budapest cohort by:**	**Assessed in the Dyne Steel cohort by:**
Depression	Brief Symptom Inventory Depression Score (1.132, 1.12)	Brief Symptom Inventory Depression Score (0.570, 0.78)	Beck Depression Inventory (6.83, 5.82), Yesavage Geriatric Depression Inventory (6.33, 5.30), Cordell Medical Index Depression Score (0.28, 0.71)
Anxiety	Brief Symptom Inventory Anxiety Score (1.015, 1.00)	Brief Symptom Inventory Anxiety Score (0.689, 0.71)	Cordell Medical Index Anxiety Score (0.82, 1.31)
Neuroticism	Big Five Inventory Neuroticism Scores (3.365, 1.00)	Big Five Inventory Neuroticism Scores (2.815, 0.84)	Eysenck Personality Questionnaire Neuroticism Scores (10.10, 5.93)
Recent life events	4 questions derived from the List of Life Threatening Experiences (1.32, 1.38)	Not assessed	Not assessed
Childhood Adversity	Sortened version of Childhood Trauma Questionnaire (3.70, 3.61)	Not assessed	Not assessed

Mean and standard deviation values are in brackets.

3.2.3 DNA sample collection, extraction and genotyping

Participants provided blood samples; the DNA was extracted with the phenol-chloroform method. The genotyping data were part of a GWAS dataset, performed on the Illumina 610 Quad microarray (Illumina, Inc. San Diego, CA, USA). Genotyping were carried out at the Wellcome Trust Clinical Research Facility, Edinburgh.

In my study I selected the serotonin receptors and serotonin transporter genes. In every case at least 10,000bp from the 5'UTR (untranslated region) and 4,000bp from the 3' UTR were also covered.

The following subsets of SNPs were used in the analyses:

- *HTR2A* gene: 37 SNPs, from rs7333412 to rs6306.
- *SLC6A4* gene: 12 SNPs, from rs1906451 to rs1487971.
- *HTR1D* gene: 3 SNPs, from rs2806566 to rs627304.
- *HTR1E* gene: 24 SNPs, from 87872689 to rs7339124.
- *HTR1F* gene: 4 SNPs, from rs7648805 to 9310061.
- *HTR2B* gene: 6 SNPs, from rs11694724 to rs6437002.
- *HTR3* gene (AB subunits): 35 SNPs, from rs869451 to rs4938066.
- *HTR3* gene (C-E subunits): 22 SNPs, from rs7613237 to rs9815292.
- *HTR4* gene: 41 SNPs, from rs4274968 to rs1820076.
- *HTR5A* gene: 7 SNPs, from rs6597451 to 732050.
- *HTR6* gene: 4 SNPs, from rs6693503 to rs6699866.
- *HTR7* gene: 20 SNPs, from 1107688 to rs4282910.

The full list of SNPs can be found in the Appendix (10.5 List of Illumina SNPs of Dyne Steel cohort).

3.3 Statistical analysis

Statistical analyses (correlation analysis and residual value calculation for the epistatic interaction analysis) were performed with SPSS for Windows Statistical Analysis Software, Version 15.0 (SPSS Inc. Chicago, Illinois, USA).

Haplotype analyses were performed with HelixTree^{TM} 6.4.1 (Golden Helix, USA) using haplotype trend regression.

PLINK v1.06 (http://pngu.mgh.harvard.edu/purcell/plink/) was used for testing association of different genetic models (dominant, recessive and additive; linear regression model, co-variation with age and sex), interactions between SNPs (epistasis) and with sex, and for calculating Hardy-Weinberg analysis.

Quanto 1.2 version (http://hydra.usc.edu/gxe) was employed to calculate the power of the recruited populations.

FDR Qvalue (http://genomics.princeton.edu/storeylab/qvalue/) was used to calculate false discovery rate. In paper 1 these q values are reported as a measure of significance of each test of many tests performed simultaneously.

In all cases, data were adjusted for age and sex.

A p value of < 0.05 was adopted for all statistical testing.

A web-based tool Gliders (http://mather.well.ox.ac.uk/Gliders) was used to evaluate LD values between SNPs (Study 2 and Study 3).

To evaluate the effects of SNPs on gene expression the Stanley Neuropathology Consortium Integrative Database (http://sncid.stanleyresearch.org) and the SNPExpress Database (Heinzen *et al.* 2008) were used (Study 2).

Broad Institute web-based tool Tagger was used to evaluate the tagger SNPs (http://www.broadinstitute.org/mpg/tagger/) (Study 2).

Individual description of statistical analyses, thresholds and parameters can be found in the manuscripts section.

Paper 1

The *HTR1A* and *HTR1B* receptor genes influence stress-related information processing

4. Study 1

The *HTR1A* and *HTR1B* receptor genes influence stress-related information processing

Krisztina Mekli[1,2,3], Antony Payton[2], Fabio Miyajima[2], Hazel Platt[2], Emma Thomas[1], Darragh Downey[5], Kathryn Lloyd-Williams[1], Diana Chase[1], Zoltan G. Toth[6], Rebecca Elliott[1], William E. Ollier[2], Ian M Anderson[1], JF William Deakin[1], Gyorgy Bagdy[3,4], Gabriella Juhasz[1]

[1]Neuroscience and Psychiatry Unit, School of Community Based Medicine, Faculty of Medical and Human Sciences, The University of Manchester, UK
[2]Centre for Integrated Genomic Medical Research, The University of Manchester, Manchester, UK
[3]Department of Pharmacodynamics, Semmelweis University, Budapest, Hungary
[4]Group of Neurochemistry, Hungarian Academy of Science and Semmelweis University, Budapest, Hungary
[5]Imaging Science and Biomedical Engineering, The University of Manchester, Manchester, UK
[6]Faculty of Life Sciences, The University of Manchester, Manchester, UK

Correspondence: Dr. Gabriella Juhasz, Neuroscience and Psychiatry Unit, School of Community Based Medicine, Faculty of Medical and Human Sciences, The University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, UK.
Tel: +44 161 275 6915
Fax: +44 161 275 7429
E-mail: gabriella.juhasz@manchester.ac.uk

Abstract

The serotonergic system has been widely implicated in stress related psychiatric disorders such as depression and anxiety. We investigated the possible association between depression and anxiety scores and SNPs within the *HTR1A* and *HTR1B* genes in a population sample (n=1387). There was no direct SNP-phenotype association, but in interaction with recent stressful life events rs6295 G, rs878567 T alleles and rs6296 C alleles were associated with significantly higher symptom scores. A subset of control subjects (n=101) took part in a computerised face emotion processing task. Healthy rs6295 GG carriers did not show an affective bias to perceive more negative emotions but reacted more quickly to fearful faces. Thus we conclude that the serotonin-1A receptor conveys vulnerability to these psychiatric disorders by modulating threat-related information processing. Our results extend previous findings of an interaction between stressful life events and the serotonin transporter gene to two other genes in the serotonergic pathway and emphasise the possible role of increased threat-related information processing as an intermediate phenotype.

Keywords**:** stress, HTR1A, HTR1B, depression, anxiety

Introduction

Depression has a strong genetic basis with twin studies reporting heritability estimates of 40-50% (Levinson 2006). Investigations that have attempted to identify the biochemical and genetic background of depression and the highly correlated trait of anxiety have been extensive (Mineka *et al,* 1998). Genes within the serotonergic system, particularly the serotonergic transporter gene (*SLC6A4*) have received much attention, since the proteins they encode are the most common targets for the treatment of anxiety disorders and depression. Interaction analysis, both epistatic and environmental, has provided important insights into the development of behavioural traits. In 2003 Caspi and colleagues demonstrated that stressful life events in the presence of the short allele residing within the *SLC6A4* gene promoter (5-HTTLPR) predisposed to develop depression (Caspi *et al,* 2003). Since then several studies have aimed to replicate this finding (e.g. Cervilla *et al,* 2006; Contreras *et al,* 2009; Drachmann Bukh *et al,* 2009; Lazary *et al,* 2008; Wray *et al,* 2009; Zalsman *et al,* 2006). However, a meta-analysis, which included 14 studies, has not found evidence that 5-HTTLPR is associated with depression when analysed in interaction with stressful life events (Risch *et al,* 2009). Another more extensive recent review of 34 studies found that 17 studies replicated the original Caspi-finding, 8 were partial replications and 9 were non-replications (Uher and McGuffin, 2010). Success of replication heavily depended on the method used for evaluation of phenotypes and environmental factors (Caspi *et al,* 2010; Rutter *et al,* 2009; Uher and McGuffin, 2010). As most of the evidence supports the 5-HTTLPR findings, it can be hypothesised that other genes throughout the serotonergic pathway that modulate serotonergic activity, such as the serotonin receptor genes 1A and 1B (*HTR1A* and *HTR1B*), are potential candidates to mediate the risk of stressful life events on behaviour.

5-HT_{1A} receptors are present in high density in serotonergic cell body areas, particularly in the dorsal and median raphe nuclei, where they function as somatodendritic autoreceptors. Activation of these autoreceptors has been shown to decrease the rate of firing of serotonergic neurons and cause a reduction in the release of serotonin (Bohmaker *et al,* 1993; Kennett *et al,* 1987; Savitz *et al,* 2009). 5-HT_{1A} receptors are also postsynaptic receptors with high density in the hippocampus, septum, amygdala, hypothalamus and neocortex (Hall *et al,* 1997; Pazos and Palacios, 1985). Many of the serotonergic terminal areas are located in

the limbic system which has been shown to be involved in the modulation of emotions, raising the possibility that these receptors may regulate emotional states (Drevets *et al,* 2008; Fisher *et al,* 2006). 5-HT_{1B} receptors are also located both pre- and postsynaptically and appear to regulate the release of 5-HT from the dorsal raphe nucleus (Lanfumey and Hamon, 2004). In addition, 5-HT_{1B} receptors also modulate the release of other neurotransmitters, such as acetylcholine in the hippocampus, dopamine in the prefrontal cortex and γ-amino-butyric-acid (GABA)-throughout the forebrain (Barnes and Sharp, 1999; Drago *et al,* 2010). In summary, there is evidence that the activity of serotonergic neurons, and also other neurotransmitter pathways, depends on 5-HT_{1A} and probably 5-HT_{1B} receptor function (Lanfumey and Hamon, 2004; Savitz *et al,* 2009).

Mice lacking the 5-HT_{1A} receptors show enhanced anxiety, increased response to stress (Parks *et al,* 1998) and changes of anxiety related behaviours, such as avoidance of open spaces and novel objects, increased cover seeking and reduced exploratory behaviour (Heisler *et al,* 1998). In contrast, mice without 5-HT_{1B} receptors exhibited an increase in aggression which is consistent with reduced anxiety and with increased impulsivity (Brunner and Hen, 1997; Ramboz *et al,* 1998). Other observations have shown that *Htr1B* knockout mice are more susceptible to the behavioural and reinforcing effects of cocaine (Crabbe *et al,* 1996; Rocha *et al,* 1998), suggesting increased sensitivity to reward. However, it is important to note that in knockout animals the relevant genes are completely inactivated while in cases of human polymorphisms the physiological pattern of gene expression is altered but not totally disrupted in most instances (Plomin and Crabbe, 2000).

Studies investigating the role of *HTR1A* genetic polymorphisms in human subjects with depression have produced mixed results (Supplementary material Table 1). Whilst a number of different single nucleotide polymorphisms (SNPs) have been studied, the majority of groups have looked at a functional promoter polymorphism (rs6295) which has been shown to increase binding potential in antidepressant naive major depressive disorder (MDD) patients (Parsey *et al,* 2006b). Studies that observed an association with this polymorphism found significant interaction effects, either with other genes (Anttila *et al,* 2007; Neff *et al,* 2009; Zhang *et al,* 2009) with genes and negative life events (Zhang *et al,* 2009), with interferon-induced depression (Kraus *et al,* 2007) or with sex specific effects (Wu *et al,* 2008). Interestingly, all these studies found that the presence of the

rs6295 G allele was associated with an increased susceptibility to depression. In support of these findings human drug studies have reported that carriers of the G allele respond less well to antidepressant treatment (Hong *et al,* 2006; Lemonde *et al,* 2004; Parsey *et al,* 2006a; Yu *et al,* 2006) although one study has found that carriers of the GG genotype respond better (Kato *et al,* 2009). The G allele also appears to play a role in anxiety-related disorders. In one study the G allele showed significant association to higher neuroticism scores on the NEO personality inventory (NEO-PI-R), which was mainly due to associations with the Neuroticism facets Anxiety and Depression. G allele carriers also showed higher Tridimensional Personality Questionnaire (TPQ) Harm Avoidance scores (Strobel *et al,* 2003). There was also significant association between this gene and panic disorder with agoraphobia (Rothe *et al,* 2004). In addition, GG homozygous panic disorder patients showed poorer response to selective serotonin reuptake inhibitor (SSRI) treatment (Yevtushenko *et al,* 2010).

Investigation of the *HTR1B* gene is much less extensive with one report describing an association between depression and the synonymous SNP rs6296 (Huang *et al,* 2003) and another report finding no association with two promoter SNPs rs130058 and rs11568817 (Zhang *et al,* 2009).

Depression has been associated with a general perceptual deficit for face emotions with a mood-congruent response bias towards negative emotions (Gur *et al,* 1992; Surguladze *et al,* 2004) and anxiety with an increased threat-related emotion processing (Mogg and Bradley, 2002). Negative and threat-related biases in perception, attention and memory are believed to play a key role in maintaining symptoms of depression and anxiety (Harmer *et al,* 2004; Harmer, 2008). The serotonergic system is involved in the face emotion recognition (Merens *et al,* 2007). In control subjects acute tryptophan depletion has been shown to impair emotional processing similar to that seen in acute depression, while acute administration of SSRIs showed the opposite effect (Harmer, 2008). Differences in emotional processing were apparent without measurable mood changes suggesting that face emotion recognition tasks are more sensitive to serotonergic changes than self-reported mood (Harmer *et al,* 2003; Harmer, 2008). Furthermore it has been demonstrated that the functional rs6295 polymorphism of the *HTR1A* gene influences threat-related face emotion processing and through modification of amygdala reactivity may drive trait anxiety (Fakra *et al,* 2009; Le Francois *et al,* 2008).

In our study we used a Caucasian cohort from the UK consisting of 1387 individuals who had provided information on depression, anxiety and recent negative life events. *HTR1A/1B* genotypes derived from haplotype tagging (htSNPs) and functional SNPs were analysed independently against depression and anxiety scores and for interaction with stressful life events. Gender specific effects that have been previously reported were also investigated. In addition the effect of the known functional rs6296 polymorphism (*HTR1A*) was investigated on mood- and threat-related emotional face processing in a subset of control subjects. This intermediate phenotype is correlated with depression and anxiety as we mentioned above, and may be an indication of altered serotonergic function well before symptoms occur. We hypothesised that carriers of the rs6296 G allele would show more depressive and anxiety symptoms especially in relation to life stresses and that they would be biased towards threat-related information. We also hypothesized that other polymorphisms in the *HTR1A* and *HTR1B* gene would show similar associations with depression and anxiety as rs6296.

Experimental procedures

Participants

This study was part of the EU funded NewMood (New Molecules for Mood Disorders) Integrated Program that aims to identify new molecular mechanisms of vulnerability to depression. In the first phase (Level1) we recruited and obtained DNA and completed questionnaires from 1387 unrelated Caucasian participants aged between 18-60 years from Greater Manchester, UK through general practices, and a web-site (http://www.newmood.co.uk). In the second phase (Level2) a subgroup of Level1 participants supplemented by new recruits took part in face-to-face interviews and computerized tasks. We report here on results from Level2 healthy control volunteers (55 from Level1 and 46 supplemental recruits). Details of our recruitment strategy and responses are described elsewhere (Juhasz *et al,* 2009). The study was approved by the local Ethics Committees (North Manchester Local Research Ethics Committee (LREC) (ref. 05/Q1406/26), the University of Manchester's Ethics Committee (ref. 05056)) and was carried out in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Phenotype assessments

The Level1 assessments consisted of brief or adapted versions of standard questionnaires that were designed for participants to complete and return to us by post. To properly test gene x environment interaction we followed the method suggested by Moffitt et al (Moffitt *et al,* 2005). We used the same background questionnaire as we used in our previous studies (Juhasz *et al,* 2009; Lazary *et al,* 2008). This structured self-rating questionnaire consists of 22 items and collects information about medical history including psychiatric history (with special interest on depression), family psychiatric history, and socioeconomic background. The Brief Symptom Inventory (BSI) questionnaire (Derogatis L, 1993) was used for depressive symptoms (six items) and for anxiety symptoms (six items). A continuous weighted dimension score was calculated for both Depression and Anxiety symptoms, which were used in the analysis. The questionnaire data on depression and anxiety scores have been validated at a second level of the study, described in detail elsewhere (Juhasz *et al,* 2009). Our Recent Negative Life Events questionnaire was based on the validated List of Life Threatening Experiences (LTE) (Brugha *et al,* 1985; Rijsdijk *et al,* 2001) and was previously used to replicate a 5-HTTLPR x stressful life events interaction (Lazary *et al,* 2008) and demonstrate cannabinoid receptor 1 gene (*CNR1*) x stressful life events interaction (Juhasz *et al,* 2009). This questionnaire provided the total number of negative life events in the previous 12 months period and this sum was used in the interaction analysis. For figures and to exclude scaling effect we grouped the participants into three groups based on our previous studies: 0-1 life event, 2 life events and 3-more life events. Questions based on the Childhood Trauma Questionnaire (CTQ) (Bernstein *et al,* 1994) were used to determine Childhood Adversity as a Likert score (ranging from never true to very often true) related to emotional and physical abuse, and emotional and physical neglect. An additional question asked about parental loss during childhood. The sum of the Likert score plus parental loss was used in the interaction analysis. These questionnaire data were validated against the original CTQ (Pearson $R=0.75$, $p<0.001$, $n=142$) at a second level of the study, and were used to replicate significant interaction between rs6265 (*BDNF* Val66Met) and childhood adversity on depression (ACNP, Hollywood, Florida, USA, 6-10 December 2009).

Level2 participants provided background questionnaire, BSI data and took part in a face-to-face diagnostic interview and a computerised emotion recognition task. The participants in this study were free from any current or previous axis I psychiatric disorders based on Structured Clinical Interview for DSM-IV (SCID), (First *et al,* 2002)

Face emotion recognition task

The task contains six varieties of emotions (anger, disgust, fear, happiness, sadness and surprise) plus neutral. (Ekman and Friesen, 1976). These emotions are represented on faces, acted out by four actors on three different levels (30%, 50% and 70%). During the practice session participants were shown 21 faces (each emotion three times) at 100% intensity. In the main task each of the six expressions was shown at each intensity four times, one presentation of each actor. A neutral expression of each individual was shown once in the task, giving a total of four neutral images. Therefore in the main task there were 76 stimuli presentations=6 expressions (anger, disgust, fear, happiness, sadness surprise) x four actors x three intensities (30, 50 and 70%) plus 4 neutral. Each face was displayed for 1000 milliseconds on the screen and was followed by a 4500 millisecond interstimulus interval. Participants were asked to identify the emotion by pressing the appropriate computer key from the seven possible choices. We recorded the percentage of the correctly identified emotions and we calculated an average reaction time for each correctly recognised emotion for each patient and these values were used for analysis. Because our hypothesis focused on intermediate phenotypes to depression and anxiety, for genetic analysis we only used data for happiness, sad, and fear emotions, which activate specific neural networks that are important for mood and threat-related information processing (Fusar-Poli *et al,* 2009; Harmer, 2008).

A summary of demographic and phenotypic data is given in Table 1.

DNA extraction and genotyping

Participants received a cytology brush (Cytobrush plus C0012, Durbin PLC) and a 15-mL plastic tube containing 2.0 ml of collection buffer. Buccal mucosa cells were collected and genomic DNA was extracted according to a protocol previously described (Freeman *et al,* 2003). HaploView software (www.broad.mit.edu/personal/jcbarret/haploview/) was employed to identify htSNPs using the confidence interval method (Barrett *et al,* 2005; Gabriel *et al,*

2002). Specifications: confidence interval minima for strong LD, upper: 0.98, lower: 0.7, upper confidence interval maximum for strong recombination: 0.9, fraction of strong LD in informative comparisons at least: 0.95, markers excluded below 5% minor allele frequency, r^2=0.8, pairwise method.

The tagging was based on the CEPH population genotype data that were available at the International HapMap Project Phase I. June 2005 release (www.hapmap.org. We also examined possibly functional htSNPs previously identified (Peters *et al*, 2004, Duan *et al,* 2003; Lemonde *et al,* 2003). Allele frequencies can be seen in Table 2.

SNPs were genotyped using the Sequenom® MassARRAY technology (Sequenom®, San Diego, http://www.sequenom.com) using 25ng of DNA. Primer sequences are available upon request. Genotyping was performed blind with regard to phenotype. All laboratory work was performed under the ISO 9001:2000 quality management requirements. In Level1 we genotyped six SNPs (See Table 2.); in Level2 we only investigated rs6295 on *HTR1A*, as only this polymorphism of our candidates has a well established function (Lemonde *et al,* 2003) with suggested role in face emotion recognition (Fakra *et al,* 2009; Le Francois *et al,* 2008).

Statistical Analysis

Allelic association (using linear regression analysis with 1000 permutations), Hardy-Weinberg equilibrium and linkage disequlibrium (LD) calculations were performed using HelixTree™ 6.4.3 (Golden Helix, USA). PLINK v1.06 (http://pngu.mgh.harvard.edu/purcell/plink/) was used for testing association of different genetic models (dominant, recessive and additive; linear regression model) in both cohorts. Interactions were calculated with recent life events and childhood adversity. All association analyses were adjusted for age and sex. Correlation calculations and repeated measure ANOVA (emotion as within-subjects factor, genotype as between-subjects factor) were performed using SPSS 15.0 for Windows software (SPSS Inc. Chicago, Illinois, USA). For PLINK analysis we used false discovery rate calculation at level of 5% (FDR; Qvalue: http://genomics.princeton.edu.storeylab/qvalue/) to adjust p values according to the number of hypotheses tested (Storey and Tibshirani, 2003). We report q value which is a measure of significance of each test of many tests performed simultaneously.

Results

All *HTR1A/1B* SNPs were in Hardy-Weinberg equilibrium in both Level1 and Level2 participants. Strong LD was observed within both the *HTR1A* and *HTR1B* genes (r^2>0.4), which is consistent with the HapMap data.

The Pearson correlation value between depression scores and anxiety scores was R=0.778, (p<0.001). The recent life events were in moderate correlation with depression (R=0.251, p<0.001) and with anxiety (R=0.229, p<0.001) scores. Childhood adversity showed stronger but still moderate correlation with depression (R=0.366, p<0.001) and anxiety (R=0.313, p<0.001) scores.

Allelic association test

None of the alleles showed significant association to either BSI depression scores or BSI anxiety scores using allelic association test for Level1 (data not shown). In Level2 significant association was observed between rs6295 G allele and decreased recognition time of identification of fearful faces (β=-343.67, permuted p=0.014), but not to the other emotions. Rs6295 G allele carriers also recognised less sad faces correctly compared to C allele carriers (β=-14.61, permuted p=0.044) (data not shown).

Genotypic association test

In the Level1 participant group none of the genotypes showed association with depression or anxiety scores using genotypic association test, additive, dominant or recessive analytical models (data not shown).

When recent negative life events were included as an interacting factor, three (rs6295, rs878567 and rs6296) out of the six investigated SNPs showed significant interaction on current depression state in the additive and dominant model even after correction for multiple testing (Table 2). The same SNPs plus

HTR1A rs749098 C also showed significant interaction on the current anxiety state in the additive model but only rs878567 and rs6296 effects remained significant after correction for multiple testing. These two SNPs also showed significant interaction with RLE on anxiety in the recessive model (Table 2 and Figure 1 a-d).

Significant gene x RLE interactions were not due to association between RLE and SNPs as a preliminary analysis revealed no significant association between the *HTR1A/1B* SNPs and RLE scores, or childhood adversity scores, in any mathematical models (data not shown). This observation supports the role of effects of negative life events on the *HTR1A* and *HTR1B* genes.

No significant interactions were observed between childhood adversity scores and genotype for any analytical model. No sex x SNP interactions were observed (data not shown).

In the Level2 study, rs6295 GG carriers had reduced accuracy in identifying emotions independently of the valence of emotion (F=4.749, df=2,96 p=0.011; Figure 2a); for emotions considered separately this effect was only significant for sad faces (Table 3a). In general, rs6295 GG carriers were not significantly quicker to recognise emotions (F=2.283, df=2,93, p=0.107) but within-subjects contrasts showed significant interaction between happy and fear (F=3.113, df=2,93 p=0.049; Figure 2b). Thus, omitting happiness from the model rs6295 GG carriers showed significantly shorter reaction times for negative emotions (F=3.915, df=2,94 p=0.023). As for separate emotions, this effect was significant for fear and a trend for sad (Table 3b, Figure 2b).

Discussion

Our results emphasise that genetic variations not only in the *HTR1A* but also in the *HTR1B* genes confer susceptibility to depression and anxiety in adults in the presence of exposure to stressful life events. We could not find a direct association between either the *HTR1A* or *HTR1B* SNPs and depressive or anxiety symptoms. However, interaction analysis with recent stressful life events showed significant associations between two *HTR1A* SNPs (rs878567 T and rs6295 G) and one *HTR1B* SNPs (rs6296 C) and both phenotypes. The number of life events was not significantly different according to genotypes which suggests that the main contributor to these phenotypes is the effect of negative life events on the *HTR1A* and *HTR1B* genes. Furthermore, we demonstrated that the effect of *HTR1A*

rs6295 G allele is correlated with enhanced threat-related information processing based on a face emotion recognition study. Thus our results extend previous findings of 5-HTTLPR and stressful life events interaction for two other genes in the serotonergic pathway and emphasise the possible role of increased threat-related information processing as an intermediate phenotype.

The association between these genes with both depression and anxiety also supports previous findings from twin studies, namely that these two traits share common genetic factors (Kendler, 1996; Mosing *et al,* 2009; Roy *et al,* 1995). These shared genetic factors partially mediated their effects through neuroticism but in the present study this was not directly associated with the investigated SNPs (data not shown), although previously Strobel et al reported significant association with neuroticism and *HTR1A* rs6295 (Strobel *et al,* 2003). However, our findings suggest the relevance of other non-neuroticism related shared mechanisms (Hettema, 2008; Kendler *et al,* 2007). Mixed presentation of anxiety and depressive symptoms is usual in population samples and categorisation into major depressive disorder, anxiety disorder or mixed anxiety-depressive disorder mainly reflects the severity and predominance of these dimensions (Das-Munshi *et al,* 2008). Frequent transitions between these syndromes also support common pathophysiology (Hettema, 2008). This observation has been corroborated by the strong correlation between depression scores and anxiety scores in our study.

In our study the rs6295 G allele was not only a risk factor for anxiety and depression, but the healthy control GG carriers required less time to correctly identify fearful faces. They also responded more quickly to sad faces, although this difference did not reach significance, but there was no effect on reaction time to happy faces. Thus the increased tendency to stress-induced anxiety and depression in GG carriers may involve speeded psychomotor responses to the perception of threat at an intermediate level. This is not due to an affective bias to perceive negative emotions that has been described in anxiety and depression (Harmer, 2008; Mogg *et al,* 2007) because the GG carriers were less accurate in detecting face emotions including happy. A potentially related finding is the reported association of the rs6295 G allele with increased questionnaire measures of impulsivity (Benko *et al,* 2010). Further studies are needed to replicate our findings and to understand the nature of speeded responses to threat. One possible mechanism that can mediate this genetic effect is the activation of the amygdala (Fisher *et al,* 2006), although contradictory results have been reported

about the rs6295 G allele effect on amygdala activation in different imaging studies (Domschke *et al,* 2006; Fakra *et al,* 2009; Le Francois *et al,* 2008). This may be related to the less accurate perception of the three face emotions in the GG carriers, which is suggested by our results.

HTR1A and rs6295 have previously been investigated in depression studies but many could not find a direct association with diagnosis or symptoms (Illi *et al,* 2009; Serretti *et al,* 2009) as in our study (see, Supplementary Table 1). However, other studies, taking into consideration the stressful life events, have found associations. One study reported on 272 suicide attempter families and observed an over-transmission of the G allele in a subset where prior to the suicide attempt a high level of previous traumatic and/or stressful life events had occurred (Wasserman *et al,* 2006). There is no functional study available for the *HTR1A* 3'UTR polymorphism, rs878567, but some studies have investigated its role in mental disorders and suicide. Three studies found no association between this SNP and susceptibility to human internalizing phenotypes, including major depression, a range of anxiety disorders and neuroticism (Hettema *et al,* 2008) and suicidal behaviour (Serretti *et al,* 2007; Serretti *et al,* 2009). However, including childhood physical abuse, the TT homozygote was associated with a lower risk of major depression (Brezo *et al,* 2009), a finding that we did not replicate. This contradiction to our findings may be due to the limited number of cases involved in the Brezo study or a false positive result. Of the other studies that have investigated an association between *HTR1B* and depression; one found associations between the C allele of rs6296 and MDD (Huang *et al,* 2003). Another investigated rs130058 and rs11568817 in depression and did not find an association; despite negative life events being included in the model (Zhang *et al,* 2009). In our study also these two SNPs do not show any associative or interactive influences on depression.

Animal studies support our findings as *HTR1A* or *HTR1B* knockout animals develop normally without any obvious behavioural disturbances but stressful paradigms provoke specific emotional phenotypes (Zhuang *et al,* 1999) suggesting that these receptors have major roles in coping with stress. Furthermore, these genetic variants may well influence the development of non-5-HT neural systems concerned with emotion processing. That 5-HT genes may act via development was indeed suggested by the finding in 5-HT knock out mice that impaired 5-HT_{1A} function during development was necessary for expression of the adult anxious

phenotype whereas inducing impaired 5-HT_{1A} function in adulthood did not affect anxiety (Gross *et al,* 2002). Exposure to childhood adversity is another developmental risk factor associated with greater sensitivity to later life events and the onset of depression. However, in our study none of the *HTR1A* and *HTR1B* SNPs showed an association with depression or anxiety when interaction with childhood adversity was tested. The interaction with recent life events rather than childhood adversity indicates that *HTR1A/1B* genes may act on a different pathway from the effect of childhood adversity to increase sensitivity to stressful events. However, since they act both pre- and postsynaptically, as auto- and heteroreceptors, it is difficult to make direct inferences about the mediating role of altered 5-HT function (Drago *et al,* 2010; Le Francois *et al,* 2008; Sari, 2004; Savitz *et al,* 2009).

The *HTR1A* rs6295 G allele has been shown to interrupt a nuclear deformed epidermal autoregulatory factor (Deaf-1/NUDR) binding site in the promoter region of HTR1A gene in a cell specific manner. Deaf-1/NUDR is a repressor transcription factor in raphe cells but acts as an enhancer in postsynaptic cells expressing 5-HT_{1A} receptors. Abrogation of its function leads to both the enhanced expression of *HTR1A* at the raphe and therefore reduced serotonergic tone, and to decreased postsynaptic *HTR1A* expression. Furthermore, the *HTR1A* rs6295 G allele, by interrupting the Hes5 (hairy and enhancer of split 5) transcription factor binding site, decreases *HTR1A* expression uniformly in raphe and cortical cell cultures (Le Francois *et al,* 2008; Savitz *et al,* 2009). Two PET studies in humans found increased raphe and hippocampal 5-HT_{1A} receptor binding in those with the rs6295 G allele but G alleles were over-represented in the depressed group and this is a potential confound as the depressed sample overall had greater 5-HT_{1A} binding (David *et al,* 2005; Parsey *et al,* 2006b). Further studies are needed to determine the influence of the *HTR1A* rs6295 polymorphism on the expression and function of the receptor in depression.

The C allele of *HTR1B* rs6296 also showed association with depression and anxiety in interaction with recent life events in our study. Whether or not this synonymous SNP is functional, remains unknown. However, based on previous studies the C allele is in linkage disequilibrium with variants that code transcriptionally less active versions of the gene (Conner *et al,* 2010; Duan *et al,* 2003). Animal studies suggest that the decreased expression of the *HTR1B* is linked with aggressiveness and impulsivity but not anxiety (Clark *et al,* 2004;

Drago *et al,* 2010). Moreover, one animal study found, that 24 hours after exposure to inescapable stress, 5-HT1B receptor was overexpressed in the dorsal raphe nuclei (DRN) and the rats' anxiety behaviour was elevated. Meanwhile 5-HT_{1B} overexpression in the DRN, in the absence of stress, increased exploratory behaviour (Clark *et al,* 2002) This finding indicates that the impact of 5-HT_{1B} in DRN is dependent on context i.e. stress and shows the complex role of 5-HT_{1B} in anxiety behaviour. However, the human phenotype appears to be more complex with men who possess the lower expression haplotypes reporting significantly more hostility but also significantly more anxiety and sadness (Conner *et al,* 2010).

The strength of our study is that we had relatively large number of individuals genotyped for these polymorphisms and we used continuous traits rather than diagnostic dichotomy. When using an additive linear model we had a 96% power in our population (N=1387) to detect a polymorphism (assuming a minor allele frequency of 25%, See Table 4) with an effect size of 1% at the 5% two-tailed significance level. To detect a gene x environment interaction with an effect size of 3%, in the same model as above, we had 97% power. Furthermore, we tested the rs6295 genetic effect in a face emotion task that is sensitive to serotonergic changes. The potential limitations of our study are that we have not investigated all the polymorphisms in our Level 2 face emotion recognition study, the lack of independent replication sample and the unknown functional relevance some of the genotyped SNPs. A possible major limitation of our study is the selection bias resulting from non-systematic recruitment and low response rates during the recruitment. In our study the mean values for BSI depression, anxiety and BigFive Neuroticism were 1.132, 1.015 and 3.365, respectively. These normative values for the population are 0.28, 0.35 (Derogatis 1993) and 2.0 (Srivastava *et al*. 2003), therefore our cohort may not be a true representation of the general population. Also, it has been demonstrated in the literature that results of gene x environment studies are heavily dependent on the measures used for evaluation of the environmental adversity. Self-reported measures that we used in our study are often less reliable than more objective measures (Uher and McGuffin 2010) and therefore the observed gene x environment interaction may be a false positive result.

In conclusion our results support previous studies suggesting that *HTR1A* and *HTR1B* polymorphisms are associated with stress-related neuropsychiatric conditions, such as depression and anxiety in the presence of environmental

stress factors. In addition, our results propose that threat-related emotional processing might be an intermediate phenotype to these disorders. If so, $5HT_{1A}$ and $5HT_{1B}$ receptors may be targets for preventive treatment strategies of stress-related neuropsychiatric disorders, especially in at-risk individuals. Further studies are needed to investigate this hypothesis, and to clarify the effect of *HTR1A* and the *HTR1B* genes in different brain neuronal networks and their role in human anxiety, depression and impulsivity.

References

Anttila, S., Huuhka, K., Huuhka, M., Rontu, R., Hurme, M., Leinonen, E., et al, 2007. Interaction between 5-HT1A and BDNF genotypes increases the risk of treatment-resistant depression. J Neural Transm. 114, 1065-1068.

Barnes, N.M., Sharp, T., 1999. A review of central 5-HT receptors and their function. Neuropharmacology. 38, 1083-1152.

Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 21, 263-265.

Benko, A., Lazary, J., Molnar, E., Gonda, X., Tothfalusi, L., Pap, D., et al, 2010. Significant association between the C(-1019)G functional polymorphism of the HTR(1A) gene and impulsivity. Am J Med Genet B Neuropsychiatr Genet. 153B, 592-599.

Bernstein, D.P., Fink, L., Handelsman, L., Foote, J., Lovejoy, M., Wenzel, K., et al, 1994. Initial reliability and validity of a new retrospective measure of child abuse and neglect. Am J Psychiatry. 151, 1132-1136.

Bohmaker, K., Eison, A.S., Yocca, F.D., Meller, E., 1993. Comparative effects of chronic 8-OH-DPAT, gepirone and ipsapirone treatment on the sensitivity of somatodendritic 5-HT1A autoreceptors. Neuropharmacology. 32, 527-534.

Brezo, J., Bureau, A., Merette, C., Jomphe, V., Barker, E.D., Vitaro, F., et al, 2009. Differences and similarities in the serotonergic diathesis for suicide attempts and mood disorders: a 22-year longitudinal gene-environment study. Mol Psychiatry. - Epub ahead of print.

Brugha, T., Bebbington, P., Tennant, C., Hurry, J., 1985. The List of Threatening Experiences: a subset of 12 life event categories with considerable long-term contextual threat. Psychol Med. 15, 189-194.

Brunner, D., Hen, R., 1997. Insights into the neurobiology of impulsive behavior from serotonin receptor knockout mice. Ann N Y Acad Sci. 836, 81-105.

Caspi, A., Hariri, A.R., Holmes, A., Uher, R., Moffitt, T.E., 2010. Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. Am J Psychiatry. 167, 509-527.

Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., et al, 2003. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science. 301, 386-389.

Cervilla, J.A., Rivera, M., Molina, E., Torres-Gonzalez, F., Bellon, J.A., Moreno, B., et al, 2006. The 5-HTTLPR s/s genotype at the serotonin transporter gene (SLC6A4) increases the risk for depression in a large cohort of primary care attendees: the PREDICT-gene study. Am J Med Genet B Neuropsychiatr Genet. 141B, 912-917.

Clark, M.S., Sexton, T.J., McClain, M., Root, D., Kohen, R., Neumaier, J.F., 2002. Overexpression of 5-HT1B receptor in dorsal raphe nucleus using Herpes Simplex Virus gene transfer increases anxiety behavior after inescapable stress. J Neurosci. 22, 4550-4562.

Clark, M.S., Vincow, E.S., Sexton, T.J., Neumaier, J.F., 2004. Increased expression of 5-HT1B receptor in dorsal raphe nucleus decreases fear-potentiated startle in a stress dependent manner. Brain Res. 1007, 86-97.

Conner, T.S., Jensen, K.P., Tennen, H., Furneaux, H.M., Kranzler, H.R., Covault, J., 2010. Functional polymorphisms in the serotonin 1B receptor gene (HTR1B) predict self-reported anger and hostility among young men. Am J Med Genet B Neuropsychiatr Genet. 153B, 67-78.

Contreras, J., Hare, L., Camarena, B., Glahn, D., Dassori, A., Medina, R., et al, 2009. The serotonin transporter 5-HTTPR polymorphism is associated with current and lifetime depression in persons with chronic psychotic disorders. Acta Psychiatr Scand. 119, 117-127.

Crabbe, J.C., Phillips, T.J., Feller, D.J., Hen, R., Wenger, C.D., Lessov, C.N., et al, 1996. Elevated alcohol consumption in null mutant mice lacking 5-HT1B serotonin receptors. Nat Genet. 14, 98-101.

Das-Munshi, J., Goldberg, D., Bebbington, P.E., Bhugra, D.K., Brugha, T.S., Dewey, M.E., et al, 2008. Public health significance of mixed anxiety and depression: beyond current classification. Br J Psychiatry. 192, 171-177.

David, S.P., Murthy, N.V., Rabiner, E.A., Munafo, M.R., Johnstone, E.C., Jacob, R., et al, 2005. A functional genetic variation of the serotonin (5-HT) transporter affects 5-HT1A receptor binding in humans. J Neurosci. 25, 2586-2590.

Derogatis L (1993). BSI: Brief Symptom Inventory: Administration, Scoring and Procedures Manual. National Computer Systems, Pearson Inc., Minneapolis.

Domschke, K., Braun, M., Ohrmann, P., Suslow, T., Kugel, H., Bauer, J., et al, 2006. Association of the functional -1019C/G 5-HT1A polymorphism with prefrontal cortex and amygdala activation measured with 3 T fMRI in panic disorder. Int J Neuropsychopharmacol. 9, 349-355.

Drachmann Bukh, J., Bock, C., Vinberg, M., Werge, T., Gether, U., Vedel, K.L., 2009. Interaction between genetic polymorphisms and stressful life events in first episode depression. J Affect Disord. 119, 107-115.

Drago, A., Alboni, S., Nicoletta, B., De Ronchi, D., Serretti, A., 2010. HTR1B as a risk profile maker in psychiatric disorders: a review through motivation and memory. Eur J Clin Pharmacol. 66, 5-27.

Drevets, W.C., Price, J.L., Furey, M.L., 2008. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. Brain Struct Funct. 213, 93-118.

Duan, J., Sanders, A.R., Molen, J.E., Martinolich, L., Mowry, B.J., Levinson, D.F., et al, 2003. Polymorphisms in the 5'-untranslated region of the human serotonin receptor 1B (HTR1B) gene affect gene expression. Mol Psychiatry. 8, 901-910.

Ekman P, Friesen WV (1976). Pictures of Facial Affect. Consulting Psychologists Press, Palo Alto, California.

Fakra, E., Hyde, L.W., Gorka, A., Fisher, P.M., Munoz, K.E., Kimak, M., et al, 2009. Effects of HTR1A C(-1019)G on amygdala reactivity and trait anxiety. Arch Gen Psychiatry. 66, 33-40.

First MB, Spitzer RL, Gibbon M, Williams JBW (2002). *Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version*. New York, New York State Psychiatric Institute.

Fisher, P.M., Meltzer, C.C., Ziolko, S.K., Price, J.C., Moses-Kolko, E.L., Berga, S.L., et al, 2006. Capacity for 5-HT1A-mediated autoregulation predicts amygdala reactivity. Nat Neurosci. 9, 1362-1363.

Freeman, B., Smith, N., Curtis, C., Huckett, L., Mill, J., Craig, I.W., 2003. DNA from buccal swabs recruited by mail: evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. Behav Genet. 33, 67-72.

Fusar-Poli, P., Placentino, A., Carletti, F., Landi, P., Allen, P., Surguladze, S., et al, 2009. Functional atlas of emotional faces processing: a voxel-based meta-analysis of 105 functional magnetic resonance imaging studies. J Psychiatry Neurosci. 34, 418-432.

Gabriel, S.B., Schaffner, S.F., Nguyen, H., Moore, J.M., Roy, J., Blumenstiel, B., et al, 2002. The structure of haplotype blocks in the human genome. Science. 296, 2225-2229.

Gross, C., Zhuang, X., Stark, K., Ramboz, S., Oosting, R., Kirby, L., et al, 2002. Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. Nature. 416, 396-400.

Gur, R.C., Erwin, R.J., Gur, R.E., Zwil, A.S., Heimberg, C., Kraemer, H.C., 1992. Facial emotion discrimination: II. Behavioral findings in depression. Psychiatry Res. 42, 241-251.

Hall, H., Lundkvist, C., Halldin, C., Farde, L., Pike, V.W., McCarron, J.A., et al, 1997. Autoradiographic localization of 5-HT1A receptors in the post-mortem human brain using [3H]WAY-100635 and [11C]way-100635. Brain Res. 745, 96-108.

Harmer, C.J., 2008. Serotonin and emotional processing: does it help explain antidepressant drug action? Neuropharmacology. 55, 1023-1028.

Harmer, C.J., Bhagwagar, Z., Perrett, D.I., Vollm, B.A., Cowen, P.J., Goodwin, G.M., 2003. Acute SSRI administration affects the processing of social cues in healthy volunteers. Neuropsychopharmacology. 28, 148-152.

Harmer, C.J., Shelley, N.C., Cowen, P.J., Goodwin, G.M., 2004. Increased positive versus negative affective perception and memory in healthy volunteers following selective serotonin and norepinephrine reuptake inhibition. Am J Psychiatry. 161, 1256-1263.

Heisler, L.K., Chu, H.M., Brennan, T.J., Danao, J.A., Bajwa, P., Parsons, L.H., et al, 1998. Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. Proc Natl Acad Sci U S A. 95, 15049-15054.

Hettema, J.M., 2008. The nosologic relationship between generalized anxiety disorder and major depression. Depress Anxiety. 25, 300-316.

Hettema, J.M., An, S.S., van den Oord, E.J., Neale, M.C., Kendler, K.S., Chen, X., 2008. Association study between the serotonin 1A receptor (HTR1A) gene and neuroticism, major depression, and anxiety disorders. Am J Med Genet B Neuropsychiatr Genet. 147B, 661-666.

Hong, C.J., Chen, T.J., Yu, Y.W., Tsai, S.J., 2006. Response to fluoxetine and serotonin 1A receptor (C-1019G) polymorphism in Taiwan Chinese major depressive disorder. Pharmacogenomics J. 6, 27-33.

Huang, Y.Y., Oquendo, M.A., Friedman, J.M., Greenhill, L.L., Brodsky, B., Malone, K.M., et al, 2003. Substance abuse disorder and major depression are associated with the human 5-HT1B receptor gene (HTR1B) G861C polymorphism. Neuropsychopharmacology. 28, 163-169.

Illi, A., Setala-Soikkeli, E., Viikki, M., Poutanen, O., Huhtala, H., Mononen, N., et al, 2009. 5-HTR1A, 5-HTR2A, 5-HTR6, TPH1 and TPH2 polymorphisms and major depression. Neuroreport. 20, 1125-1128.

Juhasz, G., Chase, D., Pegg, E., Downey, D., Toth, Z.G., Stones, K., et al, 2009. CNR1 gene is associated with high neuroticism and low agreeableness and interacts with recent negative life events to predict current depressive symptoms. Neuropsychopharmacology. 34, 2019-2027.

Kato, M., Fukuda, T., Wakeno, M., Okugawa, G., Takekita, Y., Watanabe, S., et al, 2009. Effect of 5-HT1A gene polymorphisms on antidepressant response in major depressive disorder. Am J Med Genet B Neuropsychiatr Genet. 150B, 115-123.

Kendler, K.S., 1996. Major depression and generalised anxiety disorder. Same genes, (partly)different environments--revisited. Br J Psychiatry Suppl. 68-75.

Kendler, K.S., Gardner, C.O., Gatz, M., Pedersen, N.L., 2007. The sources of co-morbidity between major depression and generalized anxiety disorder in a Swedish national twin sample. Psychol Med. 37, 453-462.

Kennett, G.A., Marcou, M., Dourish, C.T., Curzon, G., 1987. Single administration of 5-HT1A agonists decreases 5-HT1A presynaptic, but not postsynaptic receptor-mediated responses: relationship to antidepressant-like action. Eur J Pharmacol. 138, 53-60.

Kraus, M.R., Al Taie, O., Schafer, A., Pfersdorff, M., Lesch, K.P., Scheurlen, M., 2007. Serotonin-1A receptor gene HTR1A variation predicts interferon-induced depression in chronic hepatitis C. Gastroenterology. 132, 1279-1286.

Lanfumey, L., Hamon, M., 2004. 5-HT1 receptors. Curr Drug Targets CNS Neurol Disord. 3, 1-10.

Lazary, J., Lazary, A., Gonda, X., Benko, A., Molnar, E., Juhasz, G., et al, 2008. New evidence for the association of the serotonin transporter gene (SLC6A4) haplotypes, threatening life events, and depressive phenotype. Biol Psychiatry. 64, 498-504.

Le Francois, B., Czesak, M., Steubl, D., Albert, P.R., 2008. Transcriptional regulation at a HTR1A polymorphism associated with mental illness. Neuropharmacology. 55, 977-985.

Lemonde, S., Du, L., Bakish, D., Hrdina, P., Albert, P.R., 2004. Association of the C(-1019)G 5-HT1A functional promoter polymorphism with antidepressant response. Int J Neuropsychopharmacol. 7, 501-506.

Lemonde, S., Turecki, G., Bakish, D., Du, L., Hrdina, P.D., Bown, C.D., et al, 2003. Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. J Neurosci. 23, 8788-8799.

Levinson, D.F., 2006. The genetics of depression: a review. Biol Psychiatry. 60, 84-92.

Merens, W., Willem Van der Does AJ, Spinhoven, P., 2007. The effects of serotonin manipulations on emotional information processing and mood. J Affect Disord. 103, 43-62.

Mineka, S., Watson, D., Clark, L.A., 1998. Comorbidity of anxiety and unipolar mood disorders. Annu Rev Psychol. 49, 377-412.

Moffitt, T.E., Caspi, A., Rutter, M., 2005. Strategy for investigating interactions between measured genes and measured environments. Arch Gen Psychiatry. 62, 473-481.

Mogg, K., Bradley, B.P., 2002. Selective orienting of attention to masked threat faces in social anxiety. Behav Res Ther. 40, 1403-1414.

Mogg, K., Garner, M., Bradley, B.P., 2007. Anxiety and orienting of gaze to angry and fearful faces. Biol Psychol. 76, 163-169.

Mosing, M.A., Gordon, S.D., Medland, S.E., Statham, D.J., Nelson, E.C., Heath, A.C., et al, 2009. Genetic and environmental influences on the co-morbidity between depression, panic disorder, agoraphobia, and social phobia: a twin study. Depress Anxiety. 26, 1004-1011.

Neff, C.D., Abkevich, V., Packer, J.C., Chen, Y., Potter, J., Riley, R., et al, 2009. Evidence for HTR1A and LHPP as interacting genetic risk factors in major depression. Mol Psychiatry. 14, 621-630.

Peters EJ, Slager SL, McGrath PJ, Knowles JA, Hamilton SP (2004). Investigation of serotonin-related genes in antidepressant response. Molecular Psychiatry, 9(9):879-89.

Parks, C.L., Robinson, P.S., Sibille, E., Shenk, T., Toth, M., 1998. Increased anxiety of mice lacking the serotonin1A receptor. Proc Natl Acad Sci U S A. 95, 10734-10739.

Parsey, R.V., Olvet, D.M., Oquendo, M.A., Huang, Y.Y., Ogden, R.T., Mann, J.J., 2006a. Higher 5-HT1A receptor binding potential during a major depressive episode predicts poor treatment response: preliminary data from a naturalistic study. Neuropsychopharmacology. 31, 1745-1749.

Parsey, R.V., Oquendo, M.A., Ogden, R.T., Olvet, D.M., Simpson, N., Huang, Y.Y., et al, 2006b. Altered serotonin 1A binding in major depression: a [carbonyl-C-11]WAY100635 positron emission tomography study. Biol Psychiatry. 59, 106-113.

Pazos, A., Palacios, J.M., 1985. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. Brain Res. 346, 205-230.

Plomin, R., Crabbe, J., 2000. DNA. Psychol Bull. 126, 806-828.

Ramboz, S., Oosting, R., Amara, D.A., Kung, H.F., Blier, P., Mendelsohn, M., et al, 1998. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc Natl Acad Sci U S A. 95, 14476-14481.

Rijsdijk, F.V., Sham, P.C., Sterne, A., Purcell, S., McGuffin, P., Farmer, A., et al, 2001. Life events and depression in a community sample of siblings. Psychol Med. 31, 401-410.

Risch, N., Herrell, R., Lehner, T., Liang, K.Y., Eaves, L., Hoh, J., et al, 2009. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. JAMA. 301, 2462-2471.

Rocha, B.A., Scearce-Levie, K., Lucas, J.J., Hiroi, N., Castanon, N., Crabbe, J.C., et al, 1998. Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. Nature. 393, 175-178.

Rothe, C., Gutknecht, L., Freitag, C., Tauber, R., Mossner, R., Franke, P., et al, 2004. Association of a functional 1019C>G 5-HT1A receptor gene polymorphism with panic disorder with agoraphobia. Int J Neuropsychopharmacol. 7, 189-192.

Roy, M.A., Neale, M.C., Pedersen, N.L., Mathe, A.A., Kendler, K.S., 1995. A twin study of generalized anxiety disorder and major depression. Psychol Med. 25, 1037-1049.

Rutter, M., Thapar, A., Pickles, A., 2009. Gene-environment interactions: biologically valid pathway or artifact? Arch Gen Psychiatry. 66, 1287-1289.

Sari, Y., 2004. Serotonin1B receptors: from protein to physiological function and behavior. Neurosci Biobehav Rev. 28, 565-582.

Savitz, J., Lucki, I., Drevets, W.C., 2009. 5-HT(1A) receptor function in major depressive disorder. Prog Neurobiol. 88, 17-31.

Serretti, A., Calati, R., Giegling, I., Hartmann, A.M., Moller, H.J., Rujescu, D., 2009. Serotonin receptor HTR1A and HTR2C variants and personality traits in suicide attempters and controls. J Psychiatr Res. 43, 519-525.

Serretti, A., Mandelli, L., Giegling, I., Schneider, B., Hartmann, A.M., Schnabel, A., et al, 2007. HTR2C and HTR1A gene variants in German and Italian suicide attempters and completers. Am J Med Genet B Neuropsychiatr Genet. 144B, 291-299.

Storey, J.D., Tibshirani, R., 2003. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A. 100, 9440-9445.

Strobel, A., Gutknecht, L., Rothe, C., Reif, A., Mossner, R., Zeng, Y., et al, 2003. Allelic variation in 5-HT1A receptor expression is associated with anxiety- and depression-related personality traits. J Neural Transm. 110, 1445-1453.

Surguladze, S.A., Young, A.W., Senior, C., Brebion, G., Travis, M.J., Phillips, M.L., 2004. Recognition accuracy and response bias to happy and sad facial expressions in patients with major depression. Neuropsychology. 18, 212-218.

Uher, R., McGuffin, P., 2010. The moderation by the serotonin transporter gene of environmental adversity in the etiology of depression: 2009 update. Mol Psychiatry. 15, 18-22.

Wasserman, D., Geijer, T., Sokolowski, M., Rozanov, V., Wasserman, J., 2006. The serotonin 1A receptor C(-1019)G polymorphism in relation to suicide attempt. Behav Brain Funct. 2, 14.

Wray, N.R., James, M.R., Gordon, S.D., Dumenil, T., Ryan, L., Coventry, W.L., et al, 2009. Accurate, Large-Scale Genotyping of 5HTTLPR and Flanking Single

Nucleotide Polymorphisms in an Association Study of Depression, Anxiety, and Personality Measures. Biol Psychiatry. 66, 468-476.

Wu, Y., Xu, Y., Sun, Y., Wang, Y.F., Li, X., Lang, X.E., et al, 2008. Association between the serotonin 1A receptor C(-1019)G polymorphism and major depressive disorder in the northern Han ethnic group in China. Chin Med J (Engl). 121, 874-876.

Yevtushenko, O.O., Oros, M.M., Reynolds, G.P., 2010. Early response to selective serotonin reuptake inhibitors in panic disorder is associated with a functional 5-HT1A receptor gene polymorphism. J Affect Disord. 123, 308-311.

Yu, Y.W., Tsai, S.J., Liou, Y.J., Hong, C.J., Chen, T.J., 2006. Association study of two serotonin 1A receptor gene polymorphisms and fluoxetine treatment response in Chinese major depressive disorders. Eur Neuropsychopharmacol. 16, 498-503.

Zalsman, G., Huang, Y.Y., Oquendo, M.A., Burke, A.K., Hu, X.Z., Brent, D.A., et al, 2006. Association of a triallelic serotonin transporter gene promoter region (5-HTTLPR) polymorphism with stressful life events and severity of depression. Am J Psychiatry. 163, 1588-1593.

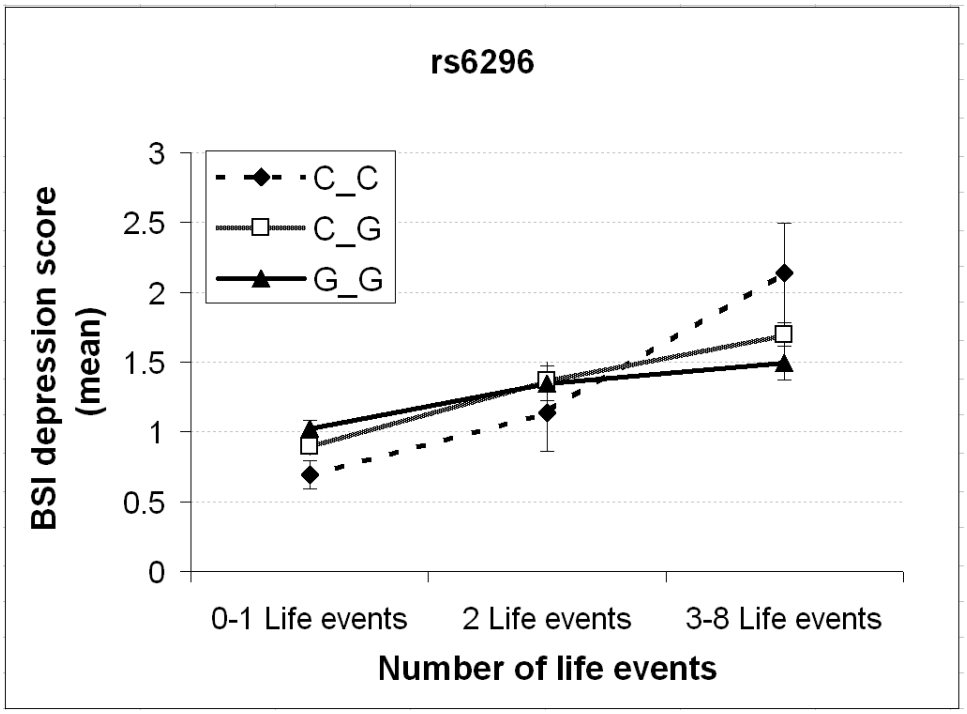
Zhang, K., Xu, Q., Xu, Y., Yang, H., Luo, J., Sun, Y., et al, 2009. The combined effects of the 5-HTTLPR and 5-HTR1A genes modulates the relationship between negative life events and major depressive disorder in a Chinese population. J Affect Disord. 114, 224-231.

Zhuang, X., Gross, C., Santarelli, L., Compan, V., Trillat, A.C., Hen, R., 1999. Altered emotional states in knockout mice lacking 5-HT1A or 5-HT1B receptors. Neuropsychopharmacology. 21, 52S-60S.

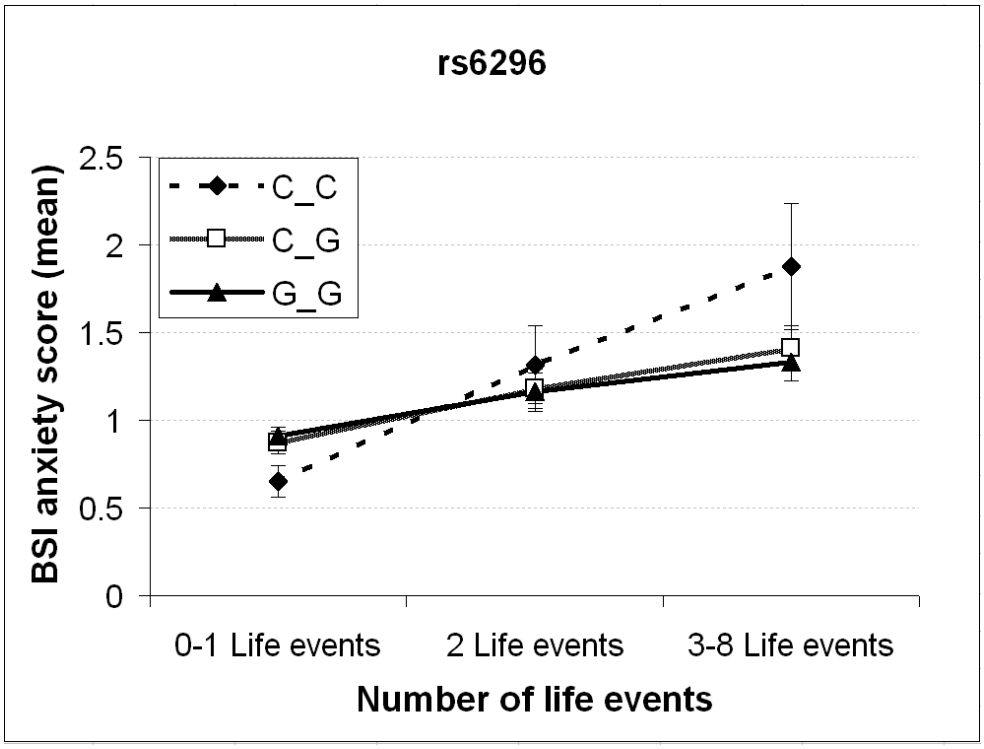
Figures

1a. *HTR1A* rs6295 and BSI depression score

1b. *HTR1A* rs6295 BSI anxiety score



1c. *HTR1B* rs6296 and BSI depression score



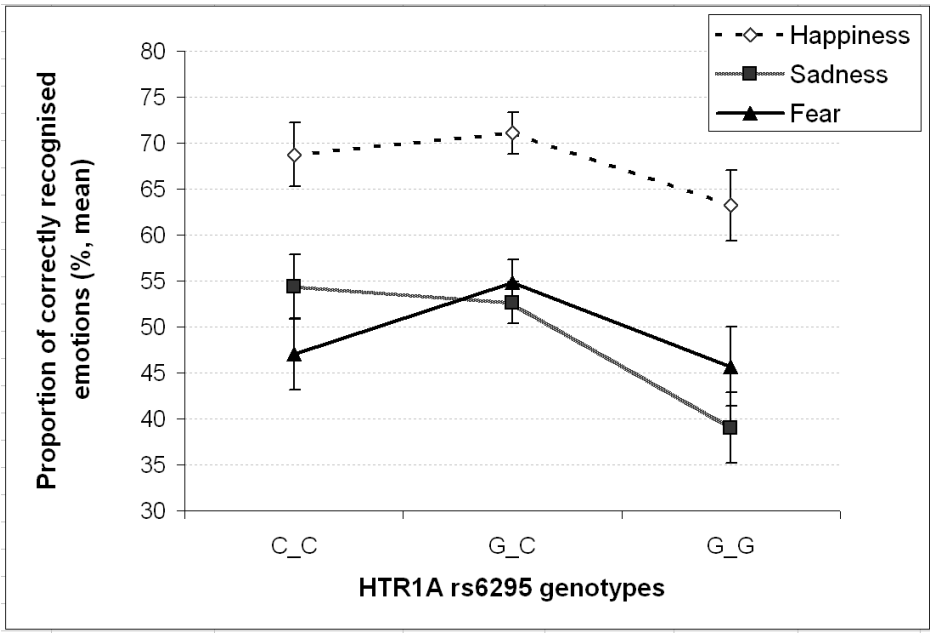
1d. *HTR1B* rs6296 and BSI anxiety score

Figure 1 Interaction of recent negative life events with *HTR1A* rs6295 and *HTR1B* rs6296 polymorphisms on depression and anxiety in Level1. Individuals were grouped into 3 groups according to the number of stressful life events experienced in the past year, shown on the X-axis. 888 people (64%) experienced 0-1 stressful

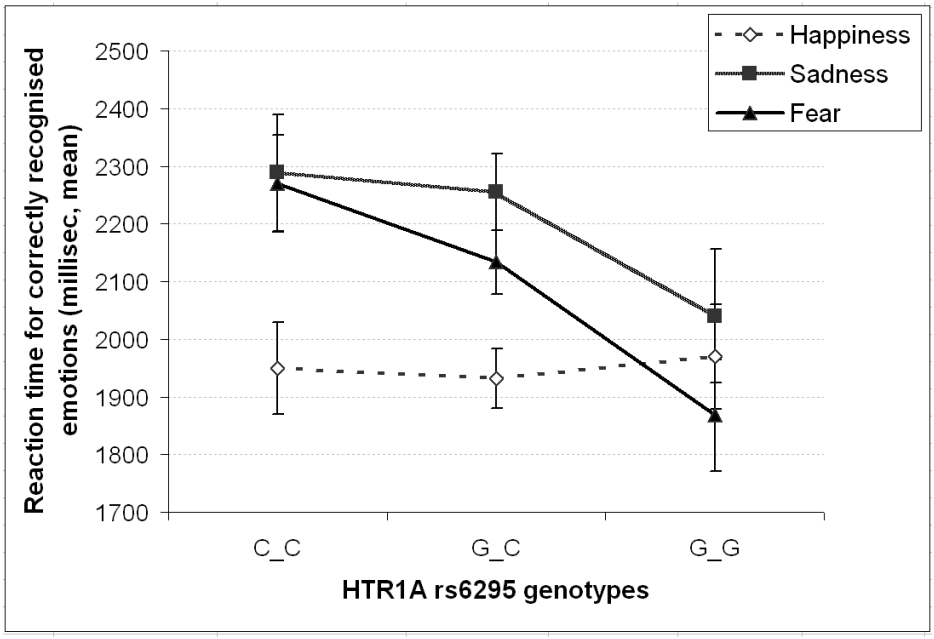
life event, 261 individuals (19%) 2 stressful life events and 238 individuals (17%) 3-8 life events. Mean values of BSI depression and anxiety scores were calculated for each group, shown on the Y axis.

BSI: Brief Symptom Inventory

For the number of individuals in each genotype group and for the mean values see Table 5.



2a *HTR1A* rs6295 and proportion of correctly recognised emotions



2b. *HTR1A* and rs6295 and reaction time for correctly recognised emotions

Figure 2 Correctly identified emotions (a, % mean) and reaction times for these (b, msec mean) according to *HTR1A* rs6295 genotypes (CC n=24; CG n=57; GG n=20). Rs6295 GG genotype carriers were less likely to correctly identify emotions but showed quicker response to negative emotions, such as sad and fear. For the mean values for each genotype group see Table 6.

Table 1 Demographic and phenotypic information of Level1 and Level2 participants

	Level1	Level2
Number of volunteers		
Women	968	58
Men	419	43
Total	1387	101
Age in years	33.97 (0.27)	30.47 (0.99)
Symptom scores:		
BSI depression	1.13 (0.03)	0.28 (0.05)
BSI anxiety	1.02 (0.03)	0.19 (0.03)
Adversities:		
Recent negative life events	1.32 (0.037)	
Childhood adversity	3.70 (0.097)	
Emotion recognition task:		
Happiness correctly identified %		68.03 (1.78)
Happiness identification RT		1950.40 (40.51)
Sadness correctly identified %,		49.44 (1.89)
Sadness identification RT		2234.35 (51.48)
Fear correctly identified %,		51.20 (1.92)
Fear identification RT		2114.85 (43.12)

Values are presented as mean (SEM).
SEM: Standard error of the mean
BSI: Brief Symptom Inventory
RT: reaction time for correctly identified emotions in msec

Table 2 Significant interaction results between SNPs and recent negative life events (RLE) on BSI depression and anxiety symptoms.

	BSI depression score						**BSI anxiety score**					
Model:	Additive		Dominant		Recessive		Additive		Dominant		Recessive	
Interaction:	RLE		RLE		RLE		RLE		RLE		RLE	
	β	P	β	P	β	P	β	P	β	P	β	P
HTR1A_rs6295 **G**	0.078	**0.009****	0.127	**0.009****	0.085	*0.088*	0.062	**0.018***	0.087	**0.042***	0.084	*0.056*
HTR1A_rs749098 **C**	-0.048	0.167	-0.047	0.278	-0.105	0.210	-0.062	**0.044***	-0.060	0.121	-0.140	*0.061*
HTR1A_rs878567 **T**	0.090	**0.012****	0.133	**0.021***	0.104	*0.076*	0.096	**0.002****	0.113	**0.026***	0.142	**0.006****
HTR1B_rs6296 **C**	0.108	**0.005****	0.133	**0.011****	0.178	**0.039***	0.091	**0.007****	0.096	**0.036***	0.192	**0.010****
HTR1B_rs130058 **T**	-0.009	0.807	-0.009	0.850	-0.023	0.786	0.001	0.982	-0.001	0.976	0.003	0.972
HTR1B_rs11568817 **G**	-0.023	0.502	-0.051	0.341	-0.007	0.916	-0.011	0.716	-0.058	0.224	0.040	0.462

Minor alleles are in bold after the rs numbers
Italic – trend in interaction ($0.05 < p < 0.1$)
Bold – significant interaction ($p < 0.05$)
* False discovery rate correction for multiple testing $0.05 < q < 0.1$
** False discovery rate correction for multiple testing $q < 0.05$

Table 3 The effect of rs6295 on correctly identified face emotions (%, table a) and on reaction time (RT) for correctly identified face emotions (table b).

a	Additive		Dominant		Recessive	
	β	P	β	P	β	P
Happiness_%	-2.437	0.348	0.247	0.951	-7.151	*0.099*
Sadness_%	-7.307	**0.005****	-5.409	0.193	-14.17	**0.001****
Fear_%	-0.117	0.968	5.361	0.233	-6.667	0.172

b	Additive		Dominant		Recessive	
	β	P	β	P	β	P
Happiness_RT	8.174	0.891	-7.224	0.937	32.14	0.750
Sadness_RT	-116.3	0.127	-88.95	0.445	-225.3	*0.082*
Fear_RT	-171.8	**0.008****	-195.4	*0.053*	-253.2	**0.020***

Italic – trend in interaction ($0.05 < p < 0.1$)
Bold – significant interaction ($p < 0.05$)
* False discovery rate correction for multiple testing $0.05 < q < 0.1$
** False discovery rate correction for multiple testing $q < 0.05$

Table 4 Allele frequencies of SNPs

Marker	**Alleles**	**Allele frequency**
*HTR1A*_rs6295	G	49.13%
*HTR1A*_rs6295	C	50.87%
*HTR1A*_rs878567	T	48.87%
*HTR1A*_rs878567	C	51.13%
*HTR1A*_rs749098	G	74.85%
*HTR1A*_rs749098	C	25.15%
*HTR1B*_rs6296	C	26.60%
*HTR1B*_rs6296	G	73.40%
*HTR1B*_rs130058	A	68.87%
*HTR1B*_rs130058	T	31.13%
*HTR1B*_rs11568817	T	53.81%
*HTR1B*_rs11568817	G	46.19%

Table 5 Genotype frequencies in the three different life events groups

Table 5a HTR1A rs6295 and mean BSI Depression scores in the three life events groups

HTR1A_rs6295	**Mean (BSI Depression)**	**Mean (BSI Depression)**	**Mean (BSI Depression)**
Genotype	**0-1 Life events**	**2 Life events**	**3-8 Life events**
C_C	0.99 (n=223)	1.28 (n=79)	1.4 (n=63)
G_C	0.89 (n=415)	1.39 (n=113)	1.59 (n=116)
G_G	1.0 (n=219)	1.3 (n=63)	1.94 (n=54)

The number of individuals in each group (n)

Table 5b HTR1A rs6295 and mean BSI Anxiety scores in the three life events groups

HTR1A_rs6295	**Mean (BSI Anxiety)**	**Mean (BSI Anxiety)**	**Mean (BSI Anxiety)**
Genotype	**0-1 Life events**	**2 Life events**	**3-8 Life events**
C_C	0.91 (n=223)	1.04 (n=79)	1.28 (n=63)
G_C	0.81 (n=415)	1.21 (n=113)	1.38 (n=116)
G_G	0.91 (n=219)	1.23 (n=63)	1.74 (n=54)

The number of individuals in each group (n)

Table 5c HTR1B rs6296 and mean BSI Depression scores in the three life events groups

HTR1B_rs6296	**Mean (BSI Depression)**	**Mean (BSI Depression)**	**Mean (BSI Depression)**
Genotype	**0-1 Life events**	**2 Life events**	**3-8 Life events**
C_C	0.69 (n=59)	1.14 (n=16)	2.14 (n=12)
C_G	0.9 (n=230)	1.37 (n=78)	1.69 (n=58)
G_G	1.03 (n=368)	1.35 (n=98)	1.49 (n=98)

The number of individuals in each group (n)

Table 5d HTR1B rs6296 and mean BSI Anxiety n scores in the three life events groups

HTR1B_rs6296	**Mean (BSI Anxiety)**	**Mean (BSI Anxiety)**	**Mean (BSI Anxiety)**
Genotype	**0-1 Life events**	**2 Life events**	**3-8 Life events**
C_C	0.65 (n=59)	1.32 (n=16)	1.87 (n=12)
C_G	0.87 (n=230)	1.18 (n=78)	1.41 (n=58)
G_G	0.91 (n=368)	1.16 (n=98)	1.33 (n=98)

The number of individuals in each group (n)

Table 6. Mean values for Level2 healthy controls in the HTR1A rs6295 genotype groups

Table 6a. Mean values for the proportion of correctly recognised emotions

			Proportion of correctly recognised emotions		
			Happy	**Sad**	**Fear**
rs6295_C_C	n=24	mean:	68.75	54.51	46.88
rs6295_G_C	n=59	mean:	70.91	51.9	54.53
rs6295_G_G	n=20	mean:	63.75	40.83	46.67

The number of individuals in each genotype group (n)

Table 6b. Mean values for the reaction times for correctly recognised emotions

			Reaction time for correctly recognised emotions		
			Happy	**Sad**	**Fear**
rs6295_C_C	n=24	mean:	1948.82	2290.37	2275.08
rs6295_G_C	n=59	mean:	1944.65	2277.06	2134.83
rs6295_G_G	n=20	mean:	1932.00	1984.12	1909.54

The number of individuals in each genotype group (n)

Manuscript 1

Evidence for epistatic interaction between *HTR2A* and *SLC6A4* genes in depression and associated phenotypes

5. Study 2

Evidence for epistatic interaction between *HTR2A* and *SLC6A4* genes in depression and associated phenotypes

Krisztina Mekli[1,2,3], Gabriella Juhasz[1], Diana Chase[1], Hazel Platt[3], Fabio Miyajima[4], Gyorgy Bagdy[2,5], William Ollier[3], Neil Pendleton[6], Mike Horan[6], Ian M Anderson[1], JF William Deakin[1], Antony Payton[3]

[1]Neuroscience and Psychiatry Unit, School of Community Based Medicine, Faculty of Medical and Human Sciences, University of Manchester, UK

[2]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, Budapest, Hungary

[3]Centre for Integrated Genomic Medical Research, School of Translational Medicine, Faculty of Medical and Human Sciences, University of Manchester, UK

[4]Department of Pharmacology and Therapeutics, School of Biomedical Sciences, University of Liverpool, Liverpool, L69 3GE

[5]Department of Pharmacodynamics, and Group of Neurochemistry, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary

[6]Department of Clinical Gerontology, University of Manchester, Greater Manchester, UK, M6 8HD

Correspondence: Krisztina Mekli, Neuroscience and Psychiatry Unit, School of Community Based Medicine, Faculty of Medical and Human Sciences, The University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, UK.

Tel: +44 161 275 7762

Fax: +44 161 275 7429

E-mail: Krisztina.Mekli@postgrad.manchester.ac.uk

Keywords: depression, polymorphism, serotonin, receptor, association

Abstract

Vulnerability to depression is a complex behavioural trait whose aetiology is likely to be influenced by both genetic and environmental factors. Gene-environment interaction has been widely reported but less information is available for interactions between polymorphisms within and between genes. Both the *SLC6A4* and *HTR2A* have been the focus of association studies in behavioural genetics but the results have been inconsistent and difficult to interpret. Here we use two large cohorts (n=1563 and 2440) of Caucasian volunteers and a haplotype tagging SNP (htSNP) approach to investigate both independent and interaction associations between *SLC6A4*/*HTR2A* genes and depression, neuroticism and anxiety. We found no compelling evidence for an independent association between any htSNPs and behavioural phenotypes. In contrast there were significant interactions (nominal p=0.04 to 0.0001) between several *HTR2A* haplotype blocks that was observed in both cohorts. Interaction was also found in both cohorts between several *HTR2A* htSNPs and the *SLC6A4* SNP rs379808 (nominal p=0.04 to 0.0004) which is an SNP previously associated in mood disorders. The nominal p values did not survive the Bonferroni-correction for multiple testing. This data is suggestive of possible biological interactions between behavioural phenotypes (depression, anxiety and neuroticism) and SNPs that are worthy of replication.

Introduction

Depression is a serious mental health problem that affects over 16% of individuals within developed countries at least once in their lives (Kessler *et al.* 2005). The biochemical mechanisms behind depression and the closely related personality traits of anxiety and neuroticism have implicated the involvement of the serotonergic system in their aetiology (Murphy *et al*. 2008, Tamminga *et al.* 2002, Quintin *et al.* 2001; Smith *et al*. 1997).

A twin study meta-analysis has estimated the heritability of major depressive disorder (MDD) to be 37% (Sullivan *et al.* 2000). Unfortunately, finding robust genetic associations with depression has proven a difficult undertaking. A candidate gene

approach has resulted in the publication of a large number of often underpowered and inconsistent reports that are difficult for the reader to interpret and draw meaningful conclusions from. Meta-analysis of MDD candidate gene studies has provided evidence of association for a small number of genes of small effect although this type of analysis tends to be hampered by inadequate gene coverage and between study heterogeneity (Lopez-Leon *et al.* 2008). A recent comparison of 57 previously associated MDD genes (78 publications) against Genome Wide Association Study (GWAS) data (1738 cases, 1802 controls) could only replicate the association with 4 genes (*TNF, SLC6A2*, *NPY* and *C5orf20*) and the authors concluded that "even these significant results may well be false positives" (Bosker *et al.* 2010). GWAS studies themselves have fared little better with several recent studies finding nothing of genome wide significance (Shi *et al*. 2010; Shyn *et al*. 2009; Muglia *et al.* 2010, Sullivan *et al.* 2009).

Whilst factors such as population stratification, phenotype classification and sample size are all possible contributors towards literature discrepancies, other possible confounders are gene-gene and gene-environment interactions (Uher and McGuffin 2008, Eley *et al.* 2004, Jokela *et al.* 2007). The importance of gene-environment interactions in depression was first shown by Caspi and colleagues who demonstrated that both a particular variant (s allele) of a functional promoter polymorphism (5-HTTLPR) within the serotonin transporter gene and exposure to stressful life events were required to observe a significant association (Caspi *et al*. 2003). The large diversity of serotonin receptors (at least 14 identified so far) suggests an evolutionary requirement for a complex fine tuning of the pathway. It is therefore not inconceivable that not only will the environment modulate the action of genes but also there may exist complex interplay between the genes themselves.

Two genes in particular have received much attention in MDD genetic association studies due to their central role in serotonergic function. Serotonin 2A receptors (5-HT2A) regulate the effects of serotonin and are located predominantly in cortical regions of the brain on γ-aminobutyric acid interneurons and glutamatergic pyramidal neurons (Jakab and Goldman-Rakic 2000; Willins *et al.* 1997). Several lines of evidence suggest that the serotonin receptor 2A gene (*HTR2A*) is a potential candidate for depression. The antidepressant Imipramine is an antagonist for 5-HT2A receptors and the mood stabiliser valproic acid has been shown to affect a signaling

cascade that involves 5-HTR2A receptors (Sullivan *et al*. 2004; Yatham *et al*. 2005). Post-mortem and positron emission tomography studies have found a significant increase in the number of 5-HT2A receptor binding sites in the frontal cortex of both suicide victims (Arora and Meltzer 1989; Arango *et al*. 1990; Turecki *et al*. 1999) and depression cases compared against controls (Yates *et al*. 1990; Hrdina *et al*. 1993; Bhagwagar *et al.* 2006) although others have found no association (Arranz *et al*. 1994; Gross-Isseroff *et al.* 1989).

Since 1999, 16 genetic studies have investigated the influence of *HTR2A* polymorphisms on depression with 6 reporting an association (Jokela *et al*. 2007, Christiansen *et al*. 2007, Choi *et al.* 2004, Eley *et al*. 2004, Jansson *et al.* 2003, Zhang *et al*. 1997) and 10 finding no association (Tencomnao *et al.* 2010, Illi *et al.* 2009, Zhang *et al.* 2009, Kishi *et al*. 2009, Yoon and Kim 2009, Shaikh *et al*. 2008, Oswald *et al.* 2003, Minov *et al*. 2001, Frisch *et al.* 1999, Tsai *et al.* 1999). Unfortunately, the populations studied and assessments used have been varied and the sample sizes were often underpowered. In addition, most groups have investigated only a single SNP. This has resulted in an array of contrasting reports that fail to conclude whether *HTR2A* is associated with depression or not.

One of the most intensively investigated genes in psychiatric genetics is the serotonin transporter gene (*SLC6A4*). This gene encodes the serotonin transporter protein (SERT) which is involved in the re-uptake of 5-HT from the synaptic cleft into the nerve endings. The selective serotonin reuptake inhibitors (SSRIs) are the most widely used agents in the treatment of depression and have a high affinity to the SERT molecule in the synaptic cleft (Murphy *et al.* 2004). The first suggestion of an interaction between *HTR2A* and *SLC6A4* came from pharmacological studies of the SSRIs citalopram and paroxetine which both show a high specificity for SERT. Two independent candidate gene studies reported associations between polymorphisms in the *HTR2A* gene and response to citalopram in MDD patients (Choi *et al.* 2005, Peters *et al.* 2009). Another study identified a *HTR2A* polymorphism (rs6314) that was associated with response to paroxetine (Wilkie *et al*. 2009). Interestingly, two of these studies investigated SNPs within *SLC6A4* and found no association with drug response (McMahon *et al*. 2006, Wilkie *et al.* 2009). A genome wide association study analyzing the same sample found that seven *HTR2A* SNPs were nominally associated with citalopram response and remission ($p<0.05$) in MDD but no

association was observed for *SLC6A4* SNPs (Garriock *et al.* 2010). In a different sample two *HTR2A* SNPs were associated with response to antidepressant treatment (escitalopram or nortriptyline) but none in the *SLC6A4* gene (Uher *et al.* 2009).

Additional evidence for interaction has come from a study of a transgenic mouse model that overexpresses SERT (Jennings *et al*. 2008). It was found that an increase in SERT expression was accompanied by an increase in 5-HT2A receptor function which the authors hypothesized may influence SERT associated phenotypes. More recently a PET study in humans found that the *HTR2A* SNPs (rs7333412, rs7997012, rs977003, rs985933 and rs594242) were significantly associated with thalamic SERT binding potential, indicating a link between *HTR2A* genetic variation and either SERT expression or central serotonin transmission that regulates the therapeutic response of SERT in depression (Laje *et al.* 2010).

Here we investigated gene-gene interactions within and between *HTR2A* and *SLC6A4* and depression, anxiety and neuroticism using two large independent cohorts.

Methods

Participants

Two independent cohorts were used in this study which allowed for replication of significant associations.

NewMood cohorts

Two cohorts were parts of the EU funded NewMood (New Molecules for Mood Disorders) research program that aims to identify new molecular mechanisms involved in vulnerability towards depression. We recruited and obtained DNA and completed questionnaires from 1387 unrelated Caucasian participants aged between 18-60 years (mean age 34 years) from Greater Manchester, UK through general practices, and a web-site (http://www.newmood.co.uk). Details of our recruitment strategy and responses are described elsewhere (Juhasz *et al*. 2009). The study was approved by the local Ethics Committees (North Manchester Local Research Ethics Committee (LREC) (ref. 05/Q1406/26), the University of Manchester's Ethics Committee (ref. 05056)) and was carried out in accordance with the Declaration of

Helsinki. All participants provided written informed consent. These participants are referred to as the NewMood Manchester cohort.

The NewMood Budapest population was recruited in Hungary, consisting of 1053 Caucasian participants. The mean age of this cohort was 31.2 years. The recruitment of participants was described elsewhere (Lazary *et al.* 2008). The method of DNA collection and extraction was identical to the Manchester group. The depression, anxiety and neuroticism questionnaires were the Hungarian translated and validated versions of the original questionnaires. All subjects were Hungarian and of Caucasian origin and they gave written informed consent before entering the study. The study was approved by the Central Ethics Committee. These participants are referred to as the NewMood Budapest cohort.

Dyne Steel Cohort

The Dyne Steel cohort is comprised of 1563 Caucasian volunteers (454 males and 1109 females; mean age 67.4 years) from the Manchester and Newcastle areas of the UK with approximately equal numbers collected from the two regions. All volunteers achieved the maximum score on the MMSE (Mini Mental State Examination). Recruitment and sample composition details are described elsewhere (Rabbitt *et al.* 2004). Volunteers gave written consent for the use of their DNA. Genetics work for the cohort is approved by University of Manchester Research Ethics Committee and Salford and Trafford Local Research Ethics Committee. The DNA bank is formed from approximately equal numbers of samples collected from Manchester and Newcastle.

Phenotype assessments

The NewMood assessments consisted of standard questionnaires that were sent to participants to complete and return to us by post. We used the same background questionnaire as we used in our previous studies (Juhasz *et al.* 2009, Lazary *et al.* 2008). This structured self-rating questionnaire consists of 22 items and collects information about medical history including psychiatric history (with special interest on depression), family psychiatric history, and socioeconomic background. The Brief Symptom Inventory (BSI) questionnaire (Derogatis 1993) was used to assess both depressive symptoms (six items) and anxiety symptoms (six items). A continuous

weighted dimension score was calculated for both depression and anxiety symptoms, which were used in the analysis. To assess personality the 44-item Big Five Inventory (BFI-44) was used (John *et al*. 1991). For the analysis a continuous weighted dimension score was calculated for Neuroticism. Validation data of neuroticism and symptom scores were published previously (Juhasz *et al*. 2009).

The Dyne Steel cohort participants completed the Beck Depression Inventory (21 items, mean 6.834, Standard Error of Mean, SEM 0.147) (Beck *et al.* 1961), the Yesavage Geriatric Depression Questionnaire (mean 6.329, SEM 0.134) (30 items) (Yesavage *et al.* 1983), the Cornell Medical Index depression (mean 0.277, SEM 0.018) and anxiety (mean 0.82, SEM 0.033) questionnaires (6 and 9 items) (Brodman *et al.* 1956), and the Eysenck Personality Questionnaire (only neuroticism was used in the analysis, mean 10.094, SEM 0.15) (23 Items) (Eysenck and Eysenck 1964).

DNA extraction and genotyping

NewMood

NewMood participants received a cytology brush (Cytobrush plus C0012, Durbin PLC) and a 15-mL plastic tube containing 2.0 ml of collection buffer. Buccal mucosa cells were collected and genomic DNA was extracted according to a protocol previously described (Freeman *et al*. 2003). DNA for the Dyne Steel cohort was obtained from blood samples and was extracted using a standard phenol-chloroform method.

HaploView software (www.broad.mit.edu/personal/jcbarret/haploview/) was used to identify htSNPs for the NewMood cohort using the confidence interval method (Barrett *et al.* 2005, Gabriel *et al.* 2002). Specifications: confidence interval minima for strong LD, upper: 0.98, lower: 0.7, upper confidence interval maximum for strong recombination: 0.9, fraction of strong LD in informative comparisons at least: 0.95, markers excluded below 5% minor allele frequency, r^2=0.8, pairwise method.

The tagging was based on the CEPH (Utah residents with ancestry from northern and western Europe) population genotype data that were available at the International HapMap Project Phase I. June 2005 release (www.hapmap.org). We also investigated functional SNPs (*HTR2A* rs6314, *SLC6A4* LPR and rs25531) that had previously been reported (Ozaki *et al*. 1997, Lesch *et al.* 1996, Nakamura *et al.* 2000). The NewMood SNPs were genotyped using the Sequenom® MassARRAY

technology (Sequenom®, San Diego, http://www.sequenom.com) using 25ng of DNA. Primer sequences are available upon request. The 5-HTTLPR was genotyped using a forward primer (5'- GCCAGCACCTAACCCCTAAT -3') labelled with 6-FAM (6-carboxyfluorescein) and a reverse primer (5'- GTAGGGTGCAAGGAGAATGC -3'), to give a product size of 366 bp and 323bp for the long and the short allele, respectively. Reactions were performed in a Thermo-Fast® 384 PCR plate (ABgene Ltd, UK) on a PTC-220 Dyad TM thermocycler (Bio-Rad, Hercules, CA). PCR amplification was performed in a final 10 µl volume containing 0.25 µM of each primer (Metabion, GmbH, Germany), 1x NH_4 buffer, 1.5 mM $MgCl_2$, 0.1 mM of each d NTP, 0.2 units of Taq DNA polymerase (all from Bioline, UK) and 20 ng DNA. Cycling conditions were as follows: initial 15 min denaturation at 95°C, then 34 cycles of denaturation at 94°C for 20 s, annealing at 64°C for 30 s and extension at 72°C for 30 s; and a final extension for 10 min at 72°C. Polymerase chain reaction products were analyzed using an ABI3100 Genetic Analyzer and GeneScan analysis software (Applied Biosystems, Nieuwerkerk aan den IJssel, the Netherlands).

Dyne Steel

Dyne Steel SNPs were selected from existing GWAS data which had been genotyped on the Illumina 610 Quad microarray (Illumina, inc. San Diego, CA, USA). These SNPs spanned the *HTR2A* gene (13:47'403'360 (rs7333412) to 47'471'761 (rs6306)) and the *SLC6A4* gene (17:28'515'479 (rs1906451) to 28'572'753 (rs1487971)). HelixTree™ 6.4.3 (Golden Helix, USA) software was used to select htSNPs from the Dyne Steel Cohort using a minimum MAF of 0.2 and a Carlson LD threshold of 0.8. Fourteen and four htSNPs were selected from the *HTR2A* and *SLC6A4* genes, respectively. In addition, *HTR2A* rs6314 and rs731779 were selected as they were also genotyped in the NewMood study and therefore could be compared directly. All Dyne Steel SNP call rates were above 98%.

All genotyping was performed blind with regard to phenotype. All laboratory work was performed under the ISO 9001:2000 quality management requirements.

Statistical Analysis

Allelic association tests (using linear regression analysis), Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) calculations were performed using HelixTree^{TM} 6.4.3 (Golden Helix, USA). PLINK v1.06 (http://pngu.mgh.harvard.edu/purcell/plink/) was used for testing association of additive genetic model (linear regression) in all both cohorts. Gene-gene interactions were calculated between *HTR2A* and *SLC6A4* SNPs with Plink. All association analyses were adjusted for age and sex. Correlation calculations between phenotypes were performed by using SPSS 15.0 for Windows software (SPSS Inc. Chicago, Illinois, USA). Statistical analyses were performed independently in the NewMood and Dyne Steel cohorts.

A web-based tool Gliders (http://mather.well.ox.ac.uk/Gliders) was used to identify SNPs in perfect or high LD to attempt direct replication between the two cohorts. The LD r^2 values for the SNPs can be seen in Table 5.

To evaluate the effects of SNPs on gene expression the Stanley Neuropathology Consortium Integrative Database (http://sncid.stanleyresearch.org) and the SNPExpress Database (Heinzen *et al.* 2008) were used.

Broad Institute web-based tool Tagger was used to evaluate the tagger SNPs (http://www.broadinstitute.org/mpg/tagger/).

Results

Allele frequencies in the three populations were similar (Tables 1 and 2) and the haplotype structures for the Manchester and Budapest NewMood samples were the same for the *HTR2A* gene (Figure1b and 1c). In the *SLC6A4* gene in the Budapest cohort the LD between rs2020934 and rs25531 was not as strong as in the Manchester cohort (Figure2b and 2c). Regardless, the Manchester and Budapest NewMood populations were combined. NewMood SNPs were in Hardy-Weinberg equilibrium (HWE) with the exception of *HTR2A* rs1928040 (p=0.024). Four out of twenty SNPs (*HTR2A* rs2770298 (p=0.024), rs731779 (p=0.036), rs927544 (p=0.043) and *SLC6A4* (rs3794808, (p=0.023)) were out of HWE in the Dyne Steel cohort using the $p<0.05$ threshold. Two of these SNPs (rs2770298 and rs731779) were in strong LD ($r^2=0.85$). SNPs out of HWE were removed from the interaction analysis with the

exception of the Dyne Steel SNP rs731779 and rs3794808 which were also genotyped in the NewMood cohort and therefore were tentatively analysed for comparison purposes.

Strong correlation existed between depression, neuroticism and anxiety scores within the NewMood cohorts (Pearson correlation R=0.61 - 0.76, p<0.01). Moderate to strong correlations were observed between depression scores (Perason correlation R=0.32 - 0.60, p<0.01), between the depression and anxiety scores (Perason correlation R=0.29 - 0.33, p<0.01) and between the depression and neuroticism scores (Perason correlation R=0.32 – 0.56, p<0.01) within the Dyne Steel cohort.

Allelic analysis (uncorrected for multiple testing) of independent SNPs for the Dyne Steel data found a single significant association between *HTR2A* rs731779 and the CMI depression score (p=0.047) (Table 1). Allelic analysis of the NewMood data identified an association between the *HTR2A* SNP rs3125 and depression (p=0.012) and neuroticism (p=0.005) with anxiety showing a non-significant trend (p=0.095) (Table 2). Significance was also observed between *HTR2A* SNPs rs2770296, rs985934 and rs6310 and anxiety (p=0.028, 0.025 and 0.016, respectively). Significance of the above results did not remain after Bonferroni correction. Allelic analysis found no associations between *SLC6A4* SNPs and behavioural phenotypes for either of the cohorts. Genotypic analysis using an additive model showed similar results to the allelic analysis.

Gene-gene interaction was investigated within and between *HTR2A* and *SLC6A4*. Supplementary Table S2 and S3 contain all possible interactions for the Dyne Steel and for the New Mood SNPs, respectively.

Interaction within HTR2A

The Dyne Steel data showed six *HTR2A* htSNPs flanking exon 3 interacting with SNPs on the opposite side of the gene (Table 3). It is noteworthy, that the LD between the interacting SNPs was weak to moderate (r^2=0.03 – 0.35, mean r^2 =0.21). The strongest interactions were observed between SNPs within haplotype blocks 1 and 3 (Table 3, Figure 1) where in particular rs1923884 and two SNPs approximately 25kbp downstream (rs972979 and rs2770304) were strongly associated with the Yesavage depression score (p=0.0001 – 0.0002, respectively). Significant interactions and non-significant trends were observed between these htSNPs and all

other measures of depression, anxiety and neuroticism with the direction of effect being the same for each trait. In the HTR2A gene there were 3 blocks in the New Mood cohort instead of four (Figure 1b and 1c). Interactions were strongest between block 1 (rs9316232) and block 3 (rs731779 and rs985934) in the NewMood cohort (Table 4). Analysis of the NewMood data also found that two exon 2 SNPs (rs2770296 and rs731779) showed significant interaction with the promoter SNP rs6311 for both depression and anxiety scores (with rs2770296: for depression p=0.007, β=0.171, for anxiety p=0.006, β=0.151; with rs731779 for depression p=0.018, β=0.182, for anxiety p=0.007, β=0.18) (Table 4). In the original data set 3 promoter SNPs were genotyped for the Dyne Steel cohort (rs6313, rs6312 and rs6306). SNP rs6312 showed interaction with rs2770296 in association with CMI depression scores (p=0.02, β=-0.246), and EPQ neuroticism scores (p=0.014, β=-1.92) and with rs731779 in association with EPQ neuroticism scores (p=0.0039, β=-2.384). This latter observation should be considered with caution as rs731779 was out of HWE in this cohort (p=0.036). SNP rs6306 interacted with rs2770296 in association with EPQ neuroticism (p=0.04, β=1.827) (Supplementary Table S2). (SNP rs2770296 is located in the third haplotype block in the original data set, data not shown.) It is noteworthy, that the direction of the interaction was not always the same between the two cohorts. Whilst direct comparisons between cohorts were difficult, two SNPs were genotyped in both the Dyne Steel and NewMood cohorts (*HTR2A* rs6314 and rs731779). Interaction analysis between these two SNPs reached significance for two measures of depression in the Dyne Steel cohort (Beck p=0.023; Yesavage p=0.015) and a non-significant trend with the same direction of effect was observed for CMI depression (p=0.149). It should be noted however, that rs731779 was not in HWE (p=0.036) in this cohort. The interaction between these two SNPs was observed in the NewMood cohort although in a different test (BSI depression p=0.045).

Table 1: Allelic association analysis of independent *HTR2A* and *SLC6A4* haplotype tagging SNPs in the Dyne Steel cohort

Gene	SNP	MAF (%)	HWE	Beck Depression	Yesavage Depression	CMI Depression	CMI Anxiety	EPQ Neuroticism
					P-Value			
HTR2A	rs7333412	22.8	0.596	0.845	0.540	0.449	0.169	0.298
HTR2A	rs6314	9.4	0.735	0.719	0.688	0.164	0.121	0.820
HTR2A	rs977003	45.3	0.940	0.767	0.416	0.671	0.140	0.610
HTR2A	rs1923884	13.1	0.299	0.428	0.715	0.537	0.996	0.578
HTR2A	rs1923886	46.5	0.675	0.507	0.643	0.709	0.865	0.982
HTR2A	rs622337	30.2	0.478	0.765	0.639	0.656	0.715	0.798
HTR2A	rs1928042	26.2	0.586	0.624	0.990	0.133	0.489	0.292
HTR2A	rs2770298	29.1	**0.024**	0.108	0.544	0.778	0.133	0.145
HTR2A	rs972979	37.5	0.501	0.073	0.920	0.986	0.425	0.377
HTR2A	rs731779	17.7	**0.036**	0.181	0.662	**0.047**	0.437	0.171
HTR2A	rs2770304	31.9	0.817	0.070	0.881	0.915	0.543	0.503
HTR2A	rs927544	28.3	**0.043**	0.068	0.966	0.365	0.762	0.229
HTR2A	rs4942587	22.6	0.060	0.103	0.684	0.226	0.999	0.410
HTR2A	rs4941573	41.4	0.198	0.063	0.842	0.514	0.165	0.997
HTR2A	rs1328684	36.2	0.434	0.639	0.847	0.106	0.239	0.426
HTR2A	rs9534511	49.5	0.896	0.178	0.504	0.816	0.726	0.680
SLC6A4	rs3794808	40.8	**0.023**	0.136	0.151	0.430	0.927	0.237
SLC6A4	rs6354	20.2	0.291	0.036	0.871	0.360	0.325	0.195
SLC6A4	rs2066713	39.9	0.111	0.982	0.129	0.664	0.520	0.284
SLC6A4	rs1487971	36.9	0.248	0.539	0.943	0.585	0.786	0.487

Minor allele frequency (MAF), Hardy-Weinberg Equilibrium (HWE), Cornell Medical Index (CMI) and Eysenck Personality Questionnaire (EPQ)

Table 2: Allelic association analysis of independent *HTR2A* and *SLC6A4* haplotype tagging SNPs in the NewMood cohort

Gene	SNP	Manchester	Budapest	HWE	Depression	Anxiety	Neuroticism
		MAF (%)	MAF (%)	(Man+Bp)		P-Value	
HTR2A	rs3125	14.1	10.3	0.832	**0.012**	0.095	**0.005**
HTR2A	rs6314	8.9	8.3	0.843	0.061	0.118	0.728
HTR2A	rs9316232	35.1	33.9	0.825	0.588	0.127	0.758
HTR2A	rs2296972	29.7	29.3	0.111	0.408	0.130	0.836
HTR2A	rs2770296	28.9	25.4	0.777	0.073	**0.028**	0.115
HTR2A	rs1928040	44.8	48.1	**0.024**	0.087	0.325	0.972
HTR2A	rs731779	18.6	16.8	0.997	0.063	0.201	0.167
HTR2A	rs985934	37.8	35.9	0.567	0.160	**0.025**	0.536
HTR2A	rs6310	4.7	3.6	0.206	0.255	**0.016**	0.448
HTR2A	rs6311	40.1	42.4	0.338	0.330	0.400	0.589
SLC6A4	rs1042173	44.9	47.3	0.961	0.491	0.454	0.607
SLC6A4	rs3794808	42.8	44.9	0.146	0.238	0.326	0.141
SLC6A4	rs140700	9.4	8.8	0.551	0.919	0.346	0.610
SLC6A4	rs2020942	38.5	37.6	0.769	0.252	0.904	0.536
SLC6A4	rs6354	19.6	18.9	0.703	0.848	0.278	0.889
SLC6A4	rs2020934	48.9	48.4	0.104	0.512	0.692	0.556
SLC6A4	LPR	43.5	41.6	0.490	0.689	0.058	0.482

Minor allele frequency (MAF), Hardy-Weinberg Equilibrium (HWE), Manchester and Budapest cohort together (Man+Bp)
Depression: BSI Depression Score
Anxiety: BSI Anxiety Score
Neuroticism: Big5 Neuroticism Score

Interaction between *HTR2A* and *SLC6A4*

Interactions were observed within the Dyne Steel cohort between a cluster of three *HTR2A* SNPs (rs9534511, rs1328684 and rs4941573) spanning exon 2 and the *SLC6A4* SNP rs3794808 (Table 3, Figures 1a and 2a). These reached significance or near significance for all measures of depression (p=0.0004 – 0.065). The third promoter SNP, rs6313 showed significant interaction with two *SLC6A4* SNPs (rs3794808 and rs4583306) decreasing the depression symptoms (Supplementary Table 2). The NewMood samples also showed significant interactions between rs3794808 (and its 3'UTR neighbour rs1042173) although in this cohort they interacted with two *HTR2A* SNPs (rs9316232 and rs2296972) located in intron 2 (p=0.007 - 0.016) (Table 4). *HTR2A* SNPs rs9316232 and rs2296972 were in strong LD (r^2=0.98) (Figure 1b and 1c) as were the *SLC6A4* SNPs rs1042173 and rs3794808 (r^2=0.92) (Figure 2b and 2c). In addition, *HTR2A* rs985934 and rs6311 interacted with the *SLC6A4* SNP rs2020934 to significantly influence depression scores (p=0.05 and 0.03, respectively) in the NewMood cohort. These interactions between *SLC6A4* and *HTR2A* were primarily observed for depression and not for anxiety or neuroticism.

Table 3: Interaction analysis between haplotype tagging SNPs within and between the *HTR2A* and *SLC6A4* genes and depression, anxiety and neuroticism scores for the Dyne Steel cohort

					Beck	Yesavage	CMI Depression	CMI Anxiety	EPQ Neuroticism
Gene	SNP1	Gene	SNP2	LD (r^2)	P-Values				
HTR2A	rs7333412	*HTR2A*	rs4942578	0.127	0.070	0.113	0.771	**0.017**	0.050
HTR2A	rs7333412	*HTR2A*	rs927544	0.105	**0.012**	**0.041**	0.323	0.090	0.149
HTR2A	rs6314	*HTR2A*	rs731779	0.032	**0.023**	**0.015**	0.149	0.556	0.690
HTR2A	rs977003	*HTR2A*	rs2770304	0.232	**0.029**	**0.019**	0.088	0.312	0.196
HTR2A	rs977003	*HTR2A*	rs4942578	0.224	**0.011**	0.090	0.127	0.050	**0.048**
HTR2A	rs1923884	*HTR2A*	rs972979	0.251	0.051	**0.00011**	0.086	0.086	**0.039**
HTR2A	rs1923884	*HTR2A*	rs2770304	0.319	**0.042**	**0.00017**	**0.019**	0.054	**0.033**
HTR2A	rs1923884	*HTR2A*	rs4942578	0.344	**0.018**	**0.002**	0.096	**0.058**	0.056
HTR2A	rs1923886	*HTR2A*	rs1928042	0.346	0.063	0.077	**0.008**	0.938	0.524
HTR2A	rs1923886	*HTR2A*	rs972979	0.089	0.911	**0.040**	0.090	**0.025**	**0.042**
HTR2A	rs1923886	*HTR2A*	rs2770304	0.224	0.762	**0.039**	0.313	**0.028**	0.053
HTR2A	rs622337	*HTR2A*	rs1928042	0.232	**0.014**	**0.016**	**0.033**	0.655	**0.012**
HTR2A	rs622337	*HTR2A*	rs582385	0.295	0.059	**0.022**	0.308	0.657	**0.014**
HTR2A	rs4941573	*SLC6A4*	rs3794808	0.012	**0.044**	**0.020**	**0.00038**	0.106	0.734
HTR2A	rs1328684	*SLC6A4*	rs3794808	0.034	**0.011**	0.065	**0.010**	**0.035**	0.900
HTR2A	rs9534511	*SLC6A4*	rs3794808	0.010	**0.036**	**0.014**	**0.002**	0.341	0.680
SLC6A4	rs3794808	*SLC6A4*	rs2066713	0.642	**0.017**	0.412	**0.006**	0.477	0.287

Linkage Disequilibrium (LD), Cornell Medical Index (CMI) and Eysenck Personality Questionnaire (EPQ)

Table 4: Interaction analysis between haplotype tagging SNPs within and between the *HTR2A* and *SLC6A4* genes and depression, anxiety and neuroticism scores for the NewMood cohort

					Depression	Anxiety	Neuroticism
Gene	SNP1	Gene	SNP2	LD (r^2)		P-Value	
HTR2A	rs6314	*HTR2A*	rs2770296	0.155	**0.012**	**0.008**	0.054
HTR2A	rs6314	*HTR2A*	rs731779	0.077	**0.045**	0.062	0.251
HTR2A	rs9316232	*HTR2A*	rs2770296	0.203	**0.016**	**0.006**	**0.003**
HTR2A	rs9316232	*HTR2A*	rs731779	0.333	**0.002**	**0.003**	**0.00013**
HTR2A	rs9316232	*HTR2A*	rs985934	0.205	**0.009**	**0.00012**	**0.002**
HTR2A	rs2296972	*HTR2A*	rs731779	0.319	**0.018**	0.071	**0.003**
HTR2A	rs2770296	*HTR2A*	rs6311	0.704	**0.007**	**0.006**	0.075
HTR2A	rs731779	*HTR2A*	rs6311	0.205	**0.018**	**0.007**	0.277
HTR2A	rs985934	*SLC6A4*	rs2020934	0.081	**0.047**	0.291	0.800
HTR2A	rs6311	*SLC6A4*	rs2020934	0.048	**0.033**	0.200	0.647
HTR2A	rs9316232	*SLC6A4*	rs1042173	0.024	**0.007**	0.315	0.167
HTR2A	rs9316232	*SLC6A4*	rs3794808	0.020	**0.014**	0.504	0.413
HTR2A	rs2296972	*SLC6A4*	rs1042173	0.029	**0.016**	0.432	0.186
HTR2A	rs2296972	*SLC6A4*	rs3794808	0.029	**0.014**	0.560	0.305
HTR2A	rs2770296	*SLC6A4*	rs2020934	0.064	**0.021**	0.190	0.751
SLC6A4	rs6354	*SLC6A4*	5-HTTLPR	0.086	**0.034**	0.060	0.130

Linkage Disequilibrium (LD)

Interaction within *SLC6A4*

Finally, the *SLC6A4* SNP rs3794808 was shown to interact in the Dyne Steel cohort with another *SLC6A4* SNP rs20667713 to influence both the Beck and CMI depression scores (p=0.017 and 0.006, respectively) although this observation was not seen for the Yesavage depression measure (p=0.412). Interactions in this region were not observed for the NewMood cohort but a significant interaction was seen between rs6354 and the promoter polymorphism for depression (p=0.034) and a non-significant trend for anxiety (p=0.060).

To estimate the number of expected observations we used three subgroup CMI measures of health used for the Dyne Steel volunteers that were unlikely to be influenced by the serotonergic pathway. These comprised questions on vision and hearing, urinary/reproduction systems and the health of teeth. For vision and hearing we observed 1 significant association at p>0.01 and for urinary/reproduction and teeth we didn't observe any significant associations either within or between *HTR2A* and *SLC6A4*. In contrast, for Beck, Yesavage and CMI depression measurements when all possible interactions were considered between the haplotype tagging SNPs, we observed 5, 3 and 7 significant observations (p>0.01), respectively (data not shown), with only weak to moderate LD between the associated SNPs (r^2 =0.03 – 0. 35). Considering all the possible number of combinations between the 20 htSNPs (190) in the Dyne Steel cohort the corrected p-value for significance is 0.000263 for each phenotype. Two of the *HTR2A* interactions (rs1923884 – rs972979 associated with Yesavage depression scores, p=0.00011 and rs1923884 – rs2770304 associated with the same phenotype, p=0.00013) survived this correction. A third interaction between HTR2A rs4941573 and SLC6A4 rs3794808 associated with CMI depression scores approaches this threshold (p=0.00038). In the New Mood cohort this corrected threshold was 0.00037 (for 17 SNPs) and once again, two interactions survived (*HTR2A* rs9316232 – rs731779 associated with Big5 neuroticism scores, p=0.00013 and rs9316232 – rs985934 associated with BSI anxiety scores, p=0.00012). In this cohort no associations between *HTR2A* and *SLCA6A4* genes were strong enough to survive correction.

Direct replication attempt

Using the Gliders software we attempted a direct replication between the two cohorts focusing on the most significant results. In the Dyne Steel cohort this was shown by rs1923884. This SNP is 4887 bp away from rs9316232 which is present in the New Mood data set. Despite the proximity there were no SNPs with adequate LD which could be used as a replication proxy. The third strongest association in the Dyne Steel cohort (between rs4941573 and rs3794808) was not replicated in the New Mood cohort (corresponding SNPs: rs6311 and rs3794808) (Supplementary Table 2 and 3). In the New Mood cohort the strongest interactions were driven by rs9316232, increasing all symptom scores. Once again, this interaction was not replicated in the Dyne Steel cohort (corresponding SNPs: rs622337 and rs985933) (supplementary Table 2 and 3). Table 5 shows the corresponding SNPs between the two cohorts.

Table 5. Corresponding SNPs between the two cohorts.

Cohort1	SNP1	Cohort2	SNP2	LD (r^2)
New Mood	rs9316232	Dyne Steel	rs622337	0.87
New Mood	rs985934	Dyne Steel	rs985933	1
New Mood	rs6311	Dyne Steel	rs6313	1
New Mood	rs6311	Dyne Steel	rs4941573	0.98

LD value between the two SNPs was determined by the Gliders web-based tool (based on HapMap phase 3 CEU population).

Evaluation of the effects of SNPs on gene expression

Using the Stanley Neuropathology Consortium website we were not able to identify SNPs within or outside *HTR2A* and *SLC6A4* genes with an effect on the expression of these genes below the p=0.00005 threshold in the cerebellum, frontal cortex or the hippocampus.

SNPExpress revealed that 4 *SLC6A4* SNPs and 1 *HTR2A* SNP had a cis-acting effect on the gene expression (Table 6).

Table 6. Cis-acting effects of polymorphisms within the *HTR2A* and *SLC6A4* genes.

SNP	Gene	Affected Transcript	p value	β coefficient
rs1042173	SLC6A4	NM_001045	0.005	4.637
rs3794808	SLC6A4	NM_001045	0.0073	-4.849
rs6354	SLC6A4	NM_001045	0.02	4.884
rs2066713	SLC6A4	NM_001045	0.058	-1.234
rs3794808	SLC6A4	NM_001045	0.05	-15.82
rs3794808	SLC6A4	NM_001045	0.046	1.754
rs6354	SLC6A4	NM_001045	0.06	-4.316
rs2066713	SLC6A4	NM_001045	0.051	-8.121
rs6311	HTR2A	NM_000621	0.053	-27.28

SLC6A4 mRNA transcript (NM_001045)
HTR2A mRNA transcript (NM_000621)

Discussion

We report a series of possible genetic interactions both within and between the *HTR2A* and *SLC6A4* genes and the behavioural traits of depression, anxiety and neuroticism. In contrast, when we analysed SNPs independently within these genes we found no strong evidence of association. However a current genome-wide study of a large UK population suggests the involvement of rs132867 (*HTR2A*) and rs1487971 (*SLC6A4*) in depression (Lewis *et al.* 2010). Our results suggest that interactions between serotonergic SNPs are important in determining phenotypic variation.

The literature concerning *HTR2A* polymorphisms and depression is inconclusive. The reasons for these inconsistencies may include population stratification effects, inadequate sample size, different phenotypic assessment methods and failure to adjust for possible environment or gene-environment effects. Indeed, previous studies have investigated 11 different populations, used sample sizes ranging from 51 to 401 cases and have accessed depression using 7 different methods (Jokela *et al*. 2007, Christiansen *et al*. 2007, Choi *et al.* 2004, Eley *et al*. 2004, Jansson *et al.* 2003, Zhang *et al*. 1997, Tencomnao *et al.* 2010, Illi *et al.* 2009, Zhang *et al.* 2009, Kishi *et al*. 2009, Yoon and Kim 2009, Shaikh *et al*. 2008, Oswald *et al.* 2003, Minov *et al*. 2001, Frisch *et al.* 1999, Tsai *et al.* 1999). Similar contrasting results have been reported between depression and *SLC6A4* polymorphisms with two

out of four meta-analysis finding no association between the promoter (5-HTTLPR) and intron 2 VNTR polymorphism (Lopez-Leon *et al.* 2008; Anguelova *et al*. 2003; Lasky-Su *et al.* 2005; Furlong *et al.* 1998). Further confounders for the commonly investigated 5-HTTLPR include the interaction with stress sensitivity (Caspi *et al*. 2010) and the tri-allelic grouping caused by the insertion of a guanine nucleotide in the long allele (Hu *et al*. 2006).

Another explanation for inconsistency between reports is that a gene or pathway may contain more than one functional polymorphism and the overall effect on the phenotype may be determined by a combination of functional polymorphisms whose independent influence may or may not show the same direction of effect. These genetic interactions are well documented and have already been reported within the *SLC6A4* gene (Hranilovic *et al.* 2004). The *HTR2A* (63kbp) and *SLC6A4* (38kbp) genes would require the genotyping of approximately 10 and 4 htSNPs, respectively, (assuming a MAF of 0.25 and a Carlson LD threshold of 0.8) for complete gene coverage. Ignoring the possibility of interaction, 11 of the 15 *HTR2A*/depression studies opted to investigate only a single SNP with the remainder analysing between 2 and 4 SNPs. For reports on *SLC6A4* the majority of groups have only investigated the 5-HTTLPR polymorphism.

Our study used both tagging SNPs and two large independent cohorts comprised of 1563 (Dyne Steel) and 2440 (NewMood) individuals. Both cohorts have over 87% power to detect a genetic interaction effect size of 1% between two SNPs assuming a MAF of 0.2 and a significance threshold of 0.005 (2-sided). Significant interactions within and between *HTR2A* and *SLC6A4* were found in both cohorts, although they were not between the same SNPs.

Within the *HTR2A* gene similar interaction patterns emerged for both cohorts with the strongest interactions occurring between haplotype blocks 1 and 3 (Figure 1) although the structures of these blocks were different between the two cohorts. Two SNPs (rs6314 and rs731779) genotyped in both cohorts allowed direct replication of their associations to increased symptom scores in both cohorts. This association did not remain significant after correction for multiple testing. The strongest interaction results in the Dyne Steel were driven by rs1923884 which has been associated with worse outcome of treatment with nortiptyline in depression in a genome-wide study (Uher *et al.* 2010).

An additional interaction was observed between SNPs within haplotype block 3 (rs2770296 and rs731779) and the promoter polymorphism rs6311 in the NewMood cohort. SNP rs6311 was not genotyped in the Dyne Steel cohort, but this SNP is in perfect LD with rs6313 (Table 5). SNP rs6313 did not show significant interaction with the HTR2A SNPs (rs2770296 and rs731779) in this cohort. Two other polymorphisms in the *HTR2A* gene also could be used for comparison (rs6312 and rs6306). SNP rs6312 showed interaction with rs2770296 and rs731779, similar to the New Mood but the direction of effect was opposite (dercreasing the symptom scores, rather than increasing it). The latter results should be considered with caution, as the rs731779 was out of HWE (p=0.036) in the Dyne Steel cohort. The third promoter SNP in this cohort was rs6306 and it was in interaction with rs2770296 with the direction being the same as in the New Mood cohort. These results indicate the existence of at least two regions of interaction with the *HTR2A* gene, between blocks 1 and 3 and between the promoter and block 3. These interactions influenced not only reported depression and anxiety symptoms but also neuroticism that is a trait personality factor for major depressive disorder and anxiety disorders. Previous twin studies demonstrated that correlations between neuroticism and these disorders largely resulte from shared genetic risk factors (Kendler *et al*. 2006, Hettema et al. 2004). Despite twin studies indicating strong heritability of neuroticism genome wide association studies were not able to find common SNP variations that strongly influence neuroticism (Calboli *et al.* 2010, Shifman *et al.* 2008).

Interactions between genes and depression were the most consistent and strongest around the *SLC6A4* SNP rs3794808 which reached significance with intron2/exon 2 *HTR2A* SNPs in both cohorts. Unfortunately, the rs3794808 SNP was marginally outside HWE in the Dyne Steel cohort, and given the Dyne Steel volunteers were not selected for depression status this makes the replication more tentative. In addition, the two cohorts showed different regions of the *HTR2A* gene that were interacting with rs3794808, with the Dyne Steel cohort showing interaction with tagging SNPs around exon 2 and the NewMood cohort showing interaction with SNPs approximately 35kb upstream within intron 2. The reasons for this difference in observation is unknown and is unlikely to be explained by LD as NewMood tagging SNPs closer to exon 2 showed no association. However, a recent study reported that rs3794808 contributed towards mood disorders via an interaction with childhood

physical abuse suggesting that a gene-environment as well as gene-gene influence may be required to determine the overall effect (Brezo *et al.* 2010). Further support for the role of rs3794808 in mood disorders comes from a recent study that showed a three SNP haplotype that included rs3794808, was associated with a 1.7 fold increase in panic disorder in European Americans (Strug *et al.* 2010). Another study supporting a relationship between *SLC6A4* and *HTR2A* used positron emission tomography to show that three SNPs spanning the *HTR2A* 3' untranslated region significantly influenced serotonin transporter binding in the thalamus (Laje *et al.* 2010). Interestingly, our between gene results appeared only to influence depression and had minimum impact on anxiety or neuroticism with the exception of an interaction between *HTR2A* rs1328684 and *SLC6A4* rs3794808 and anxiety which showed only a weak association. Unlike interactions within the *HTR2A* gene these results suggest that phenotypic specificity may reside in interactions of genetic risk variants throughout molecular pathways.

SNP interactions within *SLC6A4* were less convincing and were not replicated between cohorts. The promoter polymorphism 5-HTTLPR did show weak but significant interaction with rs6354 for depression and near significance with anxiety in the NewMood cohort but we were unable to replicate this finding in the Dyne Steel cohort which was not genotyped for 5-HTTLPR.

The functional data available is not enough at the moment to establish the exact nature of these interactions. It is possible that the observed interactions reflect an additive effect caused by allelic combinations when their effects go in the same direction. It is also possible that the observed effects are the result of interaction and are not additive in nature. An interaction effect may confound results from expression studies.

The strength of our study is the detailed phenotype assessments and the two large independent cohorts of Caucasian subjects which made it possible to detect the interaction between and within the *HTR2A* and *SLC6A4* genes. On the other hand, the results were not always directly comparable, as not exactly the same SNPs were genotyped in the two cohorts and the phenotype assessments were slightly different.

The limitations of our study are the large number of observations which have an impact on the statistical significance of the results. The four epistatic results showing the strongest association and which appeared to survive the Bonferroni-

correction lose their significance if we consider the total number of observations. For the Dyne Steel cohort the total number of observations is 950 (190 SNP x SNP interactions x 5 phenotypes) for the New Mood cohort it is 408 (137 SNP x SNP interactions x 3 phenotypes). Then the previously significant p values now show only a significance trend. It should also be noted that the haplotype block structure especially in the New Mood cohort does not reflect current knowledge. The haplotype tagging SNPs based on the HapMap Project Phase I in June 2005 do not provide adequate coverage of the *HTR2A* and the *SLC6A4* genes today. Choosing the same parameters (r^2=0.8, MAF>0.05, confidence interval method, pairwise) 43 SNPs would tag the *HTR2A* and the SNPs used in the New Mood cohort give 32% coverage of this region (Supplementary Table 4b). For the *SLC6A4* gene, 7 SNPs would tag the region, whilst SNPs selected in 2005 only cover 77% (Supplementary Table 4a). The 37 *HTR2A* SNPs and the 12 *SLC6A4* SNPs on the Illumina 610 Quad chip provide more complete coverage of these regions (77% and 82%).

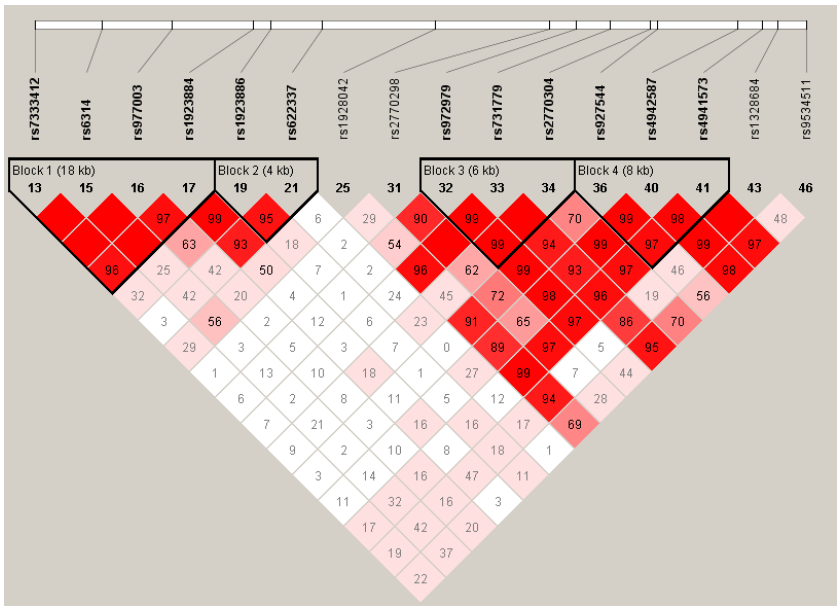
Another issue which may influence our results is the possibility of population stratification. As the allelic frequencies and haplotype structures were very similar in the New Mood Manchester and Budapest cohorts we merged them together to increase the power. However, current literature shows significant differences in allelic frequency between European Caucasian populations (Moskvina *et al.* 2010). Therefore we cannot exclude the possibility that our results are at least partially affected by population stratification. As New Mood was not a genome-wide association study we were not in the position to correct our data for population stratification.

In conclusion, we have observed a complex interplay within and between two serotonergic genes that effected depression, anxiety and neuroticism phenotypes in volunteers. We recommend that future studies genotype and analyse for epistatic interaction within and between these entire genes.

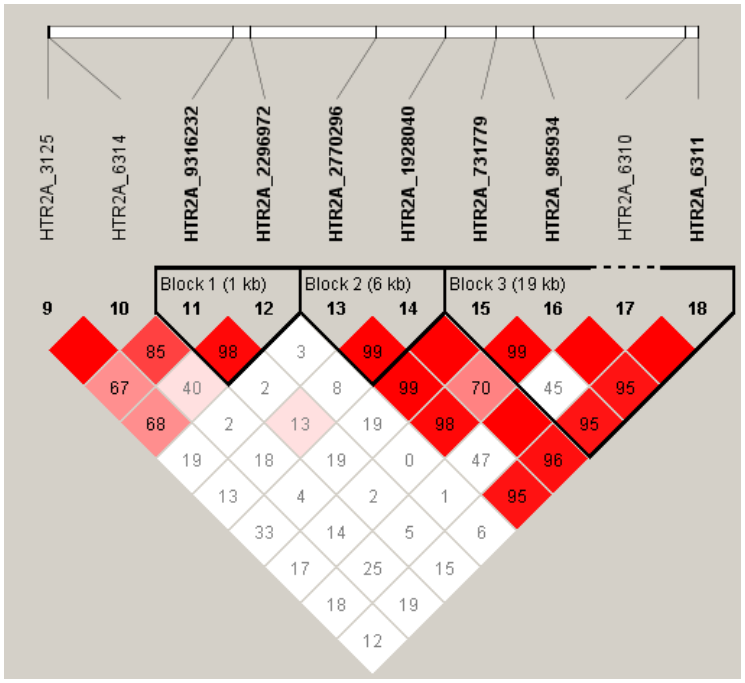
Figures

Figure 1. Serotonin Receptor 2A (*HTR2A*) haplotype tagging single nucleotide polymorphisms and linkage disequilibrium (r^2) within the gene

1a Dyne Steel cohort



1b New Mood cohort Manchester



1c New Mood cohort Budapest

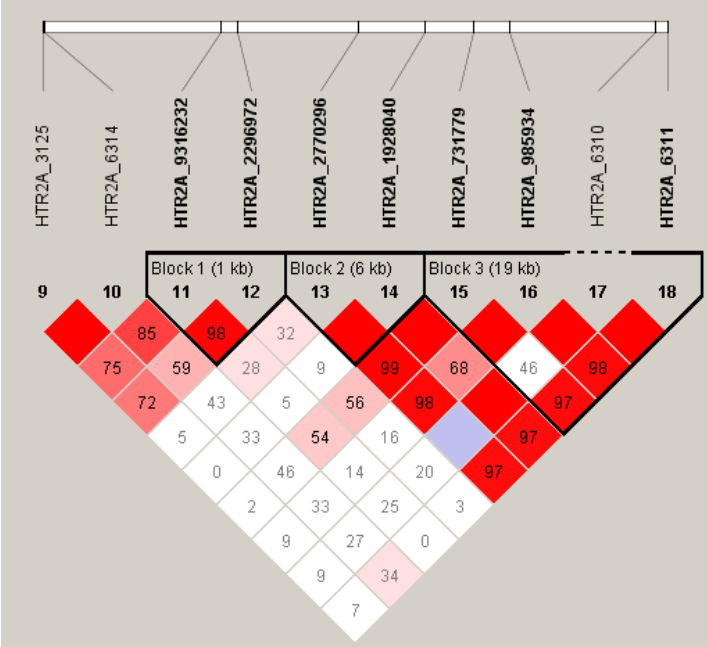
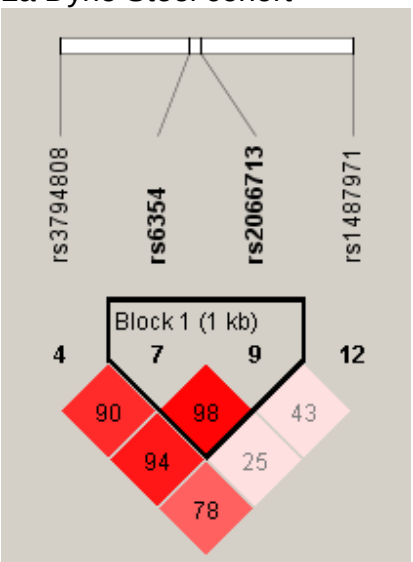
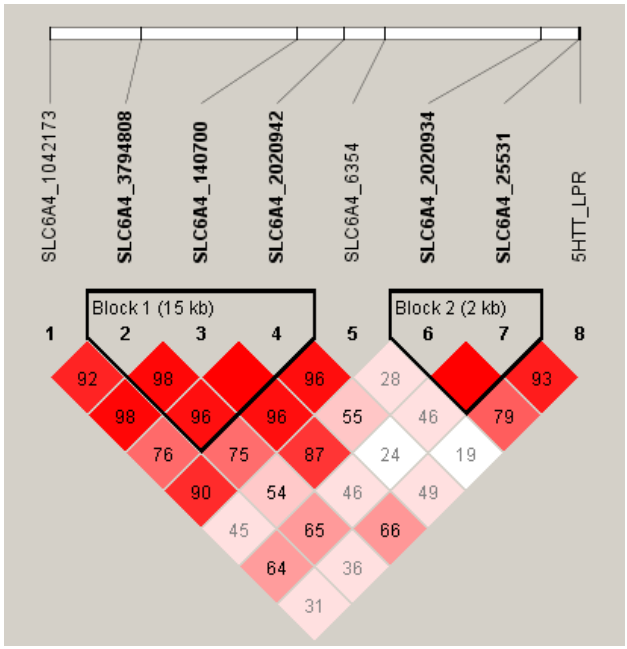


Figure 2. Serotonin Transporter (*SLC6A4*) haplotype tagging single nucleotide polymorphisms and linkage disequilibrium (r^2) within the gene

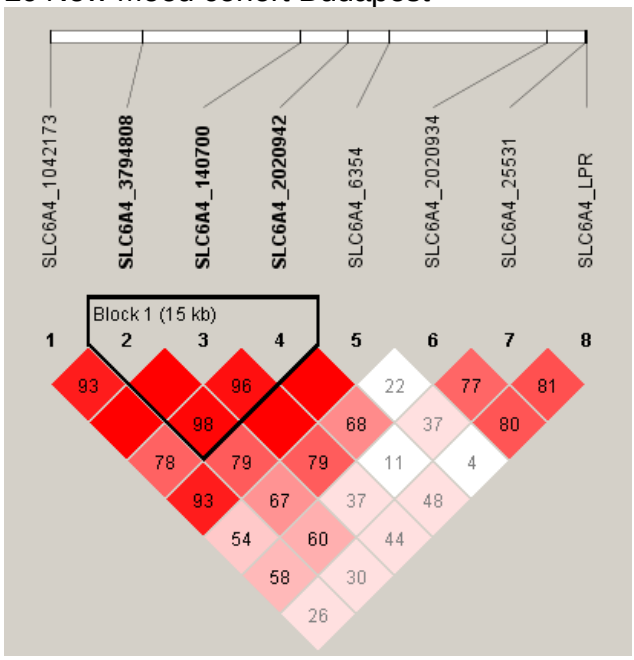
2a Dyne Steel cohort



2b New Mood cohort Manchester



2c New Mood cohort Budapest



Supplementary material for this study can be found in the Appendix (10.9 Supplementary materials for Study 2, S 1-5).

References

Arango V, Ernsberger P, Marzuk PM, Chen JS, Tierney H, Stanley M, Reis DJ, Mann JJ (1990). Autoradiographic demonstration of increased serotonin 5-HT2 and beta-adrenergic receptor binding sites in the brain of suicide victims. Arch Gen Psychiatry. 47(11):1038-47.

Arora RC, Meltzer HY (1989). Serotonergic measures in the brains of suicide victims: 5-HT2 binding sites in the frontal cortex of suicide victims and control subjects. Am J Psychiatry. 146(6):730-6.

Arranz B, Eriksson A, Mellerup E, Plenge P, Marcusson J (1994). Brain 5-HT1A, 5-HT1D, and 5-HT2 receptors in suicide victims. Biol Psychiatry. 35(7):457-63.

Anguelova M, Benkelfat C, Turecki G (2003). A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter: I. Affective disorders. Mol Psychiatry. 8: 574–591.

Barrett JC, Fry B, Maller J, Daly MJ (2005). Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 21:263-265.

Beck AT, Ward CH, Mendelsohn M, Mock J, Erbaugh J (1961). An inventory for measuring depression. Arch Gen Psych. 4:561-571.

Bhagwagar Z, Hinz R, Taylor M, Fancy S, Cowen P, Grasby P (2006). Increased 5-HT(2A) receptor binding in euthymic, medication-free patients recovered from depression: a positron emission study with [(11)C]MDL. Am J Psychiatry, 163: 1580-1587.

Bosker FJ, Hartman CA, Nolte IM, Prins BP, Terpstra P, Posthuma D, van Veen T, Willemsen G, Derijk RH, de Geus EJ, Hoogendijk WJ, Sullivan PF, Penninx BW,

Boomsma DI, Snieder H, Nolen WA (2010). Poor replication of candidate genes for major depressive disorder using genome-wide association data. Mol Psychiatry, Mar 30. Epub ahead of print

Brezo J, Bureau A, Mérette C, Jomphe V, Barker ED, Vitaro F, Hébert M, Carbonneau R, Tremblay RE, Turecki G (2010). Differences and similarities in the serotonergic diathesis for suicide attempts and mood disorders: a 22-year longitudinal gene-environment study. Mol Psychiatry. 15:831-43.

Brodman K,Erdmann AJ Jr., Wolff HG (1956). Cornell Medical Index Health Questionnaire (Manual). Cornell University Medical College, New York, NY, USA

Calboli FC, Tozzi F, Galwey NW, Antoniades A, Mooser V, Preisig M, Vollenweider P, Waterworth D, Waeber G, Johnson MR, Muglia P, Balding DJ (2010). A genome-wide association study of neuroticism in a population-based sample. PLoS One. 5(7):e11504.

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington HL, McClay J, Mill J, Martin J, Braithwaite A, Poulton R (2003). Influence of life stress on de[ression: moderation by a polymorphism in the 5-HTT gene. Science. 301:386-389.

Caspi A, Hariri AR, Holmes A, Uher R, Moffitt TE (2010). Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. Am J Psychiatry, 167(5):509-27.

Choi MJ, Kang RH, Ham BJ, Jeong HY, Lee MS (2005). Serotonin receptor 2A gene polymorphism (-1438A/G) (rs6311) and short-term treatment response to citalopram. Neuropsychobiology. 52:155-162.

Choi MJ, Lee HJ, Lee HJ, Ham BJ, Cha JH, Ryu SH, Lee MS (2004). Association between major depressive disorder and the -1438A/G polymorphism of the serotonin 2A receptor gene. Neuropsychobiology. 49(1):38-41.

Christiansen L, Tan Q, Iachina M, Bathum L, Kruse TA, McGue M, Christensen K. (2007). Candidate gene polymorphisms in the serotonergic pathway: influence on depression symptomatology in an elderly population. Biol Psychiatry. 61:223-30.

Derogatis L (1993). BSI: Brief Symptom Inventory: Administration, Scoring, and Procedures Manual. National Computer Systems Pearson Inc. Minneapolis, USA

Eley TC, Sugden K, Corsico A, Gregory AM, Sham P, McGuffin P, Plomin R, Craig IW (2004). Gene-environment interaction analysis of serotonin system markers with adolescent depression. Mol Psychiatry. 9:908-915.

Eysenck HJ, Eysenck SBG (1964). The Eysenck personality questionnaire. University of London Press, London, UK

Freeman B, Smith N, Curtis C, Huckett L, Mill J, Craig IW (2003). DNA from buccal swabs recruited by mail: evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. Behav Genet. 33: 67-72.

Frisch A, Postilnick D, Rockah R, Michaelovsky E, Postilnick S, Birman E, Laor N, Rauchverger B, Kreinin A, Poyurovsky M, Schneidman M, Modai I, Weizman R (1999). Association of unipolar major depressive disorder with genes of the serotonergic and dopaminergic pathways. Mol Psychiatry. 4:389-92.

Furlong RA, Ho L, Walsh C, Rubinsztein JS, Jain S, Paykel ES et al. (1998). Analysis and meta-analysis of two serotonin transporter gene polymorphisms in bipolar and unipolar affective disorders. Am J Med Genet. 81: 58–63

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, Defelice M, Lochner A, Faggart M et al (2002). The structure of haplotype blocks in the human genome. Science. 296:2225-2229.

Garriock HA, Kraft JB, Shyn SI, Peters EJ, Yokoyama JS, Jenkins GD, Reinalda MS, Slager SL, McGrath PJ, Hamilton SP (2010). A genome-wide association study of citalopram response in major depressive disorder. Biol Psychiatry, 67:133-137.

Gross-Isseroff R, Israeli M, Biegon A (1989). Autoradiographic analysis of tritiated imipramine binding in the human brain post mortem: effects of suicide. Arch Gen Psychiatry. 46(3):237-41.

Heinzen E, Ge D, Cronin KD, Maia J, Shianna KV, Gabriel W, Welsh-Bohmer KA, Hulette CM, Denny T, Goldstein DB (2008). Tissue specific genetic control of gene expression and alternative splicing: Implications for the study of human complex traits. PLoS Biology; 6(12): e1.

Hettema JM, Prescott CA, Kendler KS (2004). Genetic and environmental sources of covariation beyween generalized anxiety disorder and neuroticism. Am J Psych. 161:1581-1587.

Hranilovic D, Stefulj J, Schwab S, Borrmann-Hassenbach M, Albus M, Jernej B, Wildenauer D (2004). Serotonin transporter promoter and intron 2 polymorphisms: relationship between allelic variants and gene expression. Biol Psychiatry. 55:1090-4.

Hrdina PD, Demeter E, Vu TB, Sótónyi P, Palkovits M (1993). 5-HT uptake sites and 5-HT2 receptors in brain of antidepressant-free suicide victims/depressives: increase in 5-HT2 sites in cortex and amygdala. Brain Res. 614(1-2):37-44.

Hu XZ, Lipsky RH, Zhu G, Akhtar LA, Taubman J, Greenberg BD, Xu K, Arnold PD, Richter MA, Kennedy JL, Murphy DL, Goldman D (2006). Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. Am J Hum Genet. 78(5):815-26.

Illi A, Setälä-Soikkeli E, Viikki M, Poutanen O, Huhtala H, Mononen N, Lehtimäki T, Leinonen E, Kampman O (2009). 5-HTR1A, 5-HTR2A, 5-HTR6, TPH1 and TPH2 polymorphisms and major depression. Neuroreport. 20(12):1125-8.

Inkster B, Nichols TE, Saemann PG, Auer DP, Holsboer F, Muglia P, Matthews PM (2010). Pathway-based approaches to imaging genetics association studies: Wnt signaling, GSK3beta substrates and major depression. Neuroimage. 53(3):908-17.

Jakab RL, Goldman-Rakic PS (2000). Segregation of serotonin 5-HT2A and 5-HT3 receptors in inhibitory circuits of the primate cerebral cortex. J Comp Neurol. 417(3):337-48.

Jansson M, Gatz M, Berg S, Johansson B, Malmberg B, McClearn GE, Schalling M, Pedersen NL (2003). Association between depressed mood in the elderly and a 5-HTR2A gene variant. Am J Med Genet B Neuropsychiatr Genet. 120B(1):79-84.

Jennings KA, Sheward WJ, Harmar AJ, Sharp T (2008). Evidence, that genetic variation in 5-HT transporter expression is linked to changes in 5-HT2A receptor function. Neuropharmacology. 54:776-783.

John OP, Donahue EM, Kentle RL (1991). The Big Five Inventory-Versions 4a and 54. Institute of Personality and Social Research, Berkeley: University of California, Berkeley, USA

Jokela M, Keltikangas-Jarvinen L, Kivimaki M, Puttonen S, Elovainio M, Rontu R, Lehtimaki T (2007). Serotonin receptor 2A gene and influence of childhood maternal nurturance on adulthood depressive symptoms. Arch Gen Psychiatry. 64:356-360.

Juhasz G, Chase D, Pegg E, Downey D, Toth ZG, Stones K, Platt H, Mekli K, Payton A, Elliott R, Anderson IM, Deakin JFW (2009). CNR1 gene is associated with hight neuroticism and low agreeableness and interacts with recent negative life events to predict current depressive symptoms. Neuropsychopharmacology. 34:2019-2027.

Kendler KS, Gatz M, Gardner CO, Pedersen NL (2006). Personality and major depression: a Swedish longitudinal, population-based twin study. Arch Gen Psychiatry. 63:1113-1120.

Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry. 6:593-602.

Kishi T, Kitajima T, Tsunoka T, Ikeda M, Yamanouchi Y, Kinoshita Y, Kawashima K, Okochi T, Okumura T, Inada T, Ozaki N, Iwata N (2009). Genetic association analysis of serotonin 2A receptor gene (HTR2A) with bipolar disorder and major depressive disorder in the Japanese population. Neurosci Res. 64(2):231-4.

Kraft JB, Peters EJ, Slager SL, Jenkins GJ, Reinalda MS, McGrath PJ, Hamilton SP (2007). Analysis of association between the serotonin transporter and antidepressant response in a large clinical sample. Biol Psychiatry, 61:734-742.

Laje G, Cannon DM, Allen AS, Klaver JM, Peck SA, Liu X, Manji HK, Drevets WC, McMahon FJ (2010). Genetic variation in HTR2A influences serotonin transporter binding potential as measured using PET and [11C]DASB. Int J Neuropsychopharmacol. (6):715-24.

Lasky-Su JA, Faraone SV, Glatt SJ, Tsuang MT (2005). Meta-analysis of the association between two polymorphisms in the serotonin transporter gene and affective disorders. Am J Med Genet B Neuropsychiatr Genet. 133:110–115.

Lazary J, Lazary A, Gonda X, Benko A, Molnar E, Juhasz G, Bagdy G (2008). New evidence for the association of the serotonin transporter gene (SLC6A4) haplotypes, threatening life events, and depressive phenotype. Biol Psychiatry. 64:498-504.

Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science. 274:1527-1531.

Lewis CM, Ng MY, Butler AW, Cohen-Woods S, Uher R, Pirlo K, Weale ME, Schosser A, Paredes UM, Rivera M, Craddock N, Owen MJ, Jones L, Jones I, Korszun A, Aitchison KJ, Shi J, Quinn JP, Mackenzie A, Vollenweider P, Waeber G, Heath S, Lathrop M, Muglia P, Barnes MR, Whittaker JC, Tozzi F, Holsboer F, Preisig M, Farmer AE, Breen G, Craig IW, McGuffin P (2010). Genome-wide association study of major recurrent depression in the U.K. population. Am J Psychiatry, 167(8):949-957.

López-León S, Janssens AC, González-Zuloeta Ladd AM, Del-Favero J, Claes SJ, Oostra BA, van Duijn CM (2008). Meta-analyses of genetic studies on major depressive disorder. Mol Psychiatry. 13:772-85.

McMahon FJ, Buervenich S, Charney D, Lipsky R, Rush AJ, Wilson AF, Sorant AJM, GJ, Laje G, Fava M, Trivedi MH, Wisnieski SR, Manji H (2006). Variation in gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. Am J Hum Genet. 78:804-814.

Minov C, Baghai TC, Schüle C, Zwanzger P, Schwarz MJ, Zill P, Rupprecht R, Bondy B (2001). Serotonin-2A-receptor and -transporter polymorphisms: lack of association in patients with major depression. Neurosci Lett. 303(2):119-22.

Muglia P, Tozzi F, Galwey NW, Francks C, Upmanyu R, Kong XQ, Antoniades A, Domenici E, Perry J, Rothen S, Vandeleur CL, Mooser V, Waeber G, Vollenweider P, Preisig M, Lucae S, Müller-Myhsok B, Holsboer F, Middleton LT, Roses AD (2010). Genome-wide association study of recurrent major depressive disorder in two European case-control cohorts. Mol Psychiatry. 15:589-601.

Murphy DL, Fox MA, Timpano KR, Moya PR, Ren-Patterson R, Andrews AM, Holmes A, Lesch KP, Wendland JR (2008). How is the serotonin story is being rewritten by new gene-based discoveries principally related to SLC6A4, the serotonin transporter

gene, which functions to influence all cellular serotonin systems. Neuropharmacology. 55:932-960.

Murphy DL, Lerner A, Rudnick G, Lesch KP (2004). Serotonin transporter: gene, genetic disorders and pharmacogenetics. Molecular Interventions. 4:109-123.

Nakamura M, Ueno S, Sano A, Tanabe H (2000). The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. Mol Psychiatry. 5: 32–38.

Oswald P, Souery D, Massat I, Del-Favero J, Linotte S, Papadimitriou G, Dikeos D, Kaneva R, Milanova V, Oruc L, Ivezic S, Serretti A, Lilli R, Van Broeckhoven C, Mendlewicz J (2003). Lack of association between the 5HT2A receptor polymorphism (T102C) and unipolar affective disorder in a multicentric European study. Eur Neuropsychopharmacol. 13(5):365-8.

Ozaki N, Manji H, Lubierman V, Lu SJ, Lappalainen J, Rosenthal NE, Goldman D (1997). A naturally occurring amino acid substitution of the human serotonin 5-HT2A receptor influences amplitude and timing of intracellular calcium mobilization. J Neurochemistry. 68:2186-2193.

Peters EJ, Slager SL, Jenkins GD, Reinalda MS, Garriock HA, Shyn SI, Kraft JB, McGrath PJ, Hamilton SP (2009). Resequencing of serotonin-related genes and association of tagging SNPs to citalopram response. Pharmacogenetics and Genomics. 19:1-10.

Quintin P, Benkelfat C, Launay JM, Arnulf I, Pointereau-Bellenger A, Barbault S, et al. (2001). Clinical and neurochemical effect of acute tryptophan depletion in unaffected relatives of patients with bipolar affective disorder. Biol Psychiatry. 50:184 –190.

Rabbitt P, Diggle P, Horan M, Pendleton N, Bent N, Abson V, Jones M, McInnes L (2004). The University of Manchester Longitudinal Study of normal healthy old age 1983 through 2003. Aging Neuropsychol Cognit, 11, 245–279.

Shaikh SA, Strauss J, King N, Bulgin NL, Vetró A, Kiss E, George CJ, Kovacs M, Barr CL, Kennedy JL; International Consortium for Childhood-Onset Mood Disorders. (2008). Association study of serotonin system genes in childhood-onset mood disorder. Psychiatr Genet. 18(2):47-52.

Shifman S, Bhomra A, Smiley S, Wray NR, James MR, Martin NG, Hettema JM, An SS, Neale MC, van den Oord EJCG, Kendler KS, Chen X, Boomsma DI, Middeldorp CM, Hottenga JJ, Slagboom PE, Filnt J (2008). A whole genome association study of neuroticism using DNA pooling. Mol Psychiatry. 13:302-312.

Shi J, Potash JB, Knowles JA, Weissman MM, Coryell W, Scheftner WA, Lawson WB, Depaulo JR Jr, Gejman PV, Sanders AR, Johnson JK, Adams P, Chaudhury S, Jancic D, Evgrafov O, Zvinyatskovskiy A, Ertman N, Gladis M, Neimanas K, Goodell M, Hale N, Ney N, Verma R, Mirel D, Holmans P, Levinson DF (2010). Genome-wide association study of recurrent early-onset major depressive disorder. Mol Psychiatry. 2010 Feb 2. Epub ahead of print

Shyn SI, Shi J, Kraft JB, Potash JB, Knowles JA, Weissman MM, Garriock HA, Yokoyama JS, McGrath PJ, Peters EJ, Scheftner WA, Coryell W, Lawson WB, Jancic D, Gejman PV, Sanders AR, Holmans P, Slager SL, Levinson DF, Hamilton SP (2009). Novel loci for major depression identified by genome-wide association study of Sequenced Treatment Alternatives to Relieve Depression and meta-analysis of three studies. Mol Psychiatry. Epub, ahead of print

Smith KA, Fairburn CG, Cowen PJ (1997): Relapse of depression after rapid depletion of tryptophan. Lancet. 349:915–919.

Strug LJ, Suresh R, Fyer AJ, Talati A, Adams PB, Li W, Hodge SE, Gilliam TC, Weissman MM (2010). Panic disorder is associated with the serotonin transporter gene (SLC6A4) but not the promoter region (5-HTTLPR). Mol Psychiatry. 152:166-76.

Sullivan NR, Burke T, Siafaka-Kapadai A, Javors M, Hensler JG(2004). Effect of valproic acid on serotonin-2A receptor signaling in C6 glioma cells. J. Neurochem. 90 (5): 1269–1275.

Sullivan PF, Neale MC, Kendler KS (2000). Genetic epidemiology of major depression: review and meta-analysis. Am J Psychiatry. 157(10):1552-62.

Sullivan PF, de Geus EJ, Willemsen G, James MR, Smit JH, Zandbelt T, Arolt V, Baune BT, Blackwood D, Cichon S, Coventry WL, Domschke K, Farmer A, Fava M, Gordon SD, He Q, Heath AC, Heutink P, Holsboer F, Hoogendijk WJ, Hottenga JJ, Hu Y, Kohli M, Lin D, Lucae S, Macintyre DJ, Maier W, McGhee KA, McGuffin P, Montgomery GW, Muir WJ, Nolen WA, Nöthen MM, Perlis RH, Pirlo K, Posthuma D, Rietschel M, Rizzu P, Schosser A, Smit AB, Smoller JW, Tzeng JY, van Dyck R, Verhage M, Zitman FG, Martin NG, Wray NR, Boomsma DI, Penninx BW (2009). Genome-wide association for major depressive disorder: a possible role for the presynaptic protein piccolo. Mol Psychiatry. 14:359-375.

Tamminga CA, Nemeroff CB, Blakely RD, Brady L, Carter CS, Davis KL, et al. (2002). Developing novel treatments for mood disorders: Accelerating discovery. Biol Psychiatry. 52:589–609.

Tencomnao T, Thongrakard V, Phuchana W, Sritharathikhun T, Suttirat S (2010). No relationship found between -1438A/G polymorphism of the serotonin 2A receptor gene (rs6311) and major depression susceptibility in a northeastern Thai population. Genet Mol Res. 201, 9(2):1171-6.

Tsai SJ, Hong CJ, Hsu CC, Cheng CY, Liao WY, Song HL, Lai HC (1999). Serotonin-2A receptor polymorphism (102T/C) in mood disorders. Psychiatry Res. 87(2-3):233-7.

Turecki G, Brière R, Dewar K, Antonetti T, Lesage AD, Séguin M, Chawky N, Vanier C, Alda M, Joober R, Benkelfat C, Rouleau GA (1999). Prediction of level of serotonin 2A receptor binding by serotonin receptor 2A genetic variation in postmortem brain

samples from subjects who did or did not commit suicide. Am J Psychiatry. 156(9):1456-8.

Uher R, McGuffin P (2008). The moderation by the serotonin transporter gene of environmental adversity in the aetology of mental illness: review and methodological analysis. Mol Psychiatry. 13:131-146.

Uher R, Huezo-Diaz P, Perroud N, Smith R, Rietschel M, Mors O, Hauser J, Maier W, Kozel D, Henigsberg N, Barreto M, Placentino A, Dernovsek MZ, Schulze TG, Kalember P, Zobel A, Czerski PM, Larsen ER, Souery D, Giovannini C, Gray JM, Lewis CM, Farmer A, Aitchison KJ, McGuffin P, Craig I (2009). Genetic predictors of response to antidepressants in the GENDEP project. The Pharmacogenomics Journal, (4):225-233.

Wilkie MJW, Smith G, Day RK, Matthews K, Smith D, Blackwood D, Reid IC, Wolf CR (2009). Polymorphisms in the SLC6A4 and HTR2A genes influence treatment outcome following antidepressant therapy. Pharmacogenomics Journal, 9:61-70.

Willins DL, Deutch AY, Roth BL (1997). Serotonin 5-HT2A receptors are expressed on pyramidal cells and interneurons in the rat cortex. Synapse. 27(1):79-82.

Yates M, Leake A, Candy JM, Fairbairn AF, McKeith IG, Ferrier IN (1990). 5HT2 receptor changes in major depression. Biol Psychiatry. 27:489-96.

Yatham LN, Liddle PF, Lam RW, Adam MJ, Solomons K, Chinnapalli M, Ruth TJ 2005. A positron emission tomography study of the effects of treatment with valproate on brain 5-HT2A receptors in acute mania. Bipolar Disord. 7 (Suppl. 5), 53–57.

Yesavage JA, Bruik TL, Rose TL (1983). Development and validation of a geriatric depression screening scale: A preliminary report. Journal of Psychiatric Research. 17:37-49.

Yoon HK, Kim YK (2009). TPH2 -703G/T SNP may have important effect on susceptibility to suicidal behaviour in major depression Prog Neuropsychopharmacol Biol Psychiatry. 33:403-9.

Zhang HY, Ishigaki T, Tani K, Chen K, Shih JC, Miyasato K, Ohara K, Ohara K (1997). Serotonin2A receptor gene polymorphism in mood disorders. Biol Psychiatry. 41:768-73.

Zhang K, Xu Q, Xu Y, Yang H, Luo J, Sun Y, Sun N, Wang S, Shen Y (2009). The combined effects of the 5-HTTLPR and 5-HTR1A genes modulates the relationship between negative life events and major depressive disorder in a Chinese population. J Affect Disord. 114(1-3):224-31.

Manuscript 2

Investigation of serotonin receptors 1D, 1E, 1F, 2B and 3-7 in depression and related traits: a mini review and a further attempt to replicate previous findings in an elderly community dwelling population

6. Study 3

Investigation of serotonin receptors 1D, 1E, 1F, 2B and 3-7 in depression and related traits: a mini review and a further attempt to replicate previous findings in an elderly community dwelling population

Krisztina Mekli[1,2,3], Gabriella Juhasz[1], Hazel Platt[3], Fabio Miyajima[4], William Ollier[3], Neil Pendleton[5], Mike Horan[5], Antony Payton[3]

[1]Neuroscience and Psychiatry Unit, School of Community Based Medicine, Faculty of Medical and Human Sciences, University of Manchester, UK

[2]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, Budapest, Hungary

[3]Centre for Integrated Genomic Medical Research, School of Cancer and Enabling Science, Faculty of Medical and Human Sciences, University of Manchester, UK

[4]Department of Pharmacology and Therapeutics, School of Biomedical Sciences, University of Liverpool, Liverpool, L69 3GE

[5]Department of Clinical Gerontology, University of Manchester, Greater Manchester, UK, M6 8HD

Correspondence: Krisztina Mekli, Neuroscience and Psychiatry Unit, School of Community Based Medicine, Faculty of Medical and Human Sciences, The University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, UK.

Tel: +44 161 275 7762

Fax: +44 161 275 7429

E-mail: Krisztina.Mekli@postgrad.manchester.ac.uk

Keywords: depression, polymorphism, serotonin, receptor, association

Abstract

Depression is a serious health problem worldwide. This disorder often co-occurs with various anxiety disorders and a personality trait, termed neuroticism, appears to be a common background for them. Extensive research focused on the role of the genes of the serotonergic pathway, with special attention to the *HTR1A-1B* autoreceptors, *HTR2A-2C* heteroreceptors and the serotonin transporter. The aim of this study was to review the literature and investigate the possible role of other serotonergic receptors (*HTR1D*, *E*, *F*, *HTR2B*, *HTR3-7*) in depression and related phenotypes. We used haplotype tagging SNPs in a Caucasian cohort of 1563 individuals and continuous phenotypic measures of depression, anxiety and neuroticism. We found several weak associations, but after correction for multiple testing, three of them remained marginally significant. These SNPs were located in the *HTR4* and *HTR6* genes, and we found some possible indications in the literature to corroborate these findings.

Introduction

Depression is a major global public health problem that has a lifetime prevalence of between 6-30% (Waraich *et al*. 2004). Without treatment it has the tendency to assume a chronic course, to recur and to be associated with increasing disability over time. Family and twin studies suggest that major depression, anxiety disorders and related traits, such as neuroticism, have shared genetic risk factors (Hettema 2008). Despite intensive research, we still do not have a compelling genetic model for depression. Hypothesis-driven association studies and genome-wide association studies provided evidence for the role of certain serotonergic gene variants in depression; among the serotonergic pathway genes *HTR1A*, *HTR1B*, *HTR2A*, *HTR2C*, *MAO-A* and the serotonin transporter gene (*SLC6A4*) have received the most attention. Less is known about the remaining 9 serotonergic receptors. In our study, we reviewed and investigated the possible role of these lesser known receptors in depression, anxiety and neuroticism.

5-HT1D receptor

5-HT1D receptor is expressed at very low levels in the brain. Its mRNA has been found in the caudate putamen, olfactory cortex, dorsal raphe nucleus and locus coeruleus. In the human heart these receptors may modulate 5-HT release (Hannon and Hoyer 2008). Interestingly, 5-HT1D may form homodimers with itself or heterodimers with the 5-HT1B receptor (Xie *et al*. 1999). As 5-HT1B appears to have a role in depression (Drago *et al.* 2010) this heterodimerization makes 5-HT1D a possible candidate too.

No genetically modified mouse exists for this receptor to date. One study has suggested a possible involvement in substance abuse and obsessive-compulsive disorder (Gratacos *et al.* 2008). Drugs acting as antagonists at the 1D receptor were developed as a way of increasing 5-HT release by blocking pre-synaptic 5-HT1D receptors. They showed promise in animal models of depression but no clinical trials have been published.

5-HT1E receptor

In the human brain, 5-HT1E receptor mRNA is present in cortical areas, caudate, putamen, and with lower levels in the amygdala and hypothalamus (Hannon and Hoyer 2008).

The phenotype of animals with genetically modified 5-HT1E receptors has not been published. In human association studies, 5-HT1E has been implicated in attention deficit hyperactivity disorder (Lasky-Su *et al.* 2008), substance abuse and obsessive-compulsive disorder (Gratacos *et al*. 2008).

5-HT1F receptor

There is not much known about this receptor. Its expression is similar to those of 5-HT1E receptor (Filip and Bader, 2009) and no knock-out (KO) model has been described.

In humans two SNPs showed nominal association with eating disorder (rs1027689) and bipolar disorder (rs1503433 and rs1027689) (Gratacos *et al.* 2008).

5-HT2B receptor

5-HT2B receptor distribution is restricted mainly to cerebellum, hypothalamus and amygdala (Filip and Bader, 2009).

HTR2B KO leads to embryonic and neonatal death caused by heart defects, accompanied by reduction in levels of a tyrosine kinase receptor, ERBB2 (Nebigil *et al.* 2000).

Genetic studies are limited to reports of association with drug abuse (Lin et al. 2004) and obsessive-compulsive disorder (Gratacos *et al.* 2008).

5-HT3 receptor

The unique feature of this receptor family among the other serotonergic receptors is that they are ligand-gated ion channels. They trigger rapid depolarisation of the neurons by the opening of non-selective cation channels (Na^+, Ca^{2+} influx, K^+ efflux). To date, five genes have been recognised to encode 5-HT3: the 5-HT3A receptor subunit (Maricq *et al.* 1991), the 5-HT3B subunit (Dubin *et al*. 1999) and the 5-HT3C-E subunits (Niesler *et al.* 2003). 5-HT3 receptors are thought to exist as a homopentamer of A subunits or B subunits and heteropentamers of A and B subunits (Rajkumar and Mahesh 2010, Barnes *et al.* 2009). It is possible, that further subunits (C, D and E) may take part in the heteropentamer formation.

Hht3a KO mice show enhanced amygdala-mediated fear conditioning with male mice exhibiting reduced anxiety-related behaviours (Bhatnagar *et al.* 2004). Post-developmental transgenic overexpression of the 5-Ht3 receptor results in reduced voluntary ethanol self-administration (Holmes, 2008) which therefore may be important in substance abuse disorders.

Pharmacological studies showed that several commonly used antidepressants (such as tricyclic antidepressants desipramine and imipramine and the selective serotonin reuptake inhibitor fluoxetine) are functional antagonists of 5-HT3A receptor, as they effectively reduced the serotonin-induced Na^+ and Ca^{2+} -currents through this receptor. These drugs also accelerated receptor desensitization (Eisensamer *et al.* 2003). This inhibition of ion fluxes is non-competitive and requires the enrichment of drugs in raft-like domains within the cell membraine (Eisensamer *et al.* 2005).

The human *HTR3A* gene has several polymorphisms. A functional one in the 5' untranslated region (UTR) (rs1062613) which causes increased transcription in

Luciferase reporter systems (Niesler *et al.* 2001) appears to be associated with personality traits. The SNP rs1062613 T allele was associated with lower harm avoidance in females in a study that used two cohorts (Melke *et al.* 2003). Another study has reported that the C/C genotype was associated with higher harm avoidance and neuroticism (Mizuta *et al.* 2008) An fMRI study indicated that the C/C genotype was associated with increased level of neuronal activation in the right amygdala and the PFC during a face recognition task (Iidaka *et al.* 2005) and showed faster reaction time, than the C/T genotype (T/T genotype was not measured). The SNP rs10160548 was associated nominally with antisocial alcoholism (Corley *et al.* 2008).

A polymorphism in the *HTR3B* gene (rs1176744) which causes reduced cell surface expression level (Krzywkowski *et al*. 2008) was associated with anorexia nervosa in a German study in two independent samples (Hammer *et al.* 2009) and with reduced response to antidepressant escitalopram in a genome-wide association study (Uher *et al.* 2010). In a Japanese study, a haplotype block containing rs1176744 was associated with depression in females subjects (Yamada *et al.* 2006). Other studies report association with heroin addiction (Levran *at al.* 2008) and antisocial alcoholism (Corley *et al*. 2008). Another functional SNP in this gene is rs3782025; its minor allele reduced the alpha power of the electroencephalogram's rhythm and was associated with alcohol use disorders and with co-morbid antisocial personality disorders in two independent populations (Ducci *et al.* 2009). The same SNP together with rs1672717 showed nominal significance to cognitive impulsivity in a family-based attention-deficit hyperactivity disorder study (Oades *et al.* 2008). These studies suggest that variation in the 5-HT3 receptor may have a role in susceptibility to personality and addiction disorders.

HTR3C variants have been associated with autism (Rehnstroem et al. 2009) and nausea during pregnancy (Goecke *et al.* 2010) and the *HTR3E* with irritable bowel syndrome, due to a functional polymorphism (rs62625044) in the 3'UTR (Kapeller *et al.* 2008).

5-HT4 receptor

The 5-HT4 receptor is concentrated in the olfactory tubercle, substantia nigra, striatum, septum, hippocampus and amygdala, and it is also expressed in the gut (Hannon and Hoyer, 2008). The 5-HT4 receptor gene is complex and generates a

number of C-terminus variants. At least ten 5-HT4 receptor splice variants have been reported so far (5-HT4_a-5-HT4_n); one of them (5-HT4_d) seems to be unique to humans, and is limited to the gut, whereas the other isoforms are more widely distributed (Hannon and Hoyer, 2008).

Ht4 KO mice show normal feeding and motor behaviours under baseline conditions, but stress-induced hypophagia (reduced food intake) and novelty-induced exploratory activity decreases in these animals (Compan *et al.* 2004). Furthermore, a 5-HT4 receptor agonist (RS67333) augmented the acute effect of paroxetine in the ventral hippocampus (Licht *et al.* 2010).

There are not many association studies available about the 5-HT4 receptor, but one reported association between a haplotype of four *HTR4* polymorphism (around exon d) and bipolar disorder (Ohtsuki *et al.* 2002) but not to depression. The role in bipolar disorder was not confirmed in a family-based study (Shi *et al.* 2008). Importantly, a large UK genome-wide association study reported association between an intronic SNP, rs17108435 and depression (Lewis *et al.* 2010). Another genome-wide association study found that an intronic SNP rs1345697 was associated with an improved response to the antidepressant drug notriptyline (Uher *et al*. 2010). There is no evidence of this receptor's involvement in anxiety, substance abuse, panic disorder or eating disorder (Gratacos *et al.* 2008) or in chronic fatigue syndrome (Smith *et al.* 2008).

5-HT5 receptor

The function of the 5-HT5 receptor is not very well established. In humans two subtypes of this receptor exist. In the brain 5-HT5A mRNA expression is localised to the cerebral cortex, hippocampus and cerebellum (Hannon and Hoyer, 2008). *HTR5*B is considered to be a pseudogene, due to the presence of a stop codon within the gene which would result in the expression of a short, probably non-functional protein (Rees *et al.* 1994).

Htr5a KO mice show increased exploratory behaviour but no apparent changes in anxiety-like behaviours (Grailhe *et al.* 1999). In humans a variant in the 5' UTR region (-19G/C, rs79028878) of the *HTR5A* gene showed association with psychiatric phenotypes, including depression, where the G allele was protective (Birkett *et al*. 2000). In the same study 12A/T (rs6320, a transversion without amino

acid change) also showed association to depression, but this finding was not replicated by an independent study (Arias *et al.* 2001). *HTR5A* showed no association with chronic fatigue syndrome (Smith *et al*. 2008), antidepressant response (Uher *et al.* 2010) or psychiatric phenotypes (Gratacos *et al.* 2008) in a more recent study.

5-HT6 receptors

5-HT6 receptor mRNA is expressed in the striatum, amygdala, nucleus accumbens, hippocampus, cortex and olfactory tubercle (Hannon and Hoyer, 2008).

5-Ht6 KO mice have been shown to perform normally in a wide-variety of cognition and anxiety tests (Bonasera *et al.* 2006) but showed reduced response to the ataxic and sedative effects of ethanol, without differences in ethanol-induced hypothermia, compared to the wild-type. In rats, continuous intraventricular infusion of 5-HT6 receptor antisense oligonucleotides yielded increased anxiety-like behaviours (Otano *et al.* 1999). This receptor may have a role in establishing brain circuits. Riccio et al. (2009) have reported that in mice cortical regions 5-HT6 receptor blockade prevented the migratory effect of excess 5-HT on interneurons during embryonic development. In addition, several antidepressants (e.g. clomipramine, amitryptiline, doxepin and nortryptyline) are antagonists with high affinity for this receptor (Hoyer *et al.* 2002).

The *HTR6* rs1805054 showed positive heterosis effect in response to antidepressant treatment in Korean depressive patients (Lee *et al.* 2005). However a case-control study on five haplotype-tagging SNPs (including rs1805054) and meta-analysis of four studies of rs1805054 found no evidence of the association between any of these polymorphisms and MDD (Fukuo *et al.* 2010). 5-HT6 receptor showed no association to chronic fatigue syndrome (Smith *et al*. 2008) or psychiatric phenotypes (Gratacos *et al*. 2008).

5-HT7 receptors

This receptor shares relatively low sequence homology with other members of the 5-HT receptor family (lower than 50%), whereas the interspecies homology is over 90% (Hannon and Hoyer, 2008). This receptor has an extensive vascular distribution, and in non-vascular smooth muscle, but is also present in the brain, in the suprachiasmatic nucleus and amygdala (Hannon and Hoyer, 2008).

5-Ht7 KO mice show no difference in models of psychosis and anxiety states compared to wild-type animals (Guscott *et al.* 2005). In the forced swim test KO mice showed a significant decrease in immobility (Guscott *et al.* 2005). Decreased immobility was also observed in a tail suspension test (Hedlund *et al.* 2005). These phenotypes are similar to those seen in mice after antidepressant treatment. KO animals also showed indications of disturbed circadian rhythm (Guscott *et al.* 2005). They spent less time in, and had less frequent episodes of, rapid eye movement (REM) sleep which is also consistent with the antidepressant phenotype (Hedlund *et al*. 2005). KO animals also showed evidence that 5-HT induced hypothermic response may be modulated by this receptor (Hedlund *et al*. 2003).

Despite the implications from animal and pharmacological studies the role of the 5-HT7 receptor has not been investigated thoroughly, although a genome-wide association study found that rs7920627_T allele carriers response was reduced to the antidepressant drug nortriptyline (Uher *et al.* 2010). This receptor may have a role in depression (rs7916720, Lewis *et al.* 2010), antisocial alcoholism (Corley *et al.* 2008) and panic disorder (Gratacos *et al.* 2008).

The aim of this study was to investigate the role of these lesser known serotonergic receptors. We hypothesized that several of them, especially the 5-HT1D, 5-HT3, 5-HT4, 5-HT6 and 5-HT7 receptors may have a role in depression, or in related phenotypes, such as anxiety and neuroticism. We used GWAS data for a large Caucasian cohort to test this hypothesis.

Methods

Participants

The Dyne Steel cohort is comprised of 1563 Caucasian volunteers (454 males and 1109 females; mean age 67.4 years) from the Manchester and Newcastle areas of the UK with approximately equal numbers collected from the two regions. Recruitment and study details are described in detail elsewhere (Rabbitt *et al.* 2004). Volunteers gave written consent for the use of their DNA. Genetics work for the cohort is approved by University of Manchester Research Ethics Committee and Salford and Trafford Local Research Ethics Committee. The DNA bank is formed

from approximately equal numbers of samples collected from Manchester and Newcastle.

Phenotype assessments

The Dyne Steel cohort participants completed the Beck Depression Inventory (21 items, mean 6.834, Standard Error of Mean, SEM 0.147) (Beck *et al.* 1961), the Yesavage Geriatric Depression Questionnaire (mean 6.329, SEM 0.134) (30 items) (Yesavage *et al* .1983), the Cornell Medical Index depression (mean 0.277, SEM 0.018) (6 items), anxiety (mean 0.82, SEM 0.033) (9 items), cardiovascular (mean 2.24, SEM 0.059) (15 items), fatigue (mean 0.70, SEM 0.031) (7 items) and sleep disorders and habits (mean 1.30, SEM 0.028) (7 items) questionnaires (Brodman *et al.* 1956), and the Eysenck Personality Questionnaire (only EPQ neuroticism was used in the analysis, mean 10.094, SEM 0.15) (23 Items) (Eysenck and Eysenck, 1964).

DNA extraction and genotyping

DNA was obtained from blood samples and was extracted using a standard phenol-chloroform method.

SNPs were genotyped on the Illumina 610 Quad microarray (Illumina, Inc. San Diego, CA, USA). Genotyping were carried out at the Wellcome Trust Clinical Research Facility, Edinburgh.

These SNPs spanned the:

HTR1D gene (1:23,379,759 –rs2806566 to 1:23,410,142-rs627304)

HTR1E gene (6:87,672,689-rs9450576 to 6:87,806,534-rs7739124)

HTR1F gene (3:88,086,502-rs7648805 to 3:88,146,455-rs9310061)

HTR2B gene (2:231,655,711-rs11694724 to 2:231,733,528-rs6437002)

HTR3A-B gene (11:113,258,222-rs869451 to 11:113,390,622-rs4938066)

HTR3C-E gene (3:185,223,828 –rs7613237 to 3:185,317,184-rs9815292)

HTR4 gene (5:147,805,783,-rs4274968 to 5:148,025,475-rs1820076)

HTR5A gene (7:154,479,788-rs6597451 to 7:154,504,328-rs732050)

HTR6 gene (1:19,847,256-rs2314331 to 1:19,889,882-rs12127394)

HTR7 gene (10:92,484,316-rs1107688 to 10:92,621,224-rs4282910)

HelixTree™ 6.4.3 (Golden Helix, USA) software was used to select haplotype tagging SNPs (htSNPs) from the Dyne Steel Cohort using a minimum allele frequency (MAF) of 0.01 and a Carlson LD threshold of 0.9. All call rates were above 98%.

Genotyping was performed blind with regard to phenotype. All laboratory work was performed under the ISO 9001:2000 quality management requirements.

Statistical Analysis

Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) calculations were performed using HelixTree™ 6.4.3 (Golden Helix, USA). PLINK v1.06 (http://pngu.mgh.harvard.edu/purcell/plink/) was used for testing association of additive genetic model (linear regression model). To correct for multiple testings, we applied LD-based set tests with the parameters as follows: r^2=0.1, P-value=0.05, number of permutations=1000, maximum number of SNPs in the set=5, which is thought to be a moderately strict set of values. We also applied the Bonferroni-correction.

Gliders (http://mather.well.ox.ac.uk/Gliders) database was used to evaluate LDs between SNPs for replication purposes.

Results

Allele frequencies, Hardy-Weinberg Equilibrium and regression analysis p-values are shown in Table 1 for *HTR1D-F*, *HTR2B*, *HTR5A and HTR6*, in Table 2 for *HTR4* and *HTR7*, and in Table 3 for *HTR3* genes. All SNPs were in Hardy-Weinberg equilibrium with the exception of rs4912518, rs12493550 and rs1467257 (p-values: 0.029, 0.021 and 0.040, respectively) in the *HTR3CDE* subunits gene.

Relatively strong associations were observed between two SNPs in the *HTR4* gene and depression. The rs4280857_A allele was associated with an increase of Yesavage depression scores (nominal P-value 0.005) and rs1011427_T allele was associated with increased CMI depression scores (nominal P-value 0.004). Both alleles were significantly, however weakly, associated with other depression scores too (with the same effects). A third SNP in the *HTR4* gene (rs17108435_C allele) was relatively strongly associated with decreased CMI anxiety scores (nominal P-value 0.005), but with no other phenotype. Eight other SNPs within this gene were

associated at a weaker nominal significance level with decreased CMI anxiety scores (See Table 2).

Three further 5-HT receptor gene SNPs showed relatively strong association values. Rs4912138_A allele in the HTR6 gene was associated with a decrease in the Yesavage depression scores (nominal P-value 0.005) and less strongly with a decrease in the Beck depression scores (See Table1). An SNP in the *HTR3 A* subunit (rs10891611_C allele) was associated with increased Beck depression scores (nominal P-value 0.009) and with Eysenck neuroticism scores (nominal P-value 0.004) with the same effect. In the *CDE* subunit of this gene, rs939335_G allele was associated with decreased CMI anxiety scores (nominal P-value 0.005), and also associated with decreased CMI depression scores (See Table 3).

A few more, weak associations were also observed (See Tables 1, 2 and 3).

Applying LD-based set tests for multiple SNPs the following associations remained significant: rs4912138_A allele with decreased Yesavage depression scores (P=0.04), rs6693503_A with decreased CMI depression scores (P=0.042), both in *HTR6* gene and rs1011427 (P=0.041) with increased CMI depression scores in the *HTR4* gene.

None of our results remained significant after the Bonferroni correction (below 0.00009).

Association analysis of *HTR2B* SNPs with cardiovascular symptoms and *HTR7* SNPs with fatigue and with sleep disorders and habits revealed no significant results.

Table 1. Association analysis results for the *HTR1D-1F*, *2B*, *5A* and *6* genes
1a P values

					Depression			Anxiety	Neuroticism
					Beck	Yesavage	CMI	CMI	EPQ
GENE	CHR	SNP	HWE P-value	MAF	P-value	P-value	P-value	P-value	P-value
HTR1D	1	rs2806566_C	0.80	0.17	*0.10*	0.33	0.65	0.83	0.18
	1	rs641032_G	0.59	0.34	0.12	0.48	0.89	0.67	0.47
	1	rs627304_C	0.08	0.59	0.25	0.34	0.86	0.36	0.59
HTR1E	6	rs9450576_G	0.77	0.22	0.17	0.91	0.22	0.29	0.35
	6	rs12181765_G	0.83	0.10	0.43	0.57	**0.04**	0.30	0.15
	6	rs16877989_C	0.85	0.11	0.24	0.65	0.97	0.68	0.94
	6	rs828361_T	0.85	0.14	0.96	0.74	0.42	0.54	0.16
	6	rs10944288_C	0.22	0.35	0.87	0.11	0.43	0.33	0.72
	6	rs1886333_C	0.68	0.15	0.97	0.28	0.62	0.75	**0.04**
	6	rs942472_C	0.21	0.31	0.76	0.75	0.90	0.29	0.84
	6	rs1041058_T	0.70	0.13	0.46	0.59	0.15	0.45	0.57
	6	rs10806386_T	0.70	0.26	0.52	0.33	0.66	0.86	0.33
	6	rs7739124_C	0.81	0.27	0.78	0.16	0.67	0.78	0.91
HTR1F	3	rs7648805_A	0.92	0.37	0.51	0.72	0.97	0.99	0.33
	3	rs7653582_T	0.40	0.12	0.22	0.77	0.58	0.99	0.17
	3	rs7652406_T	0.56	0.40	0.19	0.43	0.44	0.43	0.87
HTR2B	2	rs11694724_G	1.00	0.08	0.63	0.68	0.71	0.85	0.79
	2	rs13394402_T	0.36	0.16	0.39	0.35	0.93	0.15	0.85
	2	rs17586405_G	0.25	0.03	0.40	0.09	0.23	0.48	0.29
	2	rs10194776_T	0.83	0.38	0.30	0.33	0.92	0.39	0.38
	2	rs4973377_A	0.29	0.15	0.20	0.80	**0.03**	0.41	0.54
	2	rs6437002_G	0.35	0.32	0.22	0.75	0.39	0.21	0.88
HTR5A	7	rs6597451_T	0.26	0.30	0.50	0.54	0.54	0.59	0.89
	7	rs1440459_G	0.88	0.35	0.99	0.23	0.31	0.54	0.53
	7	rs2241859_A	0.21	0.31	0.33	0.31	0.69	0.94	0.57
HTR6	1	rs6693503_A	0.62	0.33	0.197	0.132	**0.017***	0.38	0.20
	1	rs4912138_A	0.63	0.18	**0.01**	**0.005***	0.123	0.32	0.21
	1	rs3790756_T	0.40	0.13	0.603	0.411	0.285	0.10	0.46

1b β-coefficients

			Depression			Anxiety	Neuroticism
			Beck	Yesav	CMI	CMI	EPQ
GENE	CHR	SNP	β	β	β	β	β
HTR1D	1	rs2806566_C	0.459	0.250	0.016	-0.013	0.381
	1	rs641032_G	0.336	0.143	0.004	-0.021	0.161
	1	rs627304_C	0.245	0.186	-0.005	-0.044	0.118
HTR1E	6	rs9450576_G	0.340	-0.027	0.039	0.059	0.239
	6	rs12181765_G	0.268	0.180	0.089	0.080	0.511
	6	rs16877989_C	0.397	-0.138	-0.001	0.031	-0.025
	6	rs828361_T	0.014	0.091	0.031	0.042	0.427
	6	rs10944288_C	-0.035	0.314	0.022	0.047	0.077
	6	rs1886333_C	-0.011	0.282	0.018	0.021	0.604
	6	rs942472_C	-0.067	0.064	-0.003	0.053	-0.045
	6	rs1041058_T	0.227	-0.151	-0.056	-0.052	-0.177
	6	rs10806386_T	-0.150	-0.212	0.013	0.010	-0.233
	6	rs7739124_C	-0.064	-0.296	0.013	0.014	-0.025
HTR1F	3	rs7648805_A	-0.141	0.071	-0.001	-0.001	0.211
	3	rs7653582_T	-0.388	-0.087	-0.022	-0.001	-0.446
	3	rs7652406_T	-0.279	-0.153	-0.021	-0.037	0.036
HTR2B	2	rs11694724_G	-0.187	0.148	-0.018	0.017	0.105
	2	rs13394402_T	0.237	0.236	-0.003	-0.088	0.051
	2	rs17586405_G	0.528	0.980	0.096	0.099	0.682
	2	rs10194776_T	0.221	0.189	-0.003	-0.041	0.189
	2	rs4973377_A	-0.363	-0.068	-0.079	-0.053	-0.179
	2	rs6437002_G	-0.267	-0.064	-0.024	-0.062	0.035
HTR5A	7	rs6597451_T	-0.154	0.127	-0.018	0.027	0.031
	7	rs1440459_G	-0.003	0.239	-0.028	0.030	0.139
	7	rs2241859_A	-0.219	0.213	-0.011	0.004	-0.133
HTR6	1	rs6693503_A	-0.281	-0.301	-0.066	-0.043	-0.281
	1	rs4912138_A	-0.653	-0.692	-0.052	0.060	-0.337
	1	rs3790756_T	0.163	0.235	-0.042	-0.114	-0.232

Table 2 Association analysis results for the HTR4 and HTR7 genes
2a. P values

					Depression			Anxiety	Neuroticism
					Beck	Yesavage	CMI	CMI	EPQ
GENE	CHR	SNP	HWE P-value	MAF	P-value	P-value	P-value	P-value	P-value
HTR4	5	rs4274968_T	0.12	0.28	0.65	0.41	0.39	0.51	0.38
	5	rs17639006_C	0.70	0.12	0.43	0.24	0.22	**0.01**	0.68
	5	rs3995090_C	0.32	0.43	0.34	0.26	0.39	0.80	0.49
	5	rs4597955_G	0.83	0.40	*0.09*	0.23	0.12	0.25	0.11
	5	rs7702840_G	0.52	0.04	0.91	0.33	0.13	**0.01**	0.83
	5	rs7723153_A	0.87	0.42	0.52	0.40	*0.09*	0.20	0.26
	5	rs1368383_T	0.62	0.04	0.71	0.34	*0.08*	**0.03**	0.89
	5	rs1368384_C	0.45	0.23	0.57	0.90	0.44	0.29	0.65
	5	rs7725785_A	0.48	0.05	*0.06*	0.48	0.91	0.11	0.48
	5	rs12152801_G	0.71	0.35	0.57	0.61	0.26	0.92	0.12
	5	rs13359903_C	0.60	0.11	0.98	0.94	0.84	**0.04**	0.48
	5	rs2278392_A	0.54	0.09	0.52	0.44	0.97	**0.05**	0.37
	5	rs1345697_G	0.33	0.49	0.51	0.91	0.41	0.17	0.60
	5	rs17720733_C	0.51	0.09	0.50	0.55	0.28	**0.01**	0.82
	5	rs4599527_C	0.93	0.15	0.77	0.80	0.80	**0.01**	0.20
	5	rs17108435_C	0.42	0.13	0.34	0.64	0.28	**0.005**	0.56
	5	rs4336354_C	0.20	0.34	0.70	0.98	0.52	0.66	0.13
	5	rs867522_A	0.37	0.10	0.30	0.34	0.21	**0.01**	0.96
	5	rs1833710_C	0.84	0.13	0.80	0.16	0.28	0.23	0.68
	5	rs4280857_A	0.46	0.04	**0.01**	**0.005**	*0.06*	0.77	0.63
	5	rs2964276_T	0.06	0.36	0.79	0.83	0.31	0.76	0.58
	5	rs7711800_T	0.20	0.48	0.61	0.30	0.11	0.71	0.46
	5	rs1011427_T	*0.05*	0.08	0.25	**0.01**	**0.004***	0.33	0.80
	5	rs13182913_C	0.08	0.33	0.43	0.60	0.32	0.71	0.38
	5	rs2068190_T	0.08	0.43	0.68	0.27	0.52	0.87	0.32
	5	rs17706942_C	0.08	0.04	0.70	0.61	0.19	0.23	0.68
	5	rs13157587_G	0.86	0.07	0.53	0.85	0.96	0.65	0.72
	5	rs9686886_A	0.37	0.28	0.48	0.56	0.30	0.99	0.23
	5	rs5028114_T	0.13	0.43	0.77	0.97	0.63	0.17	0.26
	5	rs6865654_T	0.66	0.36	0.98	0.33	0.52	0.72	0.23
	5	rs1972644_A	0.29	0.40	0.57	0.98	0.96	0.47	0.39
	5	rs1833704_G	0.41	0.18	*0.09*	0.50	0.15	0.67	0.63
	5	rs7713886_C	0.65	0.20	0.64	0.56	0.17	0.63	*0.06*
	5	rs888961_T	0.49	0.23	0.38	0.64	0.15	0.70	0.17
HTR7	10	rs1107688_A	0.13	0.14	0.83	0.34	0.55	0.66	0.22
	10	rs17526697_A	0.31	0.10	0.62	0.73	0.26	0.17	*0.10*
	10	rs11812708_C	0.66	0.19	0.85	0.76	0.37	0.58	0.46
	10	rs11186300_C	0.31	0.48	0.60	0.23	0.91	0.48	0.13
	10	rs7086484_A	0.46	0.25	0.89	0.64	0.40	0.51	0.21
	10	rs11599921_C	0.46	0.17	0.81	0.30	0.31	0.98	0.83
	10	rs7916403_T	0.98	0.41	0.76	0.40	0.88	0.55	0.28
	10	rs10881838_G	0.48	0.28	0.50	0.90	0.21	0.58	0.32
	10	rs10785973_A	0.44	0.32	0.54	0.25	0.70	*0.07*	0.16
	10	rs7074715_A	0.56	0.11	0.33	0.20	0.66	0.79	**0.05**
	10	rs7916720_C	0.60	0.20	0.76	0.75	0.25	0.59	0.78
	10	rs4282910_A	0.67	0.16	0.81	0.42	0.27	0.94	0.66

2.b β-coefficients

			Depression			Anxiety	Neuroticism
			Beck	Yesav	CMI	CMI	EPQ
GENE	CHR	SNP	β	β	β	β	β
HTR4	5	rs4274968_T	-0.108	-0.179	-0.026	0.034	-0.207
	5	rs17639006_C	-0.247	-0.340	-0.050	-0.174	-0.133
	5	rs3995090_C	-0.200	-0.217	-0.023	0.012	-0.149
	5	rs4597955_G	0.354	0.229	0.041	0.054	0.340
	5	rs7702840_G	-0.054	-0.452	-0.098	-0.282	-0.113
	5	rs7723153_A	-0.134	-0.163	-0.046	-0.061	-0.239
	5	rs1368383_T	0.193	-0.459	-0.115	-0.260	0.072
	5	rs1368384_C	0.140	-0.030	0.024	0.058	0.112
	5	rs7725785_A	0.848	0.297	-0.006	-0.164	0.320
	5	rs12152801_G	-0.122	-0.102	-0.031	-0.005	-0.336
	5	rs13359903_C	-0.008	0.022	-0.008	-0.149	0.228
	5	rs2278392_A	0.223	0.246	0.002	-0.157	0.314
	5	rs1345697_G	-0.137	0.021	-0.022	-0.065	-0.109
	5	rs17720733_C	-0.244	-0.198	-0.050	-0.205	0.085
	5	rs4599527_C	-0.082	0.067	-0.009	-0.157	0.369
	5	rs17108435_C	-0.291	-0.130	-0.042	-0.190	0.182
	5	rs4336354_C	-0.086	-0.005	-0.018	0.022	-0.332
	5	rs867522_A	-0.347	-0.289	-0.053	-0.185	-0.019
	5	rs1833710_C	-0.076	-0.388	-0.042	-0.082	-0.127
	5	rs4280857_A	1.398	1.444	0.134	0.037	0.279
	5	rs2964276_T	-0.058	-0.044	-0.028	0.015	-0.124
	5	rs7711800_T	-0.107	-0.198	-0.042	-0.018	-0.156
	5	rs1011427_T	0.435	0.869	0.136	-0.082	-0.098
	5	rs13182913_C	-0.177	-0.107	-0.028	0.019	-0.196
	5	rs2068190_T	0.088	0.215	0.017	-0.008	-0.216
	5	rs17706942_C	-0.187	0.229	-0.083	0.132	0.204
	5	rs13157587_G	0.248	0.071	0.003	0.040	0.146
	5	rs9686886_A	-0.164	-0.126	-0.031	0.000	-0.279
	5	rs5028114_T	0.062	0.007	0.013	0.066	0.241
	5	rs6865654_T	0.005	0.192	0.018	-0.018	-0.262
	5	rs1972644_A	0.121	0.005	-0.001	-0.035	-0.186
	5	rs1833704_G	0.463	0.170	0.051	-0.026	0.135
	5	rs7713886_C	-0.120	0.138	0.045	0.028	0.490
	5	rs888961_T	-0.214	-0.104	-0.045	-0.021	-0.342
HTR7	10	rs1107688_A	-0.065	-0.271	-0.023	-0.031	-0.389
	10	rs17526697_A	0.168	0.109	0.049	0.106	0.579
	10	rs11812708_C	-0.050	-0.074	0.030	-0.033	-0.198
	10	rs11186300_C	-0.110	-0.233	-0.003	-0.034	-0.326
	10	rs7086484_A	0.034	-0.103	0.026	-0.035	-0.305
	10	rs11599921_C	-0.066	-0.262	-0.036	0.002	-0.061
	10	rs7916403_T	-0.065	-0.162	0.004	-0.028	-0.227
	10	rs10881838_G	-0.157	-0.026	0.037	-0.029	-0.238
	10	rs10785973_A	0.135	0.238	-0.011	0.091	0.313
	10	rs7074715_A	-0.321	-0.392	0.018	-0.020	-0.664
	10	rs7916720_C	0.079	0.076	0.038	-0.031	0.075
	10	rs4282910_A	0.065	0.210	0.039	0.004	0.126

Table 3 Association analysis results for the HTR3 gene
3a P values

					Depression			Anxiety	Neur
					Beck	Yesav	CMI	CMI	EPQ
Gene	Chr	SNP	HWE p value	MAF	p value	p value	p value	p value	p value
HTR3	3	rs7613237_C	0.07	0.12	0.67	0.89	0.25	0.84	0.91
CDE	3	rs939335_G	0.16	0.49	0.69	0.25	**0.04**	**0.005**	0.13
subunits	3	rs939334_G	0.94	0.33	0.51	0.73	0.37	*0.05*	0.35
	3	rs4912518_T	**0.03**	0.38	0.57	0.86	0.29	0.15	0.20
	3	rs12493550_A	**0.02**	0.07	0.65	0.62	0.63	*0.06*	0.46
	3	rs6792482_C	0.90	0.45	0.98	0.39	0.37	*0.07*	0.16
	3	rs1467257_A	**0.04**	0.43	0.27	0.52	0.41	0.76	0.81
	3	rs7621975_A	0.28	0.18	0.51	0.44	0.86	0.52	0.92
	3	rs10937160_T	0.73	0.41	0.42	0.74	0.69	0.73	0.85
	3	rs9819507_C	0.07	0.23	0.74	0.83	0.83	0.80	0.59
	3	rs6808122_G	0.65	0.38	0.13	0.12	0.30	0.21	0.34
	3	rs6766410_A	0.47	0.43	0.78	0.86	0.43	0.65	0.59
	3	rs9869582_C	0.72	0.10	**0.02**	0.19	0.12	0.33	*0.05*
	3	rs6443940_A	0.42	0.38	0.45	0.31	0.34	0.16	0.53
	3	rs4912521_T	0.56	0.11	*0.08*	0.49	0.43	1.00	0.24
	3	rs7648737_G	0.72	0.43	0.85	0.40	0.57	0.16	0.73
	3	rs6443942_A	0.38	0.47	0.44	0.87	0.97	0.16	0.61
	3	rs7627615_G	0.10	0.39	0.54	0.28	0.28	**0.03**	0.49
	3	rs7432211_C	0.64	0.39	0.70	0.74	0.73	0.95	0.62
	3	rs11718245_A	0.54	0.19	0.60	0.57	0.99	0.82	0.53
HTR3AB	11	rs869451_T	0.51	0.39	0.43	0.45	0.35	0.33	0.70
	11	rs2097078_A	0.56	0.17	0.27	0.37	0.47	0.18	*0.05*
subunits	11	rs2011249_T	0.52	0.19	0.39	0.47	0.89	0.91	*0.09*
	11	rs1176758_C	0.44	0.44	0.70	0.12	0.65	0.87	0.26
	11	rs3891484_C	0.75	0.13	0.11	0.67	0.71	0.53	0.15
	11	rs1176744_G	0.15	0.31	0.50	0.97	0.75	0.34	0.35
	11	rs2276307_G	0.12	0.22	0.63	0.38	0.60	0.62	0.68
	11	rs3782025_C	0.84	0.49	0.55	0.04	0.45	0.68	0.05
	11	rs1672717_C	0.31	0.44	0.96	0.39	0.66	0.64	0.57
	11	rs7129190_A	0.45	0.48	0.69	0.14	0.70	0.30	0.17
	11	rs7942029_G	0.84	0.17	0.12	0.20	0.96	0.17	0.12
	11	rs1150229_T	0.09	0.48	0.54	0.89	0.89	0.49	0.92
	11	rs1985242_A	0.36	0.32	*0.05*	0.19	0.84	0.35	0.19
	11	rs10891611_C	0.84	0.15	**0.01**	*0.07*	0.59	0.58	**0.004**
	11	rs909411_T	0.80	0.22	0.07	0.12	0.98	0.97	**0.03**
	11	rs10160548_G	0.09	0.31	0.61	0.74	0.611	0.65	0.53
	11	rs11214800_C	0.25	0.48	0.22	0.77	0.57	0.48	0.75
	11	rs11214806_C	0.37	0.10	0.34	0.62	0.69	0.58	0.01
	11	rs10891615_T	0.09	0.32	0.26	0.78	0.21	0.91	0.16

3b β-coefficients

			Depression			Anxiety	Neuroticism
			Beck	Yesav	CMI	CMI	EPQ
GENE	CHR	SNP	β	β	β	β	β
HTR3C	3	rs7613237_C	0.134	-0.040	-0.046	0.014	0.036
subunits	3	rs939335_G	0.080	-0.214	-0.052	-0.127	-0.307
	3	rs939334_G	0.142	-0.068	-0.025	-0.099	-0.209
	3	rs4912518_T	-0.117	0.035	0.028	0.067	0.271
	3	rs12493550_A	-0.174	0.179	0.024	0.162	0.294
	3	rs6792482_C	-0.004	-0.162	-0.024	-0.085	-0.291
	3	rs1467257_A	-0.224	-0.122	0.022	0.014	0.050
	3	rs7621975_A	0.175	0.189	-0.006	0.039	-0.027
	3	rs10937160_T	0.169	0.064	-0.010	-0.016	-0.041
	3	rs9819507_C	-0.082	-0.047	-0.007	0.014	-0.133
	3	rs6808122_G	0.327	0.306	0.028	0.061	0.210
	3	rs6766410_A	0.059	-0.035	-0.021	0.021	0.114
	3	rs9869582_C	-0.785	-0.413	-0.069	-0.077	-0.693
	3	rs6443940_A	0.163	0.200	0.026	0.068	0.136
	3	rs4912521_T	-0.589	-0.210	-0.034	0.000	-0.405
	3	rs7648737_G	0.040	0.164	0.015	0.066	0.072
	3	rs6443942_A	0.162	-0.033	-0.001	-0.066	0.109
	3	rs7627615_G	-0.130	0.214	0.030	0.104	0.153
	3	rs7432211_C	-0.083	-0.065	-0.010	0.003	-0.105
	3	rs11718245_A	0.137	0.140	0.000	-0.014	0.165
HTR3AB	11	rs869451_T	-0.164	0.146	0.025	-0.046	-0.084
subunits	11	rs2097078_A	0.300	0.225	-0.025	0.082	0.552
	11	rs2011249_T	-0.225	-0.176	0.005	-0.007	-0.467
	11	rs1176758_C	-0.078	-0.294	-0.012	-0.007	-0.236
	11	rs3891484_C	-0.488	-0.122	0.015	-0.043	-0.447
	11	rs1176744_G	-0.148	-0.007	-0.009	-0.047	-0.210
	11	rs2276307_G	-0.118	0.197	0.016	-0.027	0.101
	11	rs3782025_C	-0.122	-0.388	-0.020	-0.019	-0.411
	11	rs1672717_C	-0.010	-0.163	0.012	0.022	-0.119
	11	rs7129190_A	0.082	0.279	0.010	-0.047	0.289
	11	rs7942029_G	0.429	0.322	0.002	0.084	0.439
	11	rs1150229_T	0.124	0.025	-0.004	-0.032	0.022
	11	rs1985242_A	0.445	0.273	0.006	-0.047	0.304
	11	rs10891611_C	0.771	0.486	0.020	0.036	0.864
	11	rs909411_T	0.454	0.365	0.001	-0.002	0.548
	11	rs10160548_G	0.111	0.066	-0.014	-0.022	0.140
	11	rs11214800_C	0.246	-0.054	-0.015	-0.032	0.065
	11	rs11214806_C	0.323	0.152	-0.017	0.042	0.840
	11	rs10891615_T	0.248	-0.056	-0.035	0.005	0.313

Abbreviations for the tables
Chr: chromosome
HWE p value: Hardy-Weinberg Equilibrium P-value
MAF: minor allele frequency
Beck: Beck Depression Inventory
Yesav: Yesavage Geriatric Depression Score
CMI: Cornell Medical Index
Neur: Neuroticism
EPQ: Eysenck Personality Questionnaire
Marginally significant P-values are indicated in italics
Significant P-values are indicated in bold
Asterisk marks the P-values remaining significant after correction for LD-based multiple testing

Discussion

Here we report the results of a review and investigation of the possible role of less well investigated serotonergic receptors (*HT1D*, *1E*, *1F*, *2B* and *3-7*) in depression, anxiety and neuroticism.

Using htSNPs from a genome-wide association study, we found several weak associations between certain variants of these genes and the psychiatric phenotypes. The strongest associations were observed between the rs4912138_A allele (in the *HTR6* gene) and Yesavage depression score, three SNPs in the *HTR4* gene (rs17108435_C, rs4280857_A and rs1011427_T) respectively with CMI anxiety scores, Yesavage scores and CMI depression scores. Two SNPs in the HTR3 gene also showed relatively strong association to CMI anxiety and Beck depression scores (rs939335_G and rs10891611_C, respectively). After correction for multiple testing (LD base method) even with moderately strict parameters, only three results remained weakly significantly associated with depression, two SNPs in the HTR6 gene and one in the *HTR4* gene but none of them survived the Bonferroni-correction.

There are not many studies that have looked at *HTR4* and mental disorders and the majority of the reports found no association (Hirata *et al*. 2010, Elia *et al*. 2009 Gratacos *et al.* 2008, Shi *et al*. 2008). Using haplotype analyses its role in bipolar disorder (Ohtsuki *et al.* 2002) and schizophrenia (Suzuki *et al*. 2003) was suggested in Japanese studies. Our study confirms a reported association between rs17108435 and depression (Lewis *et al.* 2010). As this association became very weak after correction for LD-based multiple testing and lost significance after Bonferroni-correction, it should be treated cautiously. On the other hand this gene is very complex (700 Kb, with 38 exons and numerous splice variants) which may mean that one SNP has a small effect in the function of the gene. Further support for our finding is that *Ht4* KO animals show stress-induced hypophagia and reduced novelty seeking, which is co-morbid with depression in humans.

The *HTR6* gene has received some attention in depression research. The *HTR6* SNP rs1805054 showed positive heterosis effect in response to antidepressant treatment in Korean depressive patients (Lee *et al.* 2005) although the association was not observed in a Japanese study (Kishi *et al*. 2010). Once again, several studies

report that association was not found between variants of this gene and depression (Illi et al. 2009, Gratacos et al. Fukuo et al. 2010).

Despite several reports of a lack of association with depression, our positive finding may be valid. A recent study showed the importance of this receptor during embryonic development. 5-HT6 receptors are expressed in cortical interneurons and their activation decreased cortical interneuron migration in the developing brain (Riccio *et al.* 2009). It seems plausible to speculate that any mutation disturbing the normal function of this receptor can have a phenotypic effect. Indeed, our two SNPs (rs6693503 and rs4912138) are only 6191 bp away from each other (r^2=0.45), straddling two other missense SNPs (rs8192530 and rs61733141). The first one of these missense mutations replaces a basic amino acid residue with a polar, uncharged one, whereas the second mutation introduces an S-containing residue without causing charge change. It may be possible that one or both of those SNPs cause the phenotypic change, and our two SNPs are in LD with them. As rs8192530 and rs61733141 are not listed in the HapMap phase 3 CEU sample database this possibility remains a speculation.

There are findings in the literature which we were able to corroborate, although our results only showed weak nominal significance and they did not survive the Bonferroni-correction. For example, the *HTR3AB* subunits SNP rs3782025 which was associated with both CMI anxiety (p-value 0.05) and Yesavage depression (p-value 0.04) in this study has also been associated with alcohol use disorders, co-morbid personality disorder and reduced EEG alpha power in an independent study (Ducci *et al.* 2009). A weak association was also observed between an SNP (rs7627615) within the *HTR3CDE* subunits gene and anxiety (p-value 0.033). The SNP rs7627615 has also been associated with attention capacity in schizophrenia (Lennertz *et al*, 2010).However, a functional SNP in the *HTR3CDE* subunits gene, rs1176744 (Krzywkowski *et al*. 2008) did not result in any significant association in our sample. Another SNP, rs1062613 which is thought to be functional and showed association with bipolar affective disorder (Niesler et al. 2001) was not present among our SNPs. In the HapMap phase 3 CEU database this SNP was in strongest LD with rs10891611 (r^2=0.55) which in our cohort showed the strongest association with Beck depression and EPQ neuroticism among the *HTR3AB* variants. Therefore it is possible that this gene region has some function in mental disorders.

A somewhat surprising result was the lack of association between *HTR7* gene and the depressive phenotype in our sample. KO animal models suggest an important role in circadian rhythm (Guscott *et al*. 2005) and REM sleep (Hedlund *et al.* 2005), which is disturbed in depression. Also it has been associated with depression in a UK population (Lewis *et al.* 2010) and with antidepressant response (Uher *et al.* 2010). Despite these indications, we only found one SNP of this gene weakly associated with neuroticism.

In the literature we were not able to find indications of 5-HT1D, 5-HT1E, 5-HT1F and 5-HT2B receptors involvement in depression and related phenotypes which our finding corroborates. One report shows association between a SNP in the *HTR5A* gene and depression, -19G/C (rs79028878, Birkett *et al*. 2000). As our data did not contain this SNP and it is not listed on the HapMap phase 3 CEU database we cannot comment on this finding.

Finally, also based on the literature review of animal and human studies, we found that these genes have an apparent effect on vegetative symptoms - such as function of the cardiovascular system (*HTR2B*), food intake (*HTR4*), and sleep pattern (*HTR7*) - that represent core symptoms or co-morbid states with depression. The phenotypic data available for us did not confirm the involvement of the *HTR2B* gene in cardiovascular symptoms or the *HTR7* gene's role in fatigue and sleep disorders. The major strength of this study was its use of a large sample size. Our cohort of over 1500 individuals had over 87% power to detect a genetic effect size of 1% assuming a MAF of 28.5% (average MAF in this study) and a stringent significance threshold of 0.005 (2-sided). As none of the observed associations survived Bonferroni correction, it suggests that either the results occurred by chance and were false positives or that the effect sizes were small. However, if the nominally significant results had occurred by chance we may have expected to see a more random distribution across the behavioural traits rather than the observed clustering in specific domains. Whilst linkage disequilibrium may account for some of this clustering it may also be an indication of true association. Another strength of this study is that depression was measured using three different tests. This allowed us to test the robustness of an association and provided further indication of false positive results.

A weakness of this study is that the volunteers were not selected for depression but rather were a group who were used for a study of cognition. Unlike a

case control study where a comparison is made with the extreme end of the behavioural traits this study analysed the normal distribution of a continuous trait which can be a less powerful approach.

In conclusion, we report three associations between *HTR4* and *HTR6* SNPs and depression. These associations are weak and did not remain significant after Bonferroni-correction, which may indicate the small involvement of these receptors in the behavioural and emotional phenotype. As many of these receptors appear to play a role in other vegetative function, which are disturbed in depression, we suggest that future genetic association studies which investigate the effect of the whole serotonergic pathway should take into account important vegetative phenotypes together with behavioural assessments.

References

Arias B, Collier DA, Gasto C, Pintor L, Gutierrez B, Valles V et al. (2001). Genetic variation in the 5-HT5A receptor gene in patients with bipolar disorder and major depression. Neuroscience Letters, 3030:111-114.

Barnes NM, Hales TG, Lummis SC, Peters JA (2009). The 5-HT3 receptor--the relationship between structure and function. Neuropharmacology, 56(1):273-84. Review.

Beck AT, Ward CH, Mendelsohn M, Mock J, Erbaugh J (1961). An inventory for measuring depression. Arch Gen Psych. 4:561-571.

Bhatnagar S, Sun LM, Raber J, Maren S, Julius D, Dallmann MF (2004). Changes in anxiety-related behaviors and hypothalamic-pituitary-adrenal activity in mice lacking the 5-Ht3a receptor. Psychol Behav, 81:545-555.

Birkett JT, Arranz MJ, Munro J, Osbourn S, Kerwin RE, Collier DA (2000). Association analysis of the 5-HT5A gene in depression, psychosis and antipsychotic response. Neuroreport, 11(9):2017-2020.

Bonasera SJ, Chu HM, Brennan TJ, Tecott LH (2006). A null mutation of the serotonin 6 receptor alters acute responses to ethanol. Neuropsychopharmacology, 31:1801-1813.

Brodman K,Erdmann AJ Jr., Wolff HG (1956). Cornell Medical Index Health Questionnaire (Manual). Cornell University Medical College, New York, NY, USA

Compan V, Zhou M, Grailhe R, Gazzara RA, Martin R, Gingrich J, Dumuis A et al. (2004). Attenuated response to stress and novelty and hypersenzitivity to seizures in 5-HT4 receptor knock out mice. Journal of Neuroscience, 24:412-419.

Corley RP, Zeiger JS, Crowley T, Ehringer MA, Hewitt JK, Hopfer CJ, Lessem J, McQueen MB, Rhee SH, Smolen A, Stallings MC, Young SE, Krauter K (2008). Association of candidate genes with antisocial drug dependence in adolescents. Drug and alcohol dependence, 96:90-98.

Drago A, Alboni S, Brunello N, De Ronchi D, Serretti A (2010). HTR1B as a risk profile marker in psychiatric disorders: a review through motivation and memory. European Journal of Clinical Pharmacology, 66(1):5-27.

Dubin AE, Huvar R, D'Andrea MR, Pyati J, Zhu JY, Joy KC, et al. The pharmacological and functional characteristics of the serotonin 5-HT3A receptor are specifically modified by a 5-MT3B receptor subunit. J Biol Chem 1999;274:30799–810.

Ducci F, Enoch M-A, Yuan Q, Shen P-H, White KV, Hodgkinson C, Albaugh B, Virkkunen M, Goldman D (2009). HTR3B is associated with alcoholism with antisocial behavior and alpha EEG power-an intermediate phenotype for alcoholism and co-morbid behaviors. Alcohol, 43:73-84.

Eisensamer B, Rammes G, Gimpl G, Shapa M, Ferrari U, Hapfelmeier G, Bondy B, Parsons C, Gilling K, Zieglgänsberger W, Holsboer F, Rupprecht R (2003). Antidepressants are functional antagonists at the serotonin type 3 (5-HT3) receptor. Mol Psychiatry, 8(12):994-1007.

Eisensamer B, Uhr M, Meyr S, Gimpl G, Deiml T, Rammes G, Lambert JJ, Zieglgänsberger W, Holsboer F, Rupprecht R (2005). Antidepressants and antipsychotic drugs colocalize with 5-HT3 receptors in raft-like domains. J Neurosci, 25(44):10198-206.

Elia J, Capasso M, Zaheer Z, Lantieri F, Ambrosini P, Berrettini W, Devoto M, Hakonarson H (2009). Candidate gene analysis in an on-going genome-wide

association study of attention-deficit hyperactivity disorder: suggestive association signals in ADRA1A. Psychiatr Genet. 19(3):134-41.

Eysenck HJ, Eysenck SBG (1964). The Eysenck personality questionnaire. University of London Press, London, UK

Filip M, Bader M (2009). Overview on 5-HT receptors and their role in psychology and pathology of the central nervous system. Pharmacological Reports, 61:761-777.

Fukuo Y, Kishi T, Yoshimura R, Kitajima T, Okochi T, Yamanouchi Y, Kinoshita Y, Kawashima K, Naitoh H, Umene-Nakano W, Inada T, Kunugi H, Kato T, Yoshikawa T, Ujike H, Nakamura J, Ozaki N, Iwata N (2010). Serotonin 6 receptor gene and mood disorders: case-control study and meta-analysis. Neuroscience Research, 67:250-255.

Grailhe R, Waeber C, Dulawa SC, Hornung JP, Zhuang X, Brunner D, Greyer MA, Hen R (1999). Increased exploratory activity and altered response to LSD in mice lacking the 5-HT5A receptor. Neuron, 22:581-591.

Gratacos M, Costas J, De Cid R, Bayes M, Gonzales JR, Baca-Garcia E, De Diego Y, Fernandez-Aranda F, Fernandez-Piqueras F, Guitart M, Martin-Santos R, Martorell L, Menchon JM, Roca M, Saiz-Ruiz J, Sanjuan J, Torrens M, Urretavizcaya M, Valero J, Vilella E, Estivill X, Carracedo A and The Psychiatric Genetics Network Group (2008). Identification of new putative susceptibility genes for several psychiatric disorders by association analysis of regulatory and non-synonimous SNPs of 306 genes involved in neurotransmission and neurodevelopment. American Journal of Medical Genetics Part B, 150B:808-816.

Goecke TW, Ekici AB, Niesler B, Loehberg CR, Hammer C, Rappold G, Schanze D, Straub V, Altmann HH, Strissel P, Strick R, Beckmann MW, Fasching PA (2010). Two naturally occurring variants of the serotonin receptor gene HTR3C are associated with nausea in pregnancy. Acta Obstet Gynecol Scand 98(1):7-14.

Guscott M, Bristow LJ, Hadingham K, Rosahl TW, Beer MS, Stanton JA, Bromidge F, Owens AP, Huscroft I, Myers J, Rupniak NM, Patel S, Whiting PJ, Hutson PH, Fone KC, Biello, SM, Kulagowski JJ, McAllister G (2005) Genetic knockout and pharmacological blockage studies of the 5-HT7 receptor suggest therapeutic potential in depression. Neuropharmacology, 48:492-502.

Hammer C, Kapeller J, Endele M, Fischer C, Hebebrand J, Hinney A, Friedel S, Gratacos M, Estivill X, Fichter M, Fernandez-Aranda F, Ehrlich S, Rappold G, Niesler B (2009). Functional variants of the serotonin receptor type 3A and B gene are associated with eating disorders. Pharmacogentics and Genomics, 19(10):790-799.

Hannon J, Hoyer D (2008). Molecular biology of 5-HT receptors. Behavioural Brain Research, 195:198-213.

Hedlund PB,Danielson PE, Thomas EA, Slanina K, Carson MJ, Sutcliffe GJ (2003). No hypothermic response to serotonin in 5-HT7 receptor knockout mice. Proceedings of National Academy of Sciences, USA, 100(3):1375-1380.

Hedlund PB, Huitron-Resendiz S, Henriksen SJ, Sutcliffe GJ (2005). 5-HT7 receptor inhibition and inactivation induce antidepressant like behavior and sleep pattern. Biological Psychiatry, 58:831-837.

Heisler LK, Zhou L, Bajwe P, Hsu J, Tecott LH (2007). Serotonin 5-HT(2C) receptors regulate anxiety-like behavior. Genes Brain Behavior, 6:491-496.

Hettema JM (2008). What is the genetic relationship between anxiety and depression? Am J Med Genet C Semin Med Genet.;148C(2):140-6. Review.

Hirata Y, Souza RP, Lieberman JA, Meltzer HY, Kennedy JL (2010). Lack of association between HTR4 gene polymorphisms and schizophrenia in case-control and family-based samples. Psychiatry Res.175(1-2):176-8.

Holmes A (2008). Genetic variation in cortico-amygdala serotonin function and risk for stress-related disease. Neuroscience and Biobehavioral Reviews, 32:1293-1314.

Holmes A, Yang RJ, Lesch K-P, Crawley JN, Murphy DL (2003). Mice lacking the serotonin transporter exhibit 5-HT1A receptor-mediated abnormalities in tests for anxiety-like behavior. Neuropsychopharmacology, 28:2077-2088.

Hoyer D, Hannon JP, Martin GR (2002). Molecular, Pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem and Behav, 71:533-554.

Kishi T, Fukuo Y, Yoshimura R, Okochi T, Kitajima T, Naitoh H, Umene-Nakano W, Nakamura J, Ozaki N, Iwata N (2010). Pharmacogenetic study of serotonin 6 receptor gene with antidepressant response in major depressive disorder in the Japanese population. Hum Psychopharmacol. 25(6):481-6.

Iidaka Y, Ozaki N, Matsumoto A, Nogawa J, Kinoshita Y, Suzuki T, Iwata N, Yamamoto Y, Okada T, Sadato N (2005). A variant C178T in the regulatory region of the serotonin receptor gene HTR3A modulates neural activation in the human amygdala. Journal of Neuroscience, 25:6460-6466.

Illi A, Setälä-Soikkeli E, Viikki M, Poutanen O, Huhtala H, Mononen N, Lehtimäki T, Leinonen E, Kampman O (2009). 5-HTR1A, 5-HTR2A, 5-HTR6, TPH1 and TPH2 polymorphisms and major depression. Neuroreport. 20(12):1125-8.

Kapeller J, Houghton LA, Moennikes H, Walstab J, Moeller D, Boenisch H, Bruwinkel B, Autschbach F, Funke B, Lasitschka F, Gassler N, Fischer C, Whorwell PJ, Atkinson W, Fell C, Buecher KJ, Schmidtmann M, van der Voort I, Wisser AS, Berg T, Rappold G, Niesler B (2008). First evidence for an association of a functional variant in the microRNA-510 target site of the serotonin receptor-type 3E gene with diarrhea predominant irritable bowel syndrome. Hum Mol Genet, 17(19):2967-2977.

Krzywkowski K, Davies PA, Feinberg-Zadek PL, Brauner-Osborne H, Jensen AA (2008). High-frequency HTR3B variant associated with major depression

dramatically augments the signaling of the human 5-HT3AB receptor. Proceedings of National Academy of Sciences, USA, 105(2):722-727.

Lasky-Su J, Neale BM, Franke B, Anney RJ, Zhou K, Maller JB, Vasquez AA, Chen W, Asherson P, Buitelaar J, Banaschewski T, Ebstein R, Gill M, Miranda A, Mulas F, Oades RD, Roeyers H, Rothenberger A, Sergeant J, Sonuga-Barke E, Steinhausen HC, Taylor E, Daly M, Laird N, Lange C, Faraone SV (2008). Genome-wide association scan of quantitative traits for attention deficit hyperactivity disorder identifies novel associations and confirms candidate gene associations. Am J Med Genet B Neuropsychiatr Genet, 147B(8):1345-54.

Lee S-H, Lee K-J, Lee H-J, Ham B-J, Ryu S-H, Lee M-S (2005). Association between the 5-HT6 receptor C267T polymorphism and response to antidepressant treatment in major depressive disorder. Psychiatry and Clinical Neurosciences, 59:140145.

Lennertz L, Wagner M, Frommann I, Schulze-Rauschenbach S, Schuhmacher A, Kühn KU, Pukrop R, Klosterkötter J, Wölwer W, Gaebel W, Rietschel M, Häfner H, Maier W, Mössner R. A coding variant of the novel serotonin receptor subunit 5-HT3E influences sustained attention in schizophrenia patients. Eur Neuropsychopharmacol. 2010 Jun;20(6):414-20.

Lesch K-P, Zeng Y, Reif A, Gutknecht L (2003). Anxiety-related traits in mice with modified genes of the serotonergic pathway. European Journal of Pharmacology, 480:185-204.

Levran O, Londono D, O'Hara K, Nielsen DA, Peles E, Rotrosen J, Casadonte P, Linzy S, Randesi M, Ott J, Adelson M, Kreek MJ (2008). Genetic susceptibility to heroin addiction: a candidate gene association study. Genes, Brain and Behavior, 7:720-729.

Licht CL, Knudsen GM, Sharp T(2010). Effects of the 5-HT(4) receptor agonist RS67333 and paroxetine on hippocampal extracellular 5-HT levels. Neurosci Lett. 476(2):58-61.

Lin Z, Walther D, Yu X-Y, Drgon T, Uhl GR (2004). The human serotonin 2B: coding region polymorphisms and association with vulnerability to illegal drug abuse. Pharmacogenetics, 14:805-811.

Lira A, Zhou M, Castanon N, Ansorge MS, Gordon JA, Francis JH, Bradley-Moore M, Lira J, Underwood MD, Arango V, Kung HF, Hofer MA, Hen R, Gingrich JA (2003). Altered depression-related behaviors and functional changes in the dorsal raphe nucleus of serotonin transporter-deficient mice. Biological Psychiatry, 54:960-971.

Maricq AV, Peterson AS, Brake AJ, Myers RM, Julius D. Primary structure and functional expression of the 5ht3 receptor, a serotonin-gated ion channel.
Science 1991;254:432–7.

Melke J, Westberg L, Nillson S, Landen M, Soderstrom H, Baghaei F, Rosmond R, Holm G, Bjorntorp P, Nilsson LG, Adolffson R, Eriksson E. (2003) A polymorphism in the serotonin receptor 3A (HTR3A) gene and its association with harm avoidance in women. Archives of General Psychiatry, 60:1017-1023.

Mizuta N, Akiyoshi J, Sato A, Hanada H, Tanaka Y, Tsuru J, Matshushita H, Kodama K, Isogawa K (2008). Serotonin receptor 3A (HTR3A) gene is associated with personality traits, but not panic disorder. Psychiatric Genetics, 18:44.

Nebigil CG, Choi D-S, Dietrich A, Hickel P, Le Meur M, Messaddeq N, Launay J-M, Maroteaux L (2000). Serotonin 2B receptor is required for heart development. Proceedings of National Academy of Sciences, USA, 97:9508-9513.

Niesler B, Flohr T, Nothen MM, Fischer C, Rietschel M, Franzek E, Albus M, Propping P, Rappold GA (2001). Association between the 5'UTR variant C178T of the serotonin receptor gene HTR3A and bipolar affective disorder. Pharmacogenetics, 11:471-475.

Niesler B, Frank B, Kapeller J, Rappold GA (2003). Cloning, physical mapping and expression analysis of the human 5-HT3 serotonin receptor-like genes HTR3C, HTR3D and HTR3E. Gene, 310:101-111.

Oades RD, Lasky-Su J, Christiansen H, Faraone SV, Sonuga-Barke EJS, Banaschewski T, Chen W, Anney RJL, Buitelaar JK, Ebstein RP, Franke B, Gill M, Miranda A , Roeyers H, Rothenberger A, Sergeant JA, Steinhausen H-C, Eric A Taylor EA, Thompson M, Asherson P (2008). The influence of serotonin- and other genes on impulsive behavioral aggression and cognitive impulsivity in children with attention-deficit/hyperactivity disorder (ADHD): Findings from a family-based association test (FBAT) analysis. Behavioral and Brain Functions, 4:48.

Ohtsuki T, Ishiguro H, Detera-Wadleigh SD, Toyota T, Shimizu H, Yamada K, Yoshitsugu K, Hattori E, Yoshikawa T, Arinami T (2002). Association between serotonin 4 receptor gene polymorphisms and bipolar disorder in Japanes case-control samples and the NIMH Genetics Initiative Bipolar Pedigrees. Molecular Psychiatry, 7:954-961.

Otano A, Frechilla D, Cobreros A, Cruz-Orive LM, Insausti A, Insausti R et al. (1999). Anxiogenic-like effects and reduced stereolgical counting of immunolabelled 5 hydroxytriptamine 6 receptors in rat nucelus accumbens by antisense nucleotides. Neuroscience, 92:1001-1009.

Rabbit PMA, McInnes L, Diggle P, Holland F, Bent N, Abson V, Pendleton N, Horan M (2004). The University Of Manchester Longitudinal study of cognition in normal helathy old age. Aging Neuropsychology and Cognition, 11(2-3):245-279.

Rajkumar R, Mahesh R (2010). The auspicious role of the 5-HT3 receptor in depression: a probable neuronal target? J Psychopharmacol, 24(4):455-69. Review.

Rees S, den Daas I, Foord S, Goodson S, Bull D, Kilpatrick G, Lee M (1994). Cloning and characterisation of the human 5-HT5A serotonin receptor. FEBS Letters, 355:242–246.

Rehnstroem K, Ylsaukko-oja T, Nummela I, Ellonen P, Kempas E, Vanhala R, von Wendt L, Jarvela I, Peltonen L (2009). Allelic variants in HTR3C show association to autism. Am J Med Genet B Neuropsychiatr Genet, 150B(5):741-746.

Riccio O, Potter G, Walzer C, Vallet P, Szabo G, Vutskits L, Kiss JZ, Dayer AG (2009). Excess of serotonin affects embryonic interneuron migration through activation of the serotonin receptor 6. Molecular Psychiatry, 14:280-290.

Shi J, Badner JA, Hattori E, Potash JB, Willour VL, McMahon FJ (2008). Neurotransmission and bipolar disorder: a systematic family-based association study. Am J Med Genet, Part B Neuropsych Genet 147B:1270-1277.

Smith AK, Dimulescu I, Falkenberg VR, Narasimhan S, Heim C, Vernon SD, Rajeevan MS (2008). Genetic evaluation of the serotonergic system in chronic fatigue syndrome. Psychoneuroendocrinology, 33:188-197.

Suzuki T, Iwata N, Kitamura Y, Kitajima T, Yamanouchi Y, Ikeda M, Nishiyama T, Kamatani N, Ozaki N (2003) Association of a haplotype in the serotonin 5-HT4 receptor gene (HTR4) with Japanese schizophrenia. Am J Med Genet B Neuropsychiatr Genet. 121B(1):7-13.

Uher R, Perroud N, Ng MY, Hauser J, Henigsberg N, Maier W, Mors O, Placentino A, Rietschel M, Souery D, Zagar T, Czerski PM, Jerman B, Larsen ER, Schulze TG, Zobel A, Cohen-Woods S, Pirlo K, Butler AW, Muglia P, Barnes MR, Lathrop M, Farmer A, Breen G, Aitchison KJ, Craig I, Lewis CM, McGuffin P (2010). Genome-wide pharmacogenetics of antidepressant response in the GENDEP project. Am J Psychiatry, 167(5):555-564.

Waraich P, Goldner EM, Somers JM, Hsu L (2004). Prevalence and incidence studies of mood disorders: a systematic review of the literature. Can J Psychiatry. 49(2):124-38. Review.

Weisstaub NV, Zhou M, Lira A, Lambe E, Gonzalez-Maeso J, Hornung JP, Sibille E, Underwood M, Itohara S, Dauer WT, Ansorge MS, Morelli E, Mann JJ, Toth M, Aghajanian G, Sealfon SC, Hen R, Gingrich JA (2006). Cortical 5-HT2A receptor signaling modulates anxiety-like behaviors in mice. Science, 313:536–540.

Xie XD, Lee SP, O'Dowd BF, George SR. Serotonin 5-HT1B and 5-HT1D receptors form homodimers when expressed alone and heterodimers when co-expressed. FEBS Lett 1999;456:63–7.

Yamada K, Hattori E, Iwayama Y, Ohnishi T, Ohba H, Toyota T, Takao H, Minabe Y, Nakatani N, Higuchi T, Detera-Wadleigh SD, Yoshikava T (2006). Distinguishable haplotype blocks in the HTR3A and HTR3B region in japanese reveal evidence of association of HTR3B with female major depression. Biological Psychiatry, 60:192-201.

Yesavage JA, Bruik TL, Rose TL (1983). Development and validation of a geriatric depression screening scale: A preliminary report. Journal of Psychiatric Research. 17:37-49.

Zhuang X, Gross C, Santarelli L, Compan V, Trillat A-C, Hen R (1999). Altered emotional states in knockout mice lacking 5-HT1A or 5-HT1B receptors. Neuropsychopharmacology, 21:53S-60S.

7. General Discussion

Within a couple of years after the completion of the entire human genome sequencing (International Human Genome Sequencing Consortium 2001a and 2001b) and the first haplotype map (The International HapMap Consortium 2005), the first whole genome association studies started. Three years ago when the first results started to emerge, the expectations were high. GWAS performed very well in identifying new genes and confirming previous susceptibility candidates in many disorders, such as type 1 and 2 diabetes and Crohn disease (Manolio *et al.* 2008). However, GWAS gave less conclusive results in psychiatric disorders. After 9 depression GWAS we still do not have a conclusive list of genes which would account for earlier estimates of depression heritability (30-40%, Sullivan *et al.* 2000). Why is this so?

In 1918 RA Fisher wrote: 'if several genes affect a trait, the trait will be normally distributed even though each gene is inherited according to Mendel's laws' (Fisher 1918) (Figure 9).

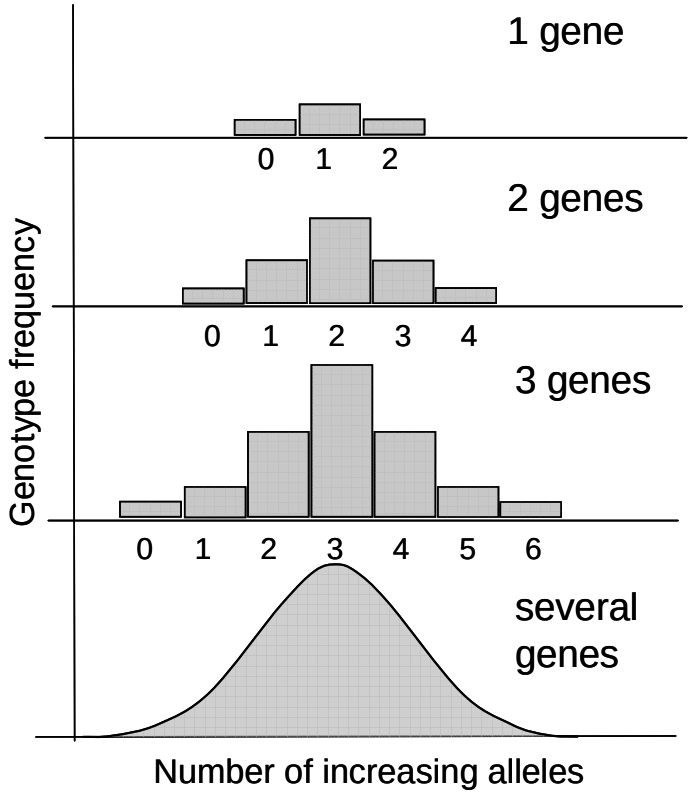


Figure 9. Fisher's bell curve. As the number of alleles increases, the distribution of the genotype reaches the perfect bell curve shape (Adapted from Plomin *et al.* 2009).

Nearly one hundred years later GWAS proved that this is indeed the case with depression, anxiety and neuroticism. Instead of a few genes with major effects, a large number of genes with very small individual effects appear to underlie these phenotypes. Genetic variations with small effects are hard to identify using GWAS. GWAS produces very large amounts of data and in the necessary correction of genome-wide significance these small significance values tend to be lost and are difficult to distinguish from the 'noise'. Also, depression and anxiety (just like neuroticism) are continuously distributed in the population, which makes it difficult to treat them in the same way as a categorical disorder. Furthermore, GWAS are not suitable to take into consideration other effects which may take part in determining the phenotype, such as environmental factors, and gene x gene interaction. Using intermediate phenotypes, such as brain imaging and computerised tasks cannot help GWAS because of the large samples required to allow true statistical significance to

emerge from the multitude of false positives in comparisons of 500,000 to over 2 million SNPs.

Smaller, hypothesis-driven studies on the other hand are only able to investigate the role of a smaller number of candidate genes in any given phenotype. Precisely because of the relatively limited amount of data these studies produce, it is possible to include interactions into the models without reducing sensitivity through severe correction for multiple comparisons. A hypothesis-driven study, containing only 847 individuals was the first to prove the logical assumption that the environment and the serotonergic system are in interaction with each other (Caspi *et al.* 2003). This relationship has been replicated several times since then (Caspi *et al*. 2010). The link between the genotype of the 5-HTTLPR and emotional behaviour was provided with a small study (Hariri *et al*. 2002). With a simple perceptual task known to reliably engage the amygdala, the researchers showed that s allele carriers of the 5-HTTLPR exhibited fivefold greater amygdala response to emotionally provocative stimuli (Hariri *et al.* 2002). This finding again has been replicated many times since (Scharinger *et al.* 2010).

It seems reasonable to suggest that the future of genetic research is the combination of the GWAS and the hypothesis-driven, gene or pathway investigating studies, which instead of competing, complement each other.

My study as a part of a large European study (New Molecules in Mood Disorders or New Mood) was a hypothesis-driven, pathway investigating study. The aim was to determine the role of the known 14 serotonergic receptors (*HTR1A-F, 2A-C*, *3A*, *B*, *4,5,6* and *7*) and the transporter molecule (SERT) in the depression, trait anxiety and neuroticism phenotypes. I also investigated the possibility that other interacting factors, such as childhood adversity, recent life events and serotonergic gene x gene interactions would influence the trait (dimensional) and the diagnostic (categorical) phenotypes.

Study 1: The *HTR1A* and *HTR1B* receptor genes influence stress-related information processing

My results showed no association between HTR1A and HTR1B genes and depression and anxiety in the allelic or genotypic association tests. However,

significant association emerged between the *HTR1A* rs6295, rs878567 and *HTR1B* rs6296 and BSI depression and anxiety scores when recent life events were included as an interacting factor in the model. A previously identified functional *HTR1A* SNP (Lemonde *et al.* 2003) was investigated in a computerised task in a subset of healthy individuals. It showed that the possession of the overexpressing allele of rs6295 was associated with a quicker response in identifying fearful face emotions. These results suggest that 5-HT1A and perhaps 5-HT1B autoreceptors mediate changes in the serotonergic system that influence the depressogenic or anxiogenic effect of recent negative life events. In the case of the 5-HT1A receptor a possible pathomechanism that may play a role in stress-related depression and anxiety could be via its involvement in threat-related information processing.

Study 2: Evidence for epistatic interaction between *HTR2A* and *SLC6A4* genes in depression and associated phenotypes

In this study I investigated the role of *HTR2A* and *SLC6A4* genes in depression, trait anxiety and neuroticism. In the New Mood cohort no association emerged in the allelic and genotypic tests. When testing for epistasis, significant interactions emerged between a promoter SNP (rs6311) and the third haplotype block, and between the second and third haplotype blocks of the *HTR2A* gene in the New Mood cohort. In this cohort interaction was observed within the *SLC6A4* gene (5-HTTLPR and rs6354), and between the *HTR2A* and *SLC6A4* genes (rs3794808 and a haplotype block in intron2).

I found a similar interaction in an independent sample (Dyne Steel cohort) between haplotype blocks two and three within the *HTR2A* gene, also between the *SLC6A4* and *HTR2A*, but with a different region of the latter (rs3794808 and a haplotype block within exon2). These observations add support for possible interactions within the *HTR2A* gene. Between the *HTR2A* and *SLC6A4* genes the findings were less convincing which may indicate the absence of such an interaction or if it is present it is very weak.

I attempted but could not replicate the original Caspi-finding in the New Mood cohort. This may be due to several reasons. First of all the phenotype assessment methods were different in the studies. In the original study participants were assessed

at age 26 with the use of the Diagnostic Interview Schedule which yielded a quantitative measure of depressive symptoms and a categorical diagnosis of a major depressive episode according to Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria. Stressful life events occurring after the 21st birthday and before the 26th birthday were assessed with the aid of a life-history calendar, thought to be a highly reliable method for ascertaining life-event histories (Caspi *et al.* 2003). In my study I used a self-administering questionnaire (Brief Symptom Inventory, Derogatis 1993) to assess depression. However, mood and anxiety symptoms of 140 participants were also rated by independent trained investigators using the Montgomery-Asberg Depression Rating Scale (Montgomery and Asberg, 1979) and the Clinical Anxiety Scale (Snaith *et al.* 1982). The results showed highly significant (p=0.001) correlations between the self-reported symptom scores and the independent ratings (Pearson's correlation: depression R=0.79; anxiety R=0.80) in the New Mood Manchester population (Juhasz *et al*. 2010, Biological Psychiatry, in press). Stressful life events were also assessed differently in my study. I used the Recent Negative Life Events questionnaire, based on the validated List of Life Threatening Experiences (LTE) (Brugha *et al.* 1985, Rijsdijk *et al.* 2001). As this questionnaire asks for recall of negative life events of the last year it is prone to recall bias (Moffit *et al.* 2005). However, it was previously used to replicate a 5-HTTLPR x stressful life events interaction in our Hungarian sample (Lazary *et al*. 2008) and to demonstrate cannabinoid receptor 1 gene (CNR1) x stressful life events interaction in the Manchester population (Juhasz *et al*. 2009) indicating its usefulness in population genetic studies. The Caspi-population was 26 years old, when assessed whilst the New Mood cohort average age was 34, which also may have a role in the lack of association. Finally, it is possible that interaction with other genes masked the effect of the 5-HTTLPR polymorphism's effect in the New Mood population.

Study 3: Investigation of serotonin receptors *1D*, *1E*, *1F*, *2B* and *3-7* in depression and related traits: a mini review and a further attempt to replicate previous findings in an elderly community dwelling population

In this study I used GWAS data to investigate the role of other, less well known serotonergic receptors in depression, anxiety and neuroticism. I found that the *HTR4*

and *HTR6* genes may play a role in depression. However the associations were weak and did not survive Bonferroni-correction for multiple testing.

As a conclusion of my studies and based on the present literature I propose the following model for depression.

The serotonergic system has a key role in brain development. In the embryo serotonergic fibres infiltrate all cortical layers and retract profoundly during the maturation of the brain (Lesch and Gutknecht 2005). 5-HT modulates the outgrowth of neurites from other neurotransmitter systems, such as glutamatergic neurons. Animal studies have demonstrated that excessive concentrations of extracellular 5-HT in *Slc6a4* and *Mao-A* KO mice disturbs the development of the somatosensory cortex resulting in axons with a decreased number of branches (Lesch and Gutknecht 2005, Gaspar *et al*. 2003). Figure 4 shows the increase in synaptic content of 5-HT associated with the less active s allele by extension from dialysis studies in *Slc6a4* KO animals. The increase in synaptic 5-HT concentration may mediate the developmental abnormalities in cortical cytoarchitecture seen in KO animals (Gaspar *et al.* 2003, Riccio *et a*l. 2009). Analogous developmental changes may occur in humans who possess the s allele as suggested by previously reviewed brain imaging studies (Hariri and Holmes 2006). 5-HT influences on brain development could also arise from less active autoreceptors (Gross *et al.* 2002) and variation in other 5-HT receptors (for example 5-HT6, Riccio *et al.* 2009). Thus some of the phenotypic effects of genetic variation in 5-HT receptors may be mediated by early influences on the development of the limbic system and its top down cognitive control (Savitz and Drevets 2009b) mechanisms that determine emotional reactivity, self-perception, memory and other cognitive biases that may be relevant to affective disorder (Wellman *et al.* 2007).

It is interesting to note that there is a specific time window in development when the maturing brain is sensitive to serotonergic disturbances, which may be due to external manipulation of 5-HT function by using drugs or genetic modifications such as conditional KO and rescue lines in the case of animal models (Gross *et al.* 2002). Brain development in humans continues into adulthood and is modified by early experience including adverse influences such as physical abuse and neglect.

Increasing evidence suggests that 5-HT and environmental factors interact during brain development to produce long-term biases in cognitive-emotional processing that underpin vulnerability to depression.

5-HT functioning is subject to many homeostatic control mechanisms that compensate for genotypic effects such that in optimal rearing conditions, minor or no phenotypic changes may be manifest. One mechanism can be seen in the adaptive changes in 5-HT receptors that have been described in the synapse of Sert KO animals that mitigate the effect of raised 5-HT content; for example down-regulation of 5-HT2A, 5-HT2C and 5-HT3 receptors (Hariri and Holmes, 2006). Whether genetic variation in 5-HT receptors can affect their ability to adapt to altered synaptic conditions is an interesting question, which has fuelled an increasing number of ongoing research studies with no definitive answer as yet. For example, the long variant of the *SLC6A4* promoter polymorphism had a threefold higher basal luciferase activity compared to the short variant in human placental choriocarcinoma cells (Heils *et al*. 1996). Whilst this finding is generally accepted, it does not explain the mechanism of SSRIs which block the transporter molecule therefore their effects correspond to the s allele. In the *HTR1A* gene the effect of rs6296 alleles is more complex. This polymorphism disrupts a binding site for the NUDR/Hes5 transcription factors. The effect of the polymorphic allele was dependent on the transcription factor and on the cell type used (Lemonde et. al 2003).

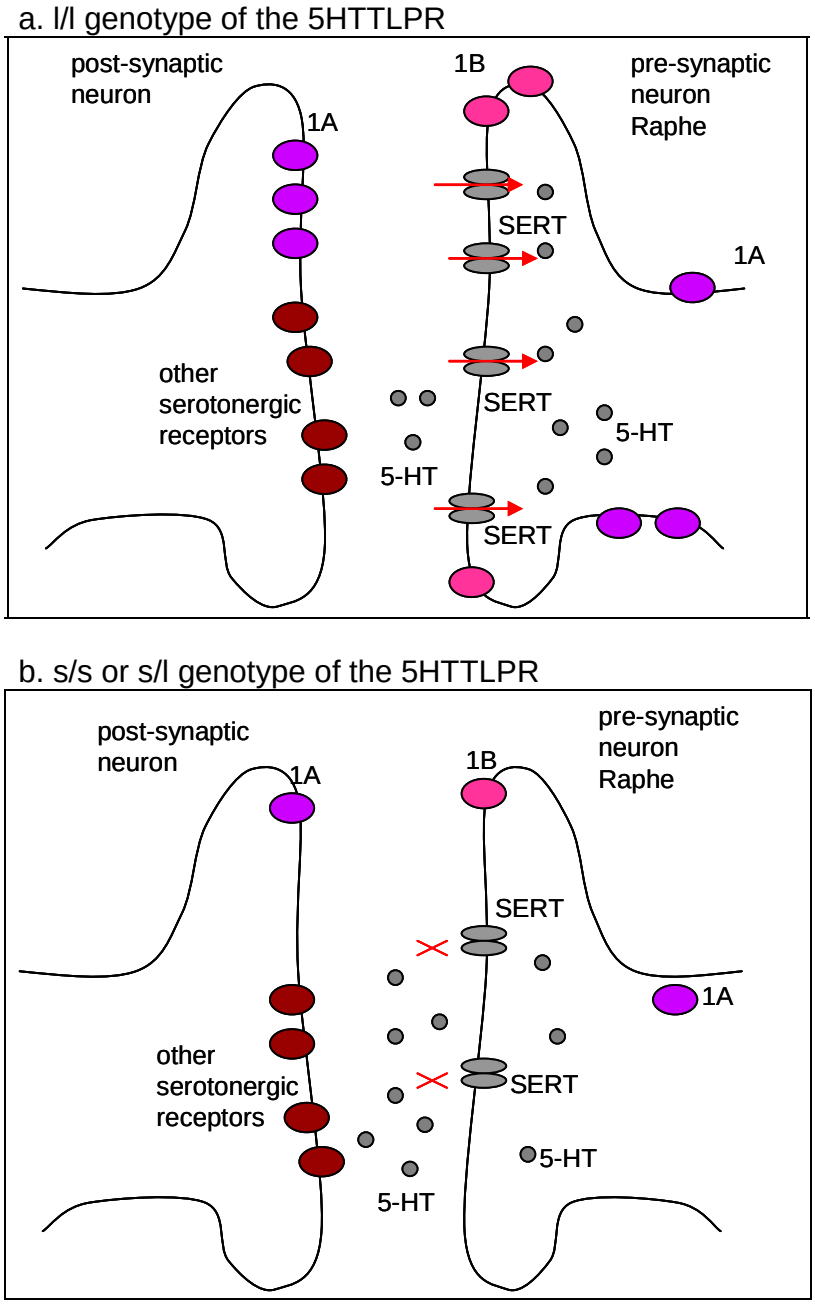


Figure 10. Function of the serotonin transporter molecules in the synaptic cleft according to the 5-HTTLPR genotype (Adapted from Hariri et al).

It is well documented that in adulthood 5-HT modifies functioning in neural systems that process emotional and stressful stimuli and control the behavioural, autonomic and endocrine response. The evidence is clear from acute 5-HT drug manipulation studies in both humans and animals on behaviour and imaging of

emotion processing. In humans for example, acute Tryptophan depletion and SSRI treatment can have opposite effects on the magnitude of amygdala fMRI response to viewing faces with fearful expressions. Because genetic variation in serotonergic genes has dual influences on 1. the development of neural systems mediating and controlling mood and emotion and 2. modulating the functioning of the same systems in adulthood, the separate contribution of these processes are difficult to disentangle. Healthy controls carrying the 5-HTTLPR s allele show increased reactivity in the emotion regulation pathway, especially in the amygdala, in fMRI studies (Hariri and Holmes 2006). Whether this is due to altered 5-HT in amygdala synapses or to developmental influences is not clear. Some evidence from connectivity and morphometric analysis suggest that the developmental effect is important (Pezawas *et al.* 2005). Similar uncertainties apply to the finding in this thesis that variation in the 5-HT1A receptor influenced face emotion processing in healthy volunteers. Multimodal pharmacological-imaging studies (PET/fMRI or pharmacological fMRI techniques which can map individual differences in behaviorally relevant brain circuit function) may be one way of unravelling the intermediate processes between genetic variation and phenotype.

The amygdala is a key structure in the organisation of behavioural and endocrine (HPA) stress responses. Serotonin controls the excitability of the amygdala (Cheng *et al.* 1998, Stutzmann *et al*. 1998, Stutzmann *et al.* 1999) and inhibits the stress responses via the 5-HT1A receptors, although 5-HT2 receptors may exert a facilitatory effect. Stress-induced activation of the HPA, increasing corticotrophin-releasing hormone (CRH), adrenocorticotroph hormone (ACTH) and peripheral cortisol secretion is partly mediated by increased 5-HT release evoked by stress (van Praag, 2004). It is likely that the magnitude and duration of the 5-HT and HPA response to stress reflects the number and sensitivity of 5-HT receptors controlling firing and release and transmitting postsynaptic signalling. However, there have been no studies of the influence of genetic variation in 5-HT genes on measures of HPA function. If stress is persistent, and inescapable, the HPA system escapes from negative feedback control and becomes chronically overactive. High levels of cortisol and CRH reduce 5-HT turnover, release and neurotransmission. As a consequence of the regulatory role of the 5-HT, every process which influences the turnover of this neurotransmitter, may lead to a dysfunctional stress response. The crucial question is

how the brain can use adaptive changes to downregulate the stress response, which is strongly influenced by our genetic make-up (Figure 10).

In addition to their role as 5-HT autoreceptors on 5-HT neurons, 5-HT1A, 5-HT1B and 5-HT1D autoreceptors also act as heteroreceptors mediating the influence of 5-HT on the release of other neurotransmitters, such as acetylcholine, GABA and glutamate (Filip and Bader 2009). Therefore any change in the expression of 5-HT1A, 5-HT1B and 5-HT1D receptors may causes changes in release of various neurotransmitters as well 5-HT itself. Many other serotonergic receptors act as heteroreceptors influencing the release of acetylcholine (5-HT3, 5-HT4, 5-HT6), GABA (5-HT2A, 5-HT2C, 5-HT3, 5-HT4, 5-HT5, 5-HT6, 5-HT7) glutamate (5-HT2A, 5-HT2C, 5-HT3, 5-HT4, 5-HT7) and dopamine (5-HT2A, 5-HT2C, 5-HT6). Variation in synaptic 5-HT content thus has a widespread influence on a number of other neurotransmitter systems. Finally, 5-HT release can be increased by the activation of the 5-HT5 and 5-HT6 receptors and they influence other neurotransmitters. The developmental and behavioural phenotype is a result of these complicated interactions. A dysfunctional or pathological phenotype only occurs if the net effect of variation in many homeostatic mechanisms at different levels of 5-HT functioning fails to compensate for the initial changes in serotonin function (Figure 11).

In summary, this model proposes:

- An increase in 5-HT release, resulting for example from a temporary environmental stressor, initiates a complicated balancing mechanism. If stress persists and/or the balancing mechanism is inadequate stress-related anxiety or depression may occur. This ‘primary’ depression or anxiety is related to the maladaptive processes in the serotonergic system (e.g. 5-HT1A, 5-HT1B and 5-HT1D autoreceptors and the transporter).
- 5-HT initiates other systems’ involvement in the psychiatric phenotype, such as the GABA, glutamate, dopamine and acetylcholine via the serotonergic heteroreceptors.
- This is a dynamic process, involving several systems, therefore possession of one polymorphism or even a few does not necessarily result in a pathological phenotype.

- The phenotype is a result of these complex interactions, which involve several neurotransmitter systems. Therefore the observed phenotype is a heterogenous set of symptoms.
- This complexity explains why one genetic variant may show only a minor effect on the depressive or anxious phenotype. This may account for the reason why GWAS studies have identified genes in basic common pathways such as the *GRM7* (metabotrobic glutamate receptor 7, Shyn *et al.* 2009), *CACNA1C* (alfa-1c subunit of the L-type voltage-gated calcium channel) (Green *et al.* 2009), and *PCLO* (protein that localises to the cytomatrix of the presynaptic active zone and has a role in monoaminergic neurotransmission in the brain) (Sullivan *et al.* 2009).

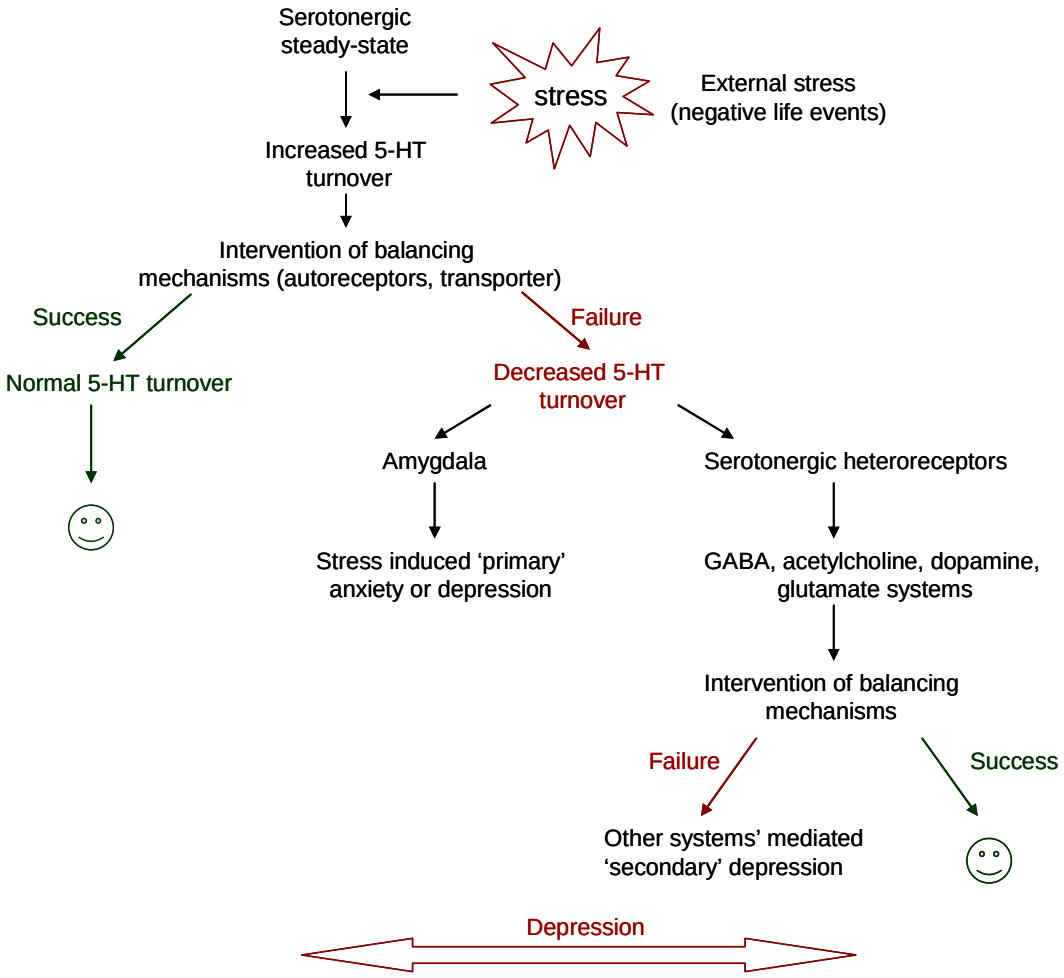


Figure 11. Schematic figure of the role of serotonergic system in the development of depression.

This model was stimulated by my results but it is set in the context of previous literature.

First, Study 1 showed that the 5-HT1A and 5-HT1B autoreceptors were positively associated with depression and anxiety when interacting with recent life events. This association was not attributable to the independent effect of life events themselves. Moreover, the rs6295 G allele which is known to cause increased transcriptional activity of the *HTR1A* gene (Lemonde *et al*. 2003) was associated with quicker amygdala response to emotionally threatening stimuli. An SNP in the *HTR1B* gene (rs6296) was also positively associated with depression and anxiety, which may be attributable to linkage disequilibrium to another transcriptionally overacting SNP allele nearby (rs13212041, Jensen *et al.* 2009).

Study 2 showed the importance of interaction of SNPs within a gene or between genes. One SNP's effect within the same gene may not lead to an altered phenotype if it is possible to compensate it with an opposite effect caused by other functional SNPs. Therefore, the pathological phenotype only occurs when two susceptibility alleles are present. This argument has some support from my study as this intragenic interaction was present in two populations, although not between exactly the same regions. Study 2 also showed that this compensation is possible between genes, as well. In my model of depression, this is a crucial argument.

On the other hand, failing to replicate the original Caspi-finding may indirectly indicate that the transporter's activity is only one component in the pathophysiology of depression and anxiety. The complex interplay between the different levels of the serotonergic pathway, such as the transporter, the autoreceptors and heteroreceptors, can balance the effect of life stresses in s allele carriers resulting in a normal phenotype.

Finally, Study 3 demonstrated other serotonergic heteroreceptors' involvement in the depressive phenotype. The weak association between *HTR3*, *HTR4* and *HTR6* and depression and associated phenotypes suggests that either the contribution to the phenotype of the individual SNPs is small or that we were observing false positive associations.

There are several strengths of my study. One of them is the use of two large independent cohorts. For example I had 96% power in the New Mood Manchester

population (N=1387) and 97% in the Dyne Steel cohort (N=1563) to detect a polymorphism (assuming a minor allele frequency of 25%) with an effect size of 1% at the 5% two-tailed significance level. To detect a gene x environment interaction with an effect size of 3%, in the same model as above, I had 97% power in the New Mood cohort (Study1). Secondly, I used continuous traits rather than diagnostic dichotomy to assess the phenotype in both cohorts. In the Dyne Steel cohort I applied three different depression measures. Finally, I used the gene-based approach with haplotype tagging SNPs instead of reporting on one or two SNPs.

On the other hand, as a 'typical' population based study, my study suffered from the 'typical' issues of other genetic association studies.

First, as life events played a crucial part in the association between the HT1A and HT1B autoreceptors and depression and anxiety, it would have been advantageous to perform analyses such as epistatic analyses between *HTR1*A, *HTR1B* and *SLC6A4* including the life events into the model, or the same with *HTR2A* and *SLC6A4*. Unfortunately, the New Mood population's size is not large enough to do that. On the other hand, life events data were only available in a small subset of the Dyne Steel cohort; therefore I could not attempt to replicate the *HTR1A*/*HTR1B* x environment interaction in that independent population. Because of the lack of replication this finding remains a possible false positive finding, especially given that a recent study failed to find the same association (Chipman *et al.* 2010).

Secondly, between-study heterogeneity was another issue. The New Mood and Dyne Steel cohorts were originated from two different study designs, and neither the phenotypes nor the majority of genotyped SNPs were the same, which made direct replication possibly only via the LD calculation method. As this method is based on probabilities the result should be considered with caution. In the case of the 5-HT3, 5-HT4, 5-HT5, 5-HT6 and 5-HT7 receptors, their genes were not investigated in the New Mood cohort, which makes the weak association findings in the Dyne Steel cohort questionable.

The failure to replicate the association between life events and the 5-HTTLPR genotype in depression is inconsistent with the majority of the literature. There could be several methodological differences that result in this non-replication, as I mentioned above. The two main differences were: 1. to assess depression self-reported questionnaire was used and 2. life events were measured retrospectively. It

may also have some importance that the New Mood population was older than the original Caspi study because of the different recruitment method. However, it is also possible that other gene x gene interactions masked or balanced the effect of 5HTTLPR x life events as my model explained above.

Thirdly, the associations I found in my study were weak ones. This however is typical of many other findings in depression, anxiety and neuroticism, but also may indicate false positives.

Finally, to build a more complete picture, other genes should have been investigated in the study, such as *TPH2* (tryptophan hydroxylase) and *MAO*-A (monoamine oxidase A).

Future directions

To understand how depression and anxiety develops, the hypothesis-testing approach will need to involve a wide range of neurotransmitter-related and neuronal development genes and analyse for possible interactions between them. It may be also important to narrow or divide the phenotypic categories (such as depression) into subgroups, with homogenous symptoms within. Multi-modal imaging studies show promise in establishing the CNS processes that intervene between genetic variation and behavioural phenotype. Also, carefully measured environmental factors have to be included into the models, taking into account their context and time course. Recently epigenetics is receiving more and more attention in the literature with a good reason. Studies have found that adult children of Holocaust survivors exhibit an increased incidence of mood disorders and multiple alterations in the HPA axis function (Neigh *et al.* 2009). One possible mechanism for this is that serotonin is an important molecule during embryonic development at a very early stage when serotonin is only produced by the maternal body, thus the genetic make-up of the mother and not the offspring could have major effects for the developing brain. However, there may be many other, more complicated mechanisms yet to be discovered, acting during development, that have a long term influence on gene expression.

8. Conclusions

In conclusion, my study provides evidence for the involvement of the serotonergic system in depression and related phenotypes, such as trait anxiety and neuroticism. I showed that recent life events take part in the development of depression, and that these are modulated via the 5-HT1A and 5-HT1B autoreceptors, but not via the SERT in the New Mood cohort. On the other hand, significant interaction emerged between the *SLC6A4* and *HTR2A* genes in depression, anxiety and neuroticism in the New Mood cohort. Interactions were also observed in an independent cohort (Dyne Steel) although not always between identical SNPs and analysis of the whole data set did not survive correction for multiple testing. Finally, my study showed the possible importance of other less investigated serotonergic receptors (5-HT4 and 5-HT6) in these phenotypes. My results shed light on the very complex interactions between the environment and the serotonergic system, as well as within and between the gene members of this neurotransmitter system.

9. References

Adamec R, Burton P, Blundell J, Murphy DL, Holmes A (2006). Vulnerability to mild predator stress in serotonin transporter knockout mice. Behavioural Brain Research, 170:126-140.

Akimova E, Lazenberger R, Kasper S (2009). The serotonin-1A receptor in anxiety disorders Biological Psychiatry, 66: 627-635.

Alexandre C, Popa D, Fabre V, Bouali S, Venault P, Lesch KP, Hamon M, Adrien J (2006). Early life blockade of 5-hydroxytryptamine 1A receptors normalizes sleep and depression-like behavior in adult knock-out mice lacking the serotonin transporter. The Journal of Neuroscience, 26:5554-5564.

Anderson WJ and Felten DL (1982). A modified Golgi-Cox technique for morphological characterization of serotonergic neurons. Journal of Histochemistry and Cytochemistry, 30:750-5.

American Psychiatric Association (1994). Diagnostic and Statistical Manual-IV. American Psychiatric Press, Inc. Washington, D.C., and London, England.

Amin N, Aulchenko YS, Dekker MC, Ferdinand RF, van Spreeken A, Temmink AH, Verhulst FC, Oostra BA, van Duijn CM (2009). Suggestive linkage of ADHD to chromosome 18q22 in a young genetically isolated Dutch population. European Journal of Human Genetics, 17(7):958-66.

Anderson IM, Ferrier IN, Baldwin RC, Howard L, Lewis G, Matthews K, McAllister-Williams RH, Peveler RC, Scott J, Tylee A (2008). Evidence-based guidelines for treating depressive disorders with antidepressants: A revision of the 2008 British Association for Psychopharmacology guidelines. Journal of Psychopharmacology, 22:343-396.

Jaffee SR, Moffitt TE, Caspi A, Fombonne E, Poulton R, Martin J (2002). Differences in early childhood risk factors for juvenile-onset and adult-onset depression. Archives of General Psychiatry, 59:215-22.

Anguelova M, Benkelfat C., Turecki G (2003a). A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter: I. Affective disorders. Molecular Psychiatry, 8:574-591.

Anguelova M, Benkelfat C., Turecki G (2003b). A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter: II. Suicidal behavior. Molecular Psychiatry, 8:646-653.

Ansorge MS, Zhou M, Lira A, Hen R, Gingrich JA (2004). Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. Science, 306:879-881.

Arias B, Collier DA, Gasto C, Pintor L, Gutierrez B, Valles V, , Fañanás L (2001). Genetic variation in the 5-HT5A receptor gene in patients with bipolar disorder and major depression. Neuroscience Letters, 3030:111-114.

Arnow BA (2004). Relationships between childhood maltreatment, adult health and psychiatric outcomes, and medical utilization. The Journal of Clinical Psychiatry, 65 Suppl 12:10-15.

Ashcroft GW, Crawford TBB, Eccleston D, Sharman DF, McDougall J, Stanton JS, Binns JK (1966). 5-hydroxyindole compounds in the cerebrospinal fluid patients with psychiatric or neurological disease Lancet, 2:1049-1052.

Ashtari M, Greenwald BS, Kramer-Ginsberg E, Hu J, Wu H, Patel M., Aupperle P, Pollack S (1999). Hippocampal/amygdala volumes in geriatric depression Psychological Medicine, 29:629-638.

Atmaca M, Yildirim BH, Ozdemir BH, Aydin BA, Tezcan AE, Ozler AS (2006). Volumetric MRI assessment of brain regions in patients with refractory obsessive-compulsive disorder. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 30 (6):1051-1057.

Atmaca M, Yildirim H, Ozdemir H, Tezcan E, Poyraz AK (2007). Volumetric MRI study of key brain regions implicated in obsessive-compulsive disorder. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 31 (1):46-52.

Azmitia EC and Gannon PJ (1986). The primate serotonergic system: a review of human and animal studies and a report on Macaca fascicularis. Advances in Neurology, 43:407-68.

Axelson DA, Doraiswamy PM, McDonald WM, Boyko OB, Tupler LA, Patterson LJ, Nemeroff CB, Ellinwood EH Jr, Krishnan KR (1993). Hypercortisolemia and hippocampal changes in depression Psychiatry Research, 47:163-173.

Ballmaier M, Toga AW, Blanton RE, Sowell ER, Lavretsky H, Peterson J, Pham D, Kumar A (2004). Anterior cingulate, gyrus rectus and orbitofrontal abnormalities in elderly depressed patients: an MRI-based parcellation of the prefrontal cortex. American Journal of Psychiatry, 161:99-108.

Barnes NM, Sharp T (1999). A review of central 5-HT receptors and their function. Neuropharmacology, 38:1083-1152.

Barr CS, Newman TK, Becker ML, Parker CC, Champoux M, Lesch K-P, Goldman D, Suomi SJ, Higley JD (2003). The utility of the non-human primate model for studying gene by environment interactions in behavioral research. Genes Brain and Behavior, 2:336-340.

Barrett JC, Fry B, Maller J, Daly MJ (2005). Haploview: analysis and

visualization of LD and haplotype maps. Bioinformatics, 21:263-265.

Baxter Jr LR, Phelps ME, Mazziotta JC, Guze BH, Schwartz JM, Selin CE (1987). Local cerebral glucose metabolic rates in obsessive-compulsive disorder. A comparison with rates in unipolar depression and in normal controls. Archives of General Psychiatry, 44:211-218.

Baxter Jr LR, Phelps ME, Mazziotta JC, Schwartz JM, Gerner RH, Selin CE, Sumida RM (1985). Cerebral metabolic rates for glucose in mood disorders. Studies with positron emission tomography and fluorordeoxyglucose F 18. Archives of General Psychiatry, 42:441-447.

Bear MF, Connors BW, Paradiso MA (2007). Neuroscience. Exploring the brain Lippincott Williams and Wilkins, USAThird ed. 2007

Beck AT, Ward CH, Mendelsohn M, Mock J, Erbaugh J (1961). An inventory for measuring depression. Archives of General Psychiatry, 4:561-571.

Bell-McGinty S, Butters MA, Meltzer CC, Greer PJ, Reynolds III CF, Becker JT (2002). Brain morphometric abnormalities in geriatric depression: long-term neurobiological effects of illness duration American Journal of Psychiatry, 159:1424-1427.

Bench CJ, Frackoviak RS, Dolan RJ (1995). Changes in regional cerebral blood flow on recovery from depression. Psychological Medicine, 25:247-261.

Bengel D, Murphy DL, Andrews AM, Wichems CH, Feltner D, Heils A,Mossner R, Westphal H, Lesch KP (1998). Altered brain serotonin homeostasis and locomotor insensitivity to 3, 4 methylenedioxymethamphetamine (‘‘Ecstasy’’) in serotonin transporter-deficient mice. Molecular Pharmacology, 53: 649-655.

Berger M, Gray JA, Roth BL (2009). The expanded biology of serotonin. Annual

Review of Medicine, 60:355-66.

Bernstein DP, Fink L, Handelsman L, Foote J, Lovejoy M, Wenzel K, Sapareto E, Ruggiero J (1994). Initial reliability and validity of a new retrospective measure of child abuse and neglect. American Journal of Psychiatry, 1132-1136.

Beskow J, Gottfries CG, Roos BE, Winblad B (1976). Determination of monoamine and monoamine metabolites in the human brain: post mortem studies in a group of suicides and in a control group Acta Psychiatrica Scandinavica, 53:7-20.

Bhagwagar Z, Hinz R, Taylor M, Fancy S, Cowen P, Grasby P (2006). Increased 5-HT(2A) receptor binding in euthymic, medication-free patients recovered from depression: a positron emission study with [(11)C]MDL. American Journal of Psychiatry, 163: 1580-1587.

Bhagwagar Z, Murthy N, Selvaraj S, Hinz R, Taylor M, Fancy S, Grasby P, Cowen P (2007). 5-HTT binding in recovered depressed patients and healthy volunteers: a positron emission tomography study with [11C]DASB. American Journal of Psychiatry, 164: 1858-1865.

Bhagwagar Z, Rabiner EA, Sargent PA, Grasby PM, Cowen PJ (2004). Persistent reduction in brain serotonin 1A receptor binding in recovered depressed men measured by positron emission tomography with [11C]Way-100635. Molecular Psychiatry, 9:386-392.

Bhatnagar S, Sun LM, Raber J, Maren S, Julius D, Dallmann MF (2004). Changes in anxiety-related behaviors and hypothalamic-pituitary-adrenal activity in mice lacking the 5-Ht3a receptor. Psychology and Behavior, 81:545-555.

Birkett JT, Arranz MJ, Munro J, Osbourn S, Kerwin RE, Collier DA (2000). Association analysis of the 5-HT5A gene in depression, psychosis and antipsychotic response. Neuroreport, 11(9):2017-2020.

Biver F, Goldman S, Delvenne V, Luxen A, De Maertalaer V, Hubain P, Mendlewicz J, Lotstra F (1994). Frontal and parietal metabolic disturbances in unipolar depression. Biological Psychiatry, 36:381-388.

Biver F, Lotstra F, Monclus M, Wikler D, Damhut P, Mendlewitz J, Goldman S (1996). Sex difference in 5HT(2) receptor in the living human brain. Neuroscience Letters, 20:25-28.

Blackshear MA, Sanders-Bush E (1982). Serotonin receptor sensitivity after acute and chronic treatment with mianserin The Journal of Pharmacology and Experimental Therapeutics, 22:303-308.

Blakely RD, De Felice LJ, Hartzell HC (1994). Molecular physiology of norepinephrine and serotonin transporters. The Journal of Experimental Biology, 196:263-81.

Blazer DG, Kessler RC, McGonagle KA, Swartz MS (1994). The Prevalence and Distribution of Major Depresson in a National Community Sample: The National Comorbid Survey American Journal of Psychiatry, 151:979-986.

Blondel O, Vandecasteele G, Gastineau M, Leclerc S, Dahmoune Y, Langlois M, Fischmeister R (1997). Molecular and functional characterization of 5-HT4 receptor cloned from human atrium. FEBS Letters, 412:465-474.

Boes AD, McCormick LM, Coryell WH, Nopoulos P (2008). Rostral anterior cingulate cortex volume correlates with depressed mood in normal healthy children. Biological Psychiatry, 63(4):391-397.

Boileau I. Warsh JJ, Guttman M, Saint-Cyr JA, McCluskey T, Rusjan P, Houle S, Wilson AA, Meyer JH, Kish SJ (2008). Elevated serotonin transporter binding in depressed patients with Parkinson's disease: a preliminary PETstudy with [11C]DASB. Movement Disorders, 23: 1776-1780.

Bonasera SJ, Chu HM, Brennan TJ, Tecott LH (2006). A null mutation of the serotonin 6 receptor alters acute responses to ethanol. Neuropsychopharmacology, 31:1801-1813.

Bosker FJ, Hartman CA, Nolte IM, Prins BP, Terpstra P, Posthuma P, van Veen T, Willemsen G, DeRijk RH, de Geus EJ, Hoogendijk WJ, Sullivan PF, Penninx BW, Boomsma DI, Sneider H, Nolen WA (2010). Poor replication of candidate genes for major depressive disorder using genome-wide association data. Molecular Psychiatry, Mar 30. E-pub, ahead of print.

Boteron KN, Raichle ME, Drevets WC, Heath AC, Todd RD (2002). Volumetric reduction in left subgenual prefrontal cortex in early-onset depression. Biological Psychiatry, 51:342-344.

Bouali S, Evrard A, Chastanet M, Lesch KP, Hamon M, Adrien J (2003). Sex hormone-dependent desensitization of 5-HT1A autoreceptors in knockout mice deficient in the 5-HT transporter. The European Journal of Neuroscience, 18:2203-2212.

Bourne HR, Bunney WE, Colburn RW, Davis JM, Davis JN, Shaw DM, Coppen A (1968). Noradrenaline, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in hindbrains of suicidal patients Lancet, 2:805-808.

Bradley CC, Blakely RD (1997). Alternative splicing of the human serotonin transporter gene. Journal of Neurochemistry, 69:1356-1367.

Bray NJ, Buckland PR, Hall H, Owen MJ, O'Donovan MC (2004). The serotonin-

2A receptor gene locus does not contain common polymorphsm affecting mRNA levels in adult brain. Molecular Psychiatry, 9:109-114.

Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL, Charney DS (2000). Hippocampal volume reduction in major depression American Journal of Psychiatry, 157:115-118.

Bremner JD, Vythilingam M, Vermetten E, Nazeer A, Adil J, Khan S, Staib LH, Charney DS (2002). Reduced volume of orbitofrontal cortex in major depression. Biological Psychiatry, 51:273-279.

Bremner JD, Vythilingam M, Vermetten E, Southwick SM, McGlashan T, Nazeer A, Khan S, Vaccarino LV, Soufer R, Garg PK, Ng CK, Staib LH,Duncan JS, Charney DS (2003). MRI and PET study of deficits in hippocampal structure and function in women with childhood sexual abuse and posttraumatic stress disorder. American Journal of Psychiatry, 160 (5): 924-932.

Briley MS, Langer SZ, Raisman R, Sechter D, Zafirian E (1980). 3H-imipramine binding sites are decreased in platelets of unreated depressed patients. Science, 209:303-305.

Brodman K, Erdmann AJ Jr., Wolff HG (1956). Cornell Medical Index Health Questionnaire (Manual). Cornell University Medical College, New York, NY, USA

Brody AL, Saxena S, Silverman DH, Alborzian S, Fairbanks LA, Phelps ME, Huang SC, Wu HM, Maidment K, Baxter LR Jr. (1999). Brain metabolic changes in patients with major depressive disorder from pre- to post-treatment with paroxetine. Psychiatry Research, 91:273-279.

Brody AL, Saxena S, Stoessel P, Gillies LA, Fairbanks LA, Alborzian S, Phelps ME, Huang SC, Wu HM, Ho ML, Ho MK, Au SC, Maidment K, Baxter LR Jr.

(2001). Regional brain metabolic changes in patients with major depression treated with either paroxetine or interpersonal therapy: preliminary findings. Archives of General Psychiatry, 58:631-640.

Brown GL, Linnoila MI (1990). CSF serotonin metabolite (5-HIAA) studies in depression, impulsivity, and violence.Journal of Clinical Psychiatry; 51 Suppl:31-41; discussion 42-3. Review.

Bruess M, Konstanian A, Boenisch H, Goethert M (2005). The naturally occuring Arg219Leu variant of the human 5-HT1A receptor: impairment of signal transduction. Pharmacogenetics and Genomics, 15:257-264.

Brugha T, Bebbington P, Tennant C, Hurry J (1985). The List of Threatening Experiences: a subset of 12 life event categories with considerable long-term contextual threat Psychological Medicine, 15:189-194.

Bundo M, Iwamoto K, Yamada K, Yoshikawa T, Kato T (2010). Mutation sreening and assessment of the effect of genetic variations on expression and RNA editing of serotonin receptor 2C in the human brain. Psychiatry and Clinical Neurosciences, 64:57-61.

Burns CM, Chu H, Rueter SM, Hutchinson LK, Canton H, Sanders-Bush E, Emeson RB (1997). Regulation of levels of serotonin-2C receptor G-protein coupling by RNA editing. Nature, 387:303-308.

Caetano SC, Hatch JP, Brambilla P, Sassi RB, Nicoletti M,Mallinger AG, Frank E, Kupfer DJ, Keshavan MS, Soares JC (2004). Anatomical MRI study of hippocampus and amygdala in patients with current and remitted major depression Psychiatry Research, 132:141-147.

Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ (1998). Maternal care during infancy regulates the development of neural systems

mediating the expression of fearfulness in the rat. Proceedings of the National Academy of Sciences USA, 95:5335-5340.

Canli T (2004). Functional barin mapping of extraversion and neuroticism: learning from individual differences in emotion processing. Journal of Personality, 72 (6):1105-1132.

Cannon DM, Ichise M, Rollis D, Klaver JM, Gandhi SK, Charney DS, Manji HK, Drevets WC (2007). Elevated serotonin transporter binding in major depressive disorder assessed using positron emission tomography and [11C]DASB; comparison with bipolar disorder. Biological Psychiatry, 62: 870-877.

Carmona S, Bassas N, Rovira M, Gispert JD, Soliva JC, Prado M, Tomas J, Bulbena A, Vilarroya O (2007). Pediatric OCD structural brain deficits in conflict monitoring circuits: a voxel-based morphometry study. Neuroscience Letters, 421 (3):218-223.

Carola V, Frazzetto G, Pascucci T, Audero E, Puglisi-Allegra S, Cabib S, Lesch KP, Gross C (2008). Identifying molecular substrates in a mouse model of the serotonin transporter environment risk factor for anxiety and depression. Biological Psychiatry, 63:840-846.

Caspi A, Hariri AR, Holmes A, Uher R, Moffitt TE (2010). Genetic sensivity to the environment: the case of the serotonin transporter gene and its implications for studiing complex diseases and traits. American Journal of Psychiatry, 167:509-527.

Caspi A, Moffitt TE, Newman DL, Silva PA (1996). Behavioral observation at age 3 years predict adult psychiatric disorders. Archives of General Psychiatry, 53:1033-1039.

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington HL, McClay J, Mill J, Martin J, Braithwaite A, Poulton R (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science, 301:386-389.

Chapman DP, Whitfield CL, Felitti VJ, Dube SR, Edwards VJ, Anda RF (2004). Adverse childhood experiences and the risk of depressive disorders in adulthood. Journal of Affective Disorders, 82:217-225.

Chen CH, Ridler K, Suckling J, Williams S, Fu CH, Merlo-Pich E, Bullmore E (2007). Brain imaging correlates of depressive symptom severity and predictors of symptom improvement after antidepressant treatment. Biological Psychiatry, 62(5):407-14.

Chen CH, Suckling J, Ooi C, Fu CH, Williams SC, Walsh ND, Mitterschiffthaler MT, Pich EM, Bullmore E (2008). Functional coupling of the amygdala in depressed patients treated with antidepressant medication Neuropharmacology, 33(8):1909-18.

Cheng LL, Wang SJ, Gean PW (1998). Serotonin depresses excitatory synaptic transmission and depolarization-evoked Ca2+ influx in rat basolateral amygdala via 5-HT1A receptors. The European Journal of Neuroscience, 10:2163-2172.

Chipman P, Jorm AF, Tan X-Y, Easteal S (2010). No association between the serotonin-1A receptor gene single nucleotide polymorphism rs6295C/G and symptoms of anxiety or depression, and no interaction between the polymorphism and environmental stressors of childhood anxiety or recent stressful life events on anxiety or depression. Psychiatric Genetics, 20:8-13.

Choi MJ, Kang RH, Ham BJ, Jeong HY, Lee MS (2005). Serotonin receptor 2A gene polymorphism (-1438A/G) (rs6311) and short-term treatment response to citalopram. Neuropsychobiology, 52:155-162.

Choi JS, Kang DH, Kim JJ, Ha TH, Lee JM, Youn T, Kim IY, Kim SI, Kwon JS (2004). Left anterior subregion of orbitofrontal cortex volume reduction and impaired organizational strategies in obsessive-compulsive disorder. Journal of Psychiatric Research, 38 (2):193-199.

Clark CP, Brown GG, Frank L, Thomas L, Sutherland AN, Gillin JC (2006). Improved anatomic delineation of the antidepressant response to partial sleep deprivation in medial frontal cortex using perfusion-weightened functional MRI. Psychiatry Research, 146:213-222.

Clark MS, Sexton TJ, McClain M, Root D, Kohen R, Neumaier JF (2002). Overexpression of 5-HT1B receptor in dorsal raphe nucleus using Herpes Simplex Virus gene transfer increases anxiety behavior after inescapable stress. The Journal of Neuroscience, 22:4550-4562.

Cloninger CR, Przybeck TR, Svrakic DM (1991). The tridimensional personality questionnaire: US normative data. Psychological Reports, 69:1047–1057.

Cloninger CR, Przybeck TR, Svrakic DM, Wetzel RD (1994). The temperament and character inventory (TCI): a guide to its development and use. Washington University, Center for Psychobiology of Personality, USA, St Louis,MO, USA

Cochran E, Robins E, Grote S (1976). Regional serotonin levels in brain: a comparision of depressive suicides and alcoholic suicides with controls. Biological Psychiatry, 3:283-294.

Cohen RM, Semple WE, Gross M, Nordahl TE, King AC, Pickar D, Post RM (1989). Evidence for common alterations in cerebral glucose metabolism in major affective disorders and schizophrenia. Neuropsychopharmacology, 2:241-254.

Comings DE, MacMurray JP (2000). Molecular heterosis: a review. Molecular Genetics and Metabolism, 71:19-31.

Compan V, Zhou M, Grailhe R, Gazzara RA, Martin R, Gingrich J, Dumuis A, Brunner D, Bockaert J, Hen R (2004). Attenuated response to stress and novelty and hypersenzitivity to seizures in 5-HT4 receptor knock out mice. The Journal of Neuroscience, 24:412-419.

Coplan JD, Smith EL, Trost RC, Scharf BA, Altemus M, Bjornson L, Owens MJ, Gorman JM, Nemeroff CB, Rosenblum LA (2000). Growth hormone response to clonidine in adversely reared young adult primates: relationship to serial cerebrospinal fluid corticotropin-releasing factor concentrations. Psychiatry Research, 95:93-102.

Coppen A, Wood K (1978). Platelet 5-Hydroxtryptamine accumulation in depressive illness Clinical Chimica Acta, 87:165-168.

Costa PT Jr, McCrae RR (1992). NEO-PI-R professional manual. Psychological Assessment Resources, Inc. Odessa, FL, USA

Crabbe JC, Phillips TJ, Feller DJ, Hen R, Wenger CD, Lessov CN, Schafer GL (1996). Elevated alcohol consumption in null mutant mice lacking 5-HT1B serotonin receptors. Nature Genetics, 14:98–101.

Craddock N, O'Donovan MC, Owen MJ (2008). Genome-wide association studies in psychiatry: lessons from early studies of non-psychiatric and psychiatric phenotypes. Molecular Psychiatry, 13:649-653.

Curzon G (1982). Transmittion amines in depression Psychological Medicine, 12:465-470.

Czesak M, Lemonde S, Peterson EA, Rogaeva A, Albert PR (2006). Cell-

specific repressor or enhancer activities of Deaf-1 at a serotonin 1A receptor gene polymorphism. The Journal of Neuroscience, 26:1864-1871.

Dahlstrom A, Fuxe K (1964). Localization of monoamines in the lower brain stem. Experientia, 20(7):398-9.

Damsa C, Kosel M, Moussaly J (2008). Current status of brain imaging in anxiety disorders. Current Opinion in Psychiatry, 22:96-110.

Davidson RJ (2002). Anxiety and affective style: role of prefrontal cortex and amygdala. Biological Psychiatry, 51:68-80.

Davidson RJ, Irwin W, Anderle MJ, Kalin NH (2003). The neural substrates of affective processing in depressed patients treated with venlafaxine. American Journal of Psychiatry, 160:64-75.

Delorme R, Betancur C, Wagner M, Krebs MO, Gorwood P, Pearl P, Nygren G, Durand CM, Buhtz F, Pickering P, Melke J, Ruhrmann S, Anckarsater H, Chabane N, Kipman A, Reck C, Millet B, Roy I, Mouren-Simeoni MC, Maier W, Rastam M, Gillberg C, Leboyer M, Bourgeron T (2005). Support for the association between the rare functional variant I425V of the serotonin transporter gene and obsessive compulsive disorder. Molecular Psychiatry, 10:1059-1061.

Derogatis L (1993). BSI: Brief Symptom Inventory: Administration, Scoring, and Procedures Manual. National Computer Systems Pearson Inc., Minneapolis, USA

Drago A, Alboni S, Brunello N, De Ronchi D, Serretti A (2010). HTR1B as a risk profile marker in psychiatric disorders: a review through motivation and memory. European Journal of Clinical Pharmacology, 66(1):5-27.

Drago A, De Ronchi D, Serretti A (2008). 5-HT1A gene variants and psychiatric disorders: a review of current literature and selection of SNPs for future studies. The International Journal of Neuropsychopharmacology, 11:701-721.
Drago A, Serretti A (2008). Focus on HTR2C: a possible suggestion for genetic studies of complex disorders. American Journal of Medical Genetics, Part B, Neuropsychiatric Genetics, 150B:601-637.

Drevets WC, Bogers W, Raichle ME (2002). Functional anatomical correlates of antidepressant drug treatment assessed using PET measures of regional glucose metabolism. European Neuropsychopharmacology, 12:527-544.

Drevets WC, Price JL, Simpson Jr JR, Todd RD, Reich T, Vannier M, Raichle ME (1997). Subgenual prefrontal cortex abnormalities in mood disorders. Nature, 386:824-827.

Drevets WC, Thase ME, Moses-Kolko EL, Price J, Frank E, Kupfer DJ, Mathis C (2007). Serotonin-1A receptor imaging in recurrent depression: replication and literature review. Nuclear Medicine and Biology, 34:865-877.

Drevets WC, Videen TO, Price JL, Preskorn SH, Carmichael ST, Raichle ME (1992). A functional anatomical study of unipolar depression. The Journal of Neuroscience, 12:3628-3641.

D'Souza UM, Craig IW (2006). Functional Polymorphsms in dopamine and serotonin pathway genes. Human Mutation, 27:1-13.

Du Y, Davisson MT, Kafadar K, Gardiner K (2006). A-to-I pre-mRNA editing of the serotonin-2C receptor: comparisions among inbred mouse strains. Gene, 382:39-46.

Duan J, Sanders AR, Vander Molen JR, Martinolich L, Mowry BJ, Levinson DF, Crowe RR, Silverman JM, Gejman PV (2003). Polymorphisms in the 5'-

untranslated region of the human serotonin receptor 1B (HTR1B) gene affect gene expression. Molecular Psychiatry, 8:901-910.

Duggan C, Sham P, Lee A, Minne C, Murray R (1995). Neuroticism: a vulnerability marker for depression evidence from a family study. Journal of Affective Disorders, 35:139-143.

Dunn RT, Kimbrell TA, Ketter TA, Frye MA, Willis MV, Luckenbaugh DA, Post RM (2002). Principal components of the Beck depression inventory and regional cerebral metabolism in unipolar and bipolar depression. Biological Psychiatry, 51:387-399.

Enoch MA, Greenberg BD, Murphy DL, Goldman D (2001). Sexually dimorphic relationship of a 5-HT2A promoter polymorphsm with obsessive-compulsive disorder. Biological Psychiatry, 49:385-388.

Enoch MA, Kaye WH, Rotondo A, Greenberg BD, Murphy DL, Goldman D (1998). 5-HT2A promoter polymorphism -1438G/A, anorexia nervos and obsessive-compulsive disorder. Lancet, 351:1785-1786.

Etkin A, Wagner TD (2007). Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social enxiety disorder and specific phobia. American Journal of Psychiatry, 164(10):1476-1488.

Eysenck HJ, Eysenck SBG (1964). The Eysenck personality questionnaire. University of London Press, UK, London, UK

Fabre V, Beaufour C, Evrard A, Rioux A, Hanoun N, Lesch KP, Murphy DL, Lanfumey L, Hamon M, Martres MP (2000). Altered expression and functions of serotonin 5-HT1A and 5-HT1B receptors in knock-out mice lacking the 5-HT transporter. The European Journal of Neuroscience, 12:2299-2310.

Fales CL, Barch DM, Rundle MM, Mintum MA, Mathews J, Snyder AZ, Sheline YI (2009). Antidepressant treatment normalizes hypoactivity in dorsolateral prefrontal cortex during emotional interference processing in major depression. Journal of Affective Disorders, 112(1-3):206-11.

Fatemi SH, Earle JA, Stary JM, Sedgewick J (2001). Altered levels of the synaptosomal associated protein SNAP-25 in hippocampus of subjects with mood disorders and schizophrenia Neuroreport, 12(15):3257-3262.

Fava M, Kendler KS (2000). Major depressive disorder Neuron, Vol. 28. 335-341.

Fehr C, Grintschuk N, Szegedi A, Anghelescu I, Klawe C, Singer P, Hiemke C, Dahmen N (2000). The HTR1B 861G>C receptor polymorphism among patients suffering from alcoholism, major depression, anxiety disorders and narcolepsy. Psychiatry Research, 97:1-10.

Filip M, Bader M (2009). Overview on 5-HT receptors and their role in psychology and pathology of the central nervous system. Pharmacological Reports, 61:761-777.

Fisher RA (1918). The correlation between relatives on the supposition of Mendelian inheritance. Transactions of Royal Society, Edinborough, 52:399-433.

Fitzgerald LW, Iyer G, Conklin D, Ktause CM, Marshall A, Patterson JP, Tran DP, Jonak GJ, Hartig PR (1999). Messenger RNA editing of the humen serotonin 5-HT2C receptor. Neuropsychopharmacology, 21:82S-90S.

Flint J, Munafo MR (2007). The endophenotypes concept in psychiatric genetics. Psychological Medicine, 37:163-180.

Flugge G, Kramer M, Rensing S, Fuchs E (1998). 5-HT1A receptors and behavior under chronic stress: selective counteraction by testosterone. The European Journal of Neuroscience, 10:2685-2693.

Fostick L, Silberman A, Beckman M, Spivak B, Amital D (2010). The economic impact of depression: resistance or severity? European Neuropsychopharmacology, 20(10):671-675.

Fox MA, Jensen CL, French HT, Stein AR, Huang SJ, Tolliver TJ, Murphy DL (2008). Neurochemical, behavioral and physiological effects of pharmacologically enhanced serotonin levels in serotonin transporter (SERT)-deficient mice. Psychopharmacology (Berlin), 201(2):203-18.

Fox MA, Jensen CL, Gallagher PS, Murphy DL (2007). Receptor mediation of exaggerated responses to serotonin-enhancing drugs in serotonin transporter (SERT)-deficient mice. Neuropharmacology, 53:643-656.

Freeman B, Smith N, Curtis C, Huckett L, Mill J, Craig IW (2003). DNA from buccal swabs recruited by mail: evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. Behavior Genetics, 33:67-72.

Freis E (1954). Mental depression in hypertensive patients treated for long periods with large doses of reserpine The New England Journal of Medicine, 251:1006-1008.

Frisch A, Postilnick D, Rockah R, Michaelovsky E, Postilnick S, Birman E, Laor N, Rauchverger B, Kreinin A, Poyurovsky M, Schneidman M, Modai I, Weizman R (1999). Association of unipolar major depressive disorder with genes of the serotonergic and dopamninergic pathways. Molecular Psychiatry, 4:389-392.

Frodl T, Meisenzahl EM, Zetzsche T, Hohne T, Banac S, Schorr C, Jäger M, Leinsinger G, Bottlender R, Reiser M, Möller HJ (2004). Hippocampal and amygdala changes in patients with major depressive disorder and healthy controls during a 1-year follow-up The Journal of Clinical Psychiatry, 65:492-499.

Frodl T, Moeller HJ, Meisenzahl E (2008). Neuroimaging genetics: new perspectives in research on major depression? Acta Psychiatrica Scandinavica, 118:363-372.

Frodl T, Schaub A, Banac S, Charypar M, Jager M, Kummler P, Bottlender R, Zetzsche T, Born C, Leinsinger G, Reiser M, Möller HJ, Meisenzahl EM (2006). Reduced hippocampal volume correlates with executive dysfunctioning in major depression Journal of Psychiatry and Neuroscience, 31:316-323.

Frodl T, Schule C, Schmitt G, Born C, Baghai T, Zill P, Bottlender R, Rupprecht R, Bondy B, Reiser M, Möller HJ, Meisenzahl EM (2007). Association of the brain-derived neurotrophic factor Val66Met polymorphism with reduced hippocampal volumes in major depression Archives of General Psychiatry, 64:410-416.

Fu CH, Williams SC, Cleare AJ, Brammer MJ, Walsh ND, Kim J, Andrew CM, Pich EM, Williams PM, Reed LJ, Mitterschiffthaler MT, Suckling J, Bullmore ET (2004). Attenuation of the neural response to sad faces in major depression by antidepressant treatment: a prospective, event-related functional magnetic resonance imaging study Archives of General Psychiatry, 61:877-889.

Fukuo Y, Kishi T, Yoshimura R, Kitajima T, Okochi T, Yamanouchi Y, Kinoshita Y, Kawashima K, Naitoh H, Umene-Nakano W, Inada T, Kunugi H, Kato T, Yoshikawa T, Ujike H, Nakamura J, Ozaki N, Iwata N (2010). Serotonin 6 receptor gene and mood disorders: case-control study and meta-analysis. Neuroscience Research, 67:250-255.

Fuxe K, Ogren SO, Agnati FL, Eneroth P, Hol AC, Andersson K (1981). Long-term treatment with zimelidine leads to a reduction in 5-hydroxytryptamine transmission within the central nervous system of the mouse and rat Neuroscience Letters, 21:57-62.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Alshuler D (2002). The structure of haplotype blocks in the human genome. Science, 296:2225-2229.

Garriock HA, Kraft JB, Shyn SI, Peters EJ, Yokoyama JS, Jenkins GD, Reinalda MS, Slager SL, McGrath PJ, Hamilton (2010). A genomwide association study of citalopram response in major depressive disorder Biological Psychiatry, 67:133-138.

Gaspar P, Cases O, Maroteaux L (2003). The developmental role of serotonin: news from mouse molecular genetics. Nature Reviews, Neuroscience, 4:1002-1012.

Gelder M, Mayou R, Cowen P (2001). Shorter Oxford Textbook of Psychiatry Oxford University Press, UK, 4th edition, pages 217-248, 271-325.

Gessa GL, Tagliamonte A (1974). Role of brain monoamines in male sexual behavior. Life Sciences, 14:425-36. Review.

Geyer MA, Puerto A, Dawsey WJ, Knapp S, Bullard WP, Mandell AJ (1976). Histologic and enzymatic studies of the mesolimbic and mesostriatal serotonergic pathways. Brain Research, 106:241-56.

Gloor P, Olivier A, Quesney LF, Andermann F, Horowitz S (1982). The role of the limbic system in experiential phenomena of temporal lobe epilepsy Annals of Neurology, 12:129-144.

Gobbi G, Murphy DL, Lesch KP, Blier P (2001). Modifications of the serotonergic system in mice lacking serotonin transporters. An in vivo electrophysiological study. The Journal of Pharmacology and Experimental Therapeutics, 296:987-995.

Goldapple K, Segal Z, Garson C, Lau M, Bieling P, Kennedy S, Mayberg H (2004). Modulation of cortical-limbic pathways in major depression: treatment-specific effects of cognitive behavior therapy Archives of General Psychiatry, 61:34-41.

Gosso MF, de Geus EJ, van Belzen MJ, Polderman TJ, Heutink P, Boomsma DI, Posthuma D (2006). The SNAP-25 gene is associated with cognitive ability: evidence from a family-based study in two independent Dutch cohorts. Molecular Psychiatry, 11(9):878-886.

Goswami D, May WL, Stockmeier CA, Austin MC (2010). Transcriptional expression of serotonergic regulators in laser-captured microdissected dorsal raphe neurons of subjects with major depressive disorder: sex-specific differences. Journal of Neurochemistry, 112:397-409.

Gottesman II, Gould TD (2003). The endophenotype concept in psychiatry: etymology and atrategic intenstions. American Journal of Psychiatry, 160:636-645.

Grailhe R, Waeber C, Dulawa SC, Hornung JP, Zhuang X, Brunner D, Greyer MA, Hen R (1999). Increased exploratory activity and altered response to LSD in mice lacking the 5-HT5A receptor. Neuron, 22:581-591.

Gratacos M, Costas J, De Cid R, Bayes M, Gonzales JR, Baca-Garcia E, De Diego Y, Fernandez-Aranda F, Fernandez-Piqueras F, Guitart M, Martin-Santos R, Martorell L, Menchon JM, Roca M, Saiz-Ruiz J, Sanjuan J, Torrens M, Urretavizcaya M, Valero J, Vilella E, Estivill X, Carracedo A and The Psychiatric Genetics Network Group (2008). Identification of new putative susceptibility genes for several psychiatric disorders by association analysis of regulatory and non-synonimous SNPs of 306 genes involved in neurotransmission and neurodevelopment. American Journal of Medical Genetics, Part B, Neuropsychiatric Genetics, 150B:808-816.

Green AR, Heal DJ, Johnson P, Laurence BE, Nimgaonkar VL (1983). Antidepression treatments: effects in rodents on dose-response curves of 5-hydroxytryptamine and dopamine-mediated behaviours and 5-HT2 receptor number in frontal cortex British Journal of Pharmacology, 80:377-385.

Green AR, Costain DW (1979). The biochemistry of depression In: Psychopharmacology of affective disorders, ed. ES Paykel, A Coppen, Oxford University Press

Green AR, Costain DW (1981). Pharmacology and biochemistry of psychiatric disorders John Wiley and Sons, 111 River Street, Hoboken, NJ07030, USA1981, 1st ed.

Green EK, Grozeva D, Jones I, Jones L, Kirov G, Caesar S, Gordon-Smith K, Fraser C, Forty L, Russell E, Hamshere ML, Moskvina V, Nikolov I, Farmer A, McGuffin P; Wellcome Trust Case Control Consortium5, Holmans PA, Owen MJ, O'Donovan MC, Craddock N (2009). The bipolar disorder risk allele at CACNA1C also confers risk of recurrent major depression and of schizophrenia. Molecular Psychiatry, E-pub, ahead of print.

Greenwald BS, Kramer-Ginsberg E, Bogerts B, Ashtari M, Aupperle P, Wu H, Allen L, Zeman D, Patel M (1997). Qualitative magnetic resonance imaging

findings in geriatric depression. Possible link between later-onset depression and Alzheimer's disease? Psychological Medicine, 27:421-431.

Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, Santarelli L, Beck S (2002). Serotonin 1A receptor acts during development to establish normal anxiety-like behavior in the adult. Nature, 416:396-400.

Gurevich I, Tamir H, Arango V, Dwork A, Mann JJ, Schmauss C (2002). Altered editing of serotonin 2C receptor pre-mRNA in the prefrontal cortex of depressed suicide victims. Neuron, 34:349-356.

Guscott M, Bristow LJ, Hadingham K, Rosahl TW, Beer MS, Stanton JA, Bromidge F, Owens AP, Huscroft I, Myers J, Rupniak NM, Patel S, Whiting PJ, Hutson PH, Fone KC, Biello, SM, Kulagowski JJ, McAllister G (2005). Genetic knockout and pharmacological blockage studies of the 5-HT7 receptor suggest therapeutic potential in depression. Neuropharmacology, 48:492-502.

Hadjighassem MR, Austin MC, Szewczyk B, Daigle M, Stockmeier CA, Albert PR (2009). Human Freud-2/CC2D1B: a novel repressor of postsynaptic serotonin-1A receptor expression. Biological Psychiatry, 66;214-222.

Hajek T, Kozeny M, Alda M, Hoschl C (2008). Reduced subgenual cingulate volumes in mood disorders: a meta-analysis. Journal of Psychiatry and Neuroscience, 33:91-99.

Hamilton J, Gotlib IH (2008). Neural substrates of increased memory sensitivity for negative stimuli in major depression Biological Psychiatry, 63(12):1155-62.

Hannestad J, Taylor WD, McQuoid DR, Payne ME, Krishnan KR, Steffens DC, Macfall JR (2006). White matter lesion volumes and caudate volumes in late-life depression. International Journal of Geriatric Psychiatry, 21:1193-1198.

Hannon J, Hoyer D (2002). Serotonin receptors and systems: endless diversity? Acta Biologica Szegediensis, 46:1-12.

Hannon J, Hoyer D (2008). Molecular biology of 5-HT receptors. Behavioural Brain Research, 195:198-213.

Hariri AR, Holmes A (2006). Genetics of emotional regulation: the role of the serotonin transporter in neural function. Trends in Cognitive Sciences, 10:182-191.

Hariri AR, Mattay VS, Tessitore A, Kolachana B, Fera F, Goldman D, Egan MF, Weinberger DR (2002). Serotonin transporter genetic variation and the response of the human amygdala. Science, 297:400-403.

Hariri AR, Weinberger DR (2003). Functional neuroimaging of genetic variation in serotonergic neurotransmission. Genes Brain and Behavior, 2:341-349.

Harlow H, Harlow M (1962). Social deprivation in monkeys. Scientific American, 207:136-146.

Harvey JA, Schlosberg AJ, Yunger LM (1975). Behavioral correlates of serotonin depletion. Federation Proceedings, (9):1796-1801.

Hasler G, Drevets WC, Manji HK, Charney DS (2004). Discovering endophenotypes for major depression Neuropsychopharmacology, 29:1765-1781.

Hastings RS, Parsey RV, Oquendo MA, Arango V, Mann JJ (2004). Volumetric analysis of the prefrontal cortex, amygdala, and hippocampus in major depression. Neuropharmacology, 29:952-959.

Hedlund PB, Huitron-Resendiz S, Henriksen SJ, Sutcliffe GJ (2005). 5-HT7

receptor inhibition and inactivation induce antidepressant like behavior and sleep pattern. Biological Psychiatry, 58:831-837.

Hedlund PB, Danielson PE, Thomas EA, Slanina K, Carson MJ, Sutcliffe GJ (2003). No hypothermic response to serotonin in 5-HT7 receptor knockout mice. Proceedings of the National Academy of Sciences USA, 100(3):1375-1380.

Heils A, Teufel A, Petri S, Stöber G, Riederer P, Bengel D, Lesch KP (1996). Allelic variation of human serotonin transporter gene expression. Journal of Neurochemistry, 66(6):2621-2624.

Heim C, Nemeroff CB (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. Biological Psychiatry, 49:1023-1039.

Heim C, Newport DJ, Mletzko T, Miller AH, Nemeroff CB (2008). The link between childhood trauma and depression: insights from HPA axis studies in humans. Psychoneuroendocrinology, 33:693-710.

Heinzen E, Ge D, Cronin KD, Maia J, Shianna KV, Gabriel W, Welsh-Bohmer KA, Hulette CM, Denny T, Goldstein DB (2008). Tissue specific genetic control of gene expression and alternative splicing: Implications for the study of human complex traits. PLoS Biology; 6(12): e1.

Heisler LK, Zhou L, Bajwe P, Hsu J, Tecott LH (2007). Serotonin 5-HT(2C) receptors regulate anxiety-like behavior. Genes Brain and Behavior, 6:491-496.

Hettema JM (2008). The nosologic relationship between generalized anxiety disorder and major depression. Depression and Anxiety, 25(4):300–316

Hettema JM, Neale MC, Myers JM, Prescott CA, Kendler KS (2006). A population-based twin study of the relationship between neuroticism and

internalizing disorders. American Journal of Psychiatry, 163:857-864.

Hettema JM, Prescott CA, Kendler KS (2004). Genetic and encironmental sources of covariation beyween generalized anxiety disorder and neuroticizm. American Journal of Psychiatry, 161:1581-1587.

Higley JD, Linnoila M (1997). Low central nervous system serotonergic activity is traitlike and correlates with impulsive behaviour. Annals of the New York Acadademy of Sciences, 836:39-56.

Hirvonen J, Karlsson H, Kajander J, Lepola A, Markkula J, Rasi-Hakala H, Någren K, Salminen JK, Hietala J (2008). Decreased brain serotonin 5-HT1A receptor availability in medication-naive patients with major depressive disorder: an in-vivo imaging study using PET and [carbonyl-11C]WAY-100635. International Journal of Neuropsychopharmacology, 11:465-476.

Holmes A (2008). Genetic variation in cortico-amygdala serotonin function and risk for stress-related disease. Neuroscience and Biobehavioral Reviews, 32:1293-1314.

Holmes A, Li Q, Murphy DL, Gold E, Crawley JN (2003a). Abnormal anxietyrelated behavior in serotonin transporter null mutant mice: the influence of genetic background. Genes Brain and Behavior, 2:365-380.

Holmes A, Murphy DL, Crawley JN (2002a). Reduced aggression in mice lacking the serotonin transporter. Psychopharmacology (Berlin), 161:160-167.

Holmes A, Yang RJ, Lesch KP, Crawley JN, Murphy DL (2003b). Mice lacking the serotonin transporter exhibit 5-HT(1A) receptor-mediated abnormalities in tests for anxiety-like behavior. Neuropsychopharmacology, 28:2077-2088.

Holmes A, Yang RJ, Murphy DL, Crawley JN (2002b). Evaluation of antidepressant-related behavioral responses in mice lacking the serotonin

transporter. Neuropsychopharmacology, 27:914-923.

Honer WG, Falkai P, Bayer TA, Xie J, Hu l, Li HY, Arango V, Mann JJ, Dwork AJ, Trimble WS (2002). Abnormalities of SNARE mechanism proteins in anterior frontal cortex in severe mental illness. Cerebral Cortex (New York), 12(4):349-356.

Horstmann S, Lucae S, Menke A, Hennings JM, Ising M, Roeske D, Müller-Myhsok B, Holsboer F, Binder EB (2010). Polymorphisms in GRIK4, HTR2A, and FKBP5 show interactive effects in predicting remission to antidepressant treatment. Neuropsychopharmacology, 35(3):727-740.

Hoyer D, Hannon JP, Martin GR (2002). Molecular, Pharmacological and functional diversity of 5-HT receptors. Pharmacology, Biochemistry and Behaviour, 71:533-554.

Hu XZ, Lipsky RH, Zhu G, Akhta LA , Taubman J, Greenberg BD, Xu K, Arnold PD, Richter MA, Kennedy JL, Murphy DL, Goldman D (2006). Serotonin transporter promoter gain-of-function genotypes are linked to obsessive–compulsive disorder. American Journal of Human Genetics, 78:815-826.

Huan YY, Grailhe R, Arango V, Hen R, Mann JJ (1999). Relationship of psychopathology to the human serotonin 1B genotype and receptor binding kinetics in postmortem brain tissue. Neuropsychopharmacology, 21:238-246.

Huang YY. Oquendo MA, Friedman JM, Greenhill LL, Brodsky B, Malone KM, Khait, V, Mann JJ (2003). Substance abuse disorder and major depression are associated with the human 5-HT1B receptor gene (HTR1B) G861C polymorphism. Neuropsychopharmacology, 28:163-169.

Huot RL, Thrivikraman KV, Meaney MJ, Plotsky PM (2001). Development of adult ethanol preference and anxiety as a consequence of neonatal maternal

separation in Long Evans rats and reversal with antidepressant treatment. Psychopharmacology, 158:366-373.

Hurwitz TA, Clark C, Murphy E, Kolonoff H, Martin WR, Pate BD (1990). Regional cerebral glucose metabolism in major depressive disorder. Canadian Journal of Psychiatry, 35:684-688.

Husain MM, McDonald WM, Doraiswamy PM, Figiel GS, Na C, Escalona PR, Boyko OB, Nemeroff CB, Krishnan KR (1991). A magnetic resonance imaging study of putamen nuclei in major depression Psychiatry Research, 40:95-99.

Iidaka Y, Ozaki N, Matsumoto A, Nogawa J, Kinoshita Y, Suzuki T, Iwata N, Yamamoto Y, Okada T, Sadato N (2005). A variant C178T in the regulatory region of the serotonin receptor gene HTR3A modulates neural activation in the human amygdala. The Journal of Neuroscience, 25:6460-6466.

International Human Genome Sequencing Consortium (2001a). Initial sequencing and analysis of the human genome. Nature, 409:860-921.

International Human Genome Sequencing Consortium (2001b). A physiscal map of the human genome. Nature, 409:934-941.

Irwin W, Anderle MJ, Abercrombie HC, Schaefer SM, Kalin NH, Davidson RJ (2004). Interhemispheric functional connectivity differs between the non-depressed and depressed human brain Neuroimage, 21:674-686.

Iwamoto K, Bundo M, Kato T (2009). Serotonin 2C and mental disorders. RNA Biology, 6:248-253.

Iwamoto K, Kato T (2003). RNA editing of serotonin 2C receptor in human postmortem brains of major mental disorders. Neuroscience Letters, 346:169-172.

Iwamoto K, Nakatani N, Bundo M, Yoshikawa T, Kato T (2005). Altered RNA editing of serotonin 2C receptor in a rat model of depression. Neuroscience Research, 53:69-76.

Iwata N, Virkkunen M, Linnoila M, Goldman D (1998). Brain Research, Molecular Brain Research, 58:217-220.

Iyo AH, Kieran N, Chandran A, Albert PR, Wicks I, Bissette G, Austin MC (2009). Differential regulation of the serotonin 1A transcriptional modulators five prime repressor element under dual repression-1 and nuclear-deformed epidermal autoregulatory factor by chronic stress. Neuroscience, 163:1119-1127.

Jacobs BL, Wilkinson LO, Fornal CA (1990). The role of brain serotonin. A neurophysiologic perspective. Neuropsychopharmacology, 3:473-479.

Janssen J, Hulshoff Pol HE, Lampe IK, Schnack HG, de Leeuw FE, Kahn RS, Heeren TJ (2004). Hippocampal chenges and white matter lesions in early-onset depression. Biological Psychiatry, 56:825-831.

Jennings KA, Loder MK, Sheward WJ, Pei Q, Deacon RM, Benson MA, Olverman HJ, Hastie ND, Harmar AJ, Shen S, Sharp T (2006). Increased expression of the 5-HT transporter confers a low-anxiety phenotype linked to decreased 5-HT transmission. The Journal of Neuroscience, 26:8955-8964.

Jennings KA, Sheward WJ, Harmar AJ, Sharp T (2008). Evidence that genetic variation in 5-HT transporter expression is linked to changes in 5-HT2A receptor function. Neuropharmacology, 54:776-783.

Jensen KP, Covault J, Conner TS, Tennen H, Kranzler HR, Furneaux HM (2009). A common polymorphsm in serotonin receptor 1B mRNA modulates

regulation by miR-96 and associates with aggressive human behaviors. Molecular Psychiatry, 14(4):381-389.

John OP, Donahue EM, Kentle RL (1991). The Big Five Inventory-Versions 4a and 54. Institute of Personality and Social Research, Berkeley: University of California, Berkeley, USA

Juhasz G, Chase D, Pegg E, Downey D, Toth ZG, Stones K, Platt H, Mekli K, Payton A, Elliott R, Anderson IM, Deakin JFW (2009). CNR1 gene is associated with hight neuroticism and low agreeableness and interacts with recent negative life events to predict current depressive symptoms. Neuropsychopharmacology, 34:2019-2027.

Juhasz G, Dunham JS, McKie S, Thomas E, Downey D, Chase D, Lloyd-Williams K, Toth ZG, Platt H, Mekli K, Payton A, Elliott R, Williams SR, Anderson IM, Deakin JFW (2010). The CREB1-BDNF-TrkB pathway in depression: multiple gene-cognition-environment interactions. Biological Psychiatry, 2010, in press.

Kalin NH, Shelton SE, Fox AS, Rogers J, Oakes TR, Davidson RJ (2008). The serotonin transporter genotype is associated with intermediate brain phenotypes that depend on the context of eliciting stressor. Molecular Psychiatry, 13:1021–1027.

Kalueff AV, Fox MA, Gallagher PS, Murphy DL (2007). Hypolocomotion, anxiety and serotonin syndrome-like behavior contribute to the complex phenotype of serotonin transporter knockout mice. Genes Brain and Behavior, 6:389-400.

Kalueff AV, Olivier JDA, Nonkes LJP, Homberg JR (2010). Conserved role for the serotonin transporter gene in rat and mouse neubehavioral endophenotypes. Neuroscience and Biobehavioral Reviews, 34:373-386.

Kang DH, Kim JJ, Choi JS, Kim YI, Kim CW, Youn T, Han MH, Chang KH, Kwon JS (2004). Volumetric investigation of the frontalsubcortical circuitry in patients with obsessive-compulsive disorder. The Journal of Neuropsychiatry and Clinical Neurosciences, 16 (3):342-349.

Kasai K, Yamasue H, Gilbertson MW, Shenton ME, Rauch SL, Pitman RK (2008). Evidence for acquired pregenual anterior cingulate gray matter loss from a twin study of combat-related posttraumatic stress disorder. Biological Psychiatry, 63 (6) 550-556.

Kato M, Ikenaga Y, Wakeno M, Okugawa G, Nobuhara K, Fukuda T, Fukuda K, Azuma J, Kinoshita T (2005). Controlled clinical comparison of paroxetine and fluvoxamine considering the serotonin transporter promoter polymorphism. International Clinical Psychopharmacology, 20(3):151-156.

Kato M, Fukuda T, Wakeno M, Fukuda K, Okugawa G, Ikenaga Y, Yamashita M,Takekita Y, Nobuhara K, Azuma J, Kinoshita T (2006). Effects of serotonin type 2A, 3A and 3B receptor and the serotonin transpoerter genes on paroxetine and fluvoxamine efficacy and adverse drug reactions in depressed japanese patients. Neuropsychobiology, 53:186-195.

Kawai M, Rosen CJ (2010). Minireview: A skeleton in serotonin's closet? Endocrinology, 151:4103-4108. Review.

Keedwell P, Drapier D, Surguladze S, Giampietro V, Brammer M, Phillips M (2009). Neural markers of symptomatic improvement during antidepressant therapy in severe depression: subgenual cingulate and visual cortical responses to sad but not happy facial stimuli are correlated with changes in symptom score. Journal of Psychopharmacology, 23(7):775-88.

Kegeles LS, Malone KM, Slifstein M, Ellis SP, Xanthopoulos E, Keilp JG, Campbell C, Oquendo M, Van Heertum RL, Mann JJ (2003). Response of

cortical metabolic deficits to serotonergic challenge in familiar mood disorders. The American Journal of Psychiatry, 160:76-82.

Kellar KJ, Cascio CS, Butler JA, Kurtzke RN (1981). Differential effects of electroconvulsive shock ant anti-depressant drugs on serotonin- receptors in rat brain. European Journal of Pharmacology, 69:515-518.

Keller J, Shen L, Gomez RG, Garrett A, Solvason HB, Reiss A, Schatzberg AF (2008). Hippocampal and amygdalar volumes in psychotic and nonpsychotic unipolar depression American Journal of Psychiatry, 165:872-880.

Kendler KS, Gardner CO, Prescott CA (2002). Toward a comprehensive developmental model for major depression in women. American Journal of Psychiatry, 159:1133-1145.

Kendler KS, Gardner CO, Prescott CA (2006a). Toward a comprehensive developmental model for major depression in men. American Journal of Psychiatry, 163:115-124.

Kendler KS, Gatz M, Gardner CO, Pedersen NL (2006b). Personality and major depression: a Swedish longitudinal, population-based twin study. Archives of General Psychiatry, 63:1113-1120.

Kendler KS, Kuhn J, Prescott CA (2004). The interrelationship of neuroticism, sex and stressful life events in the prediciton of episodes of major depression. American Journal of Psychiatry, 161:631-636.

Kennedy SH, Evans KR, Kruger S, Mayberg HS, McCann S, Arifuzzman AI, Houle S, Vaccarino FJ (2001). Changes in regional brain glucose metabolism measured with positron emission tomography after paroxetine treatment of major depression American Journal of Psychiatry, 158:899-905.

Kessler RC (1997). The effects of stressful life events on depression Annual Review of Psychology, 48:191-214.

Kessler R, Berglund P, Demler O, Jin R, Merikangas K, Walters E (2005). Lifetime prevalence and age of onset distributions of DSMIV disorders in the National Comorbidity Survey Replication. Archives of General Psychiatry 62:593–602.

Kilic F, Murphy DL, Rudnick G (2003). A human serotonin transporter mutation causes constitutive activation of transport activity. Molecular Pharmacology, 64:440-446.

Kim DK, Kim BL, Sohn SE, Lim SW, Na DG, Paik CH, Krishnan KR, Carroll BJ (1999). Candidate neuroanatomic substrates of psychosis in old-aged depression. Progess in Neuro-psychopharmacology and Biological Psychiatry, 23:793-807.

Kim DK, Lim SW, Lee S, Sohn SE, Kim S, Hahn CG, Carroll BJ (2000). Serotonin transporter gene polymorphism and antidepressant response. Neuroreport, 11(1):215-219.

Kim DK, Tolliver TJ, Huang SJ, Martin BJ, Andrews AM, Wichems C,Holmes A, Lesch KP, Murphy DL (2005). Altered serotonin synthesis, turnover and dynamic regulation in multiple brain regions of mice lacking the serotonin transporter. Neuropharmacology, 49:798-810.

Kinnier WJ, Chuang DM, Costa E (1980). Down regulation of dihydroalprenolol and imipramine binding sites in brain of rats treated with imipramine European Journal of Pharmacology, 67:289-294.

Klein SB (2000). Biological psychology. Prentice Hall, Upper Sadle River, NJ, USA, First ed. 2000

Koks S, Nikopensius T, Koido K, Maron E, Altmae S, Heinaste E, Vabrit K, Tammekivi V, Hallast P, Kurg A, Shlik J, Vasar V, Metspalu A, Vasar E (2006). Analysis of SNP profiles in patients with major depressive disorder. The International Journal of Neuropsychopharmacology, 9:167-174.

Krishnan KR, McDonald WM, Escalona PR, Doraiswamy PM, Na C, Husain MM, Figiel GS, Boyko OB, Ellinwood EH, Nemeroff CB (1992). Magnetic resonance imaging of the caudate nuclei in depression. Preliminary observations. Archives of General Psychiatry, 49:553-557.

Krzywkowski K, Davies PA, Feinberg-Zadek PL, Brauner-Osborne H, Jensen AA (2008). High-frequency HTR3B variant associated with major depression dramatically augments the signaling of the human 5-HT3AB receptor. Proceedings of the National Academy of Sciences USA, 105(2):722-727.

LaBar KS, Cabeza R (2006). Cognitive neuroscience of emotional memory Nature Reviews, Neuroscience, 7:54-64.

Lai T, Payne ME, Byrum CE, Steffens DC, Krishnan KR (2000). Reduction of orbital frontal cortex volume in geriatric depression. Biological Psychiatry, 48:971-975.

Laje G, Cannon DM, AAllen AS, Klaver JM, Peck SA, Liu X, Manji HK, Drevets WC, McMahon F (2010). Genetic variation in HTR2A influences serotonin binding potential as measured using PET and [11C]DASB International Journal of Neuropsychopharmacology, E-pub, ahead of print

Lanfumey L, Hamon M (2004). 5-HT1 receptors Current Drug Targets-CNS and Neurological Disorders, 3:1-10.

Lange C, Irle E (2004). Enlarged amygdala volume and reduced hippocampal volume in young women with major depression Psychological Medicine, 34:1059-1064.

Langer SZ, Zafirian E, Briley M, Raisman R, Sechter D (1981). High-affinity binding of 3H-imipramine in brain and platelets and its relevance to the biochemistry of affective disorders Life Sciences, 29:211-220.

Lanzenberger RR, Mitterhauser M, Spindelegger C, Wadsak W, Klein N, Mien LK, Holik A, Attarbaschi T, Mossaheb N, Sacher J, Geiss-Granadia T, Kletter K, Kasper S, Tauscher J (2007). Reduced serotonin-1A receptor binding in social anxiety disorder. Biological Psychiatry, 61: 1081-1089.

Lappalainen J, Long CJ, Eggert M, Ozaki N, Robin RW, Brown GL, Naukkarinen H, Virkkunen M, Linnoila M, Goldman D (1998). Linkage of antisocal alcoholism to the serotonin 5-HT1B receptor gene in 2 populations. Archives of General Psychiatry, 55:989-994.

Lasky-Su J, Faraone SV, Glatt SJ, Tsuang MT (2005). Meta-analysis of the association between two polymorphisms in the serotonin transporter gene and affective disorders. American Journal of Medical Genetics, Part B, Neuropsychiatric Genetics, 133B:110-115.

Lasky-Su J, Neale BM, Franke B, Anney RJ, Zhou K, Maller JB, Vasquez AA, Chen W, Asherson P, Buitelaar J, Banaschewski T, Ebstein R, Gill M, Miranda A, Mulas F, Oades RD, Roeyers H, Rothenberger A, Sergeant J, Sonuga-Barke E, Steinhausen HC, Taylor E, Daly M, Laird N, Lange C, Faraone SV (2008). Genome-wide association scan of quantitative traits for attention deficit hyperactivity disorder identifies novel associations and confirms candidate gene associations. American Journal of Medical Genetics, Part B, Neuropsychiatric Genetics, 147B(8):1345-54.

Lavretsky H, Kurbanyan K, Ballmaier M, Mintz J, Toga A, Kumar A (2004). Sex differences in brain structure in geriatric depression. The American Journal of Geriatric Psychiatry, 12:653-657.

Lazary J, Lazary A, Gonda X, Benko A, Molnar E, Juhasz G, Bagdy G (2008). New evidence for the association of the serotonin transporter gene (SLC6A4) haplotypes, threatening life events, and depressive phenotype. Biological Psychiatry, 64(6):498-504.

Le Francois B, Czesak M, Steubl D, Albert PR (2008). Transcriptional regulation at a HTR1A polymorphism associated with mental illness. Neuropharmacology, 55:977-985.

Leboyer M, Bellvier F, Nosten-Bertrand M, Jouvent R, Pauls D, Mallet J (1998). Psychiatric genetics: search for phenotypes. Trends in Neurosciences, 21:102-105.

Lee S-H, Lee K-J, Lee H-J, Ham B-J, Ryu S-H, Lee M-S (2005). Association between the 5-HT6 receptor C267T polymorphism and response to antidepressant treatment in major depressive disorder. Psychiatry and Clinical Neurosciences, 59:140145.

Lemonde S, Turecki G, Bakish D, Du L, Hrdina PD, Bown CD, Sequeira A, Kushwaha N, Morris SJ, Basak A, Ou X-M, Albert PR (2003). Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. The Journal of Neuroscience, 23:8788-8799.

Lenze EJ, Sheline YI (1999). Absence of striatal volume differences between depressed subjects with no comorbid medical illness and matched comparison subjects. American Journal of Psychiatry, 156:1989-1991.

Lenze S, Xiong C, Sheline YI (2008). Childhood adversity predicts earlier onset

of major depression but not reduced hippocampal volume. Psychiatry Research: Neuroimaging, 162:39-49.

Lerer B, Macciardi F, Segman RH, Adolffson R, Blackwood D, Blairy S, Del Favero J, Dikeos DG, Kaneva R, Lilli R, Massat I, Milanova V, Muir W, Noethen M, Oruc L, Petrova T, Papadimitriou GN, Rietschel M, Serretti A, Souery D, Van Gestel S, Van Broeckhoven C, Mendlewicz J (2001). Variability of 5-HT2C receptor cys23ser polymorphism among European populations and vulnerability to affective disorder. Molecular Psychiatry, 6:579-585.

Lesch KP (2005). Alcohol dependence and gene x environment interaction in emotion regulation: is serotonin the link? European Journal of Pharmacology, 526:113-124.

Lesch KP, Balling U, Gross J, Strauss K, Wolozin BL, Murphy DL, Riederer P (1994). Organization of the human transporter gene. Journal of Neural Transmission, General section, 95:157-162.

Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science, 274:1527-1531.

Lesch KP, Gutknecht L (2005). Pharmacogenetics of the serotonin transporter. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 29(6):1062-73.

Lesch KP, Meyer J, Glatz K, Flügge G, Hinney A, Hebebrand J, Klauck SM, Poustka A, Poustka F, Bengel D, Mössner R, Riederer P, Heils A (1997). The 5-HT transporter gene-linked polymorphic region (5-HTTLPR) in evolutionary perspective: alternative biallelic variation in rhesus monkeys. Rapid communication. Journal of Neural Transmission, 104(11-12):1259-66.

Levinson, D.F., 2006. The genetics of depression: a review. Biol Psychiatry. 60, 84-92.

Lewis CM, Ng MY, Butler AW, Cohen-Woods S, Uher R, Pirlo K, Weale ME, Schosser A, Paredes UM, Rivera M, Craddock N, Owen MJ, Jones L, Jones I, Korszun A, Aitchison KJ, Shi J, Quinn JP, Mackenzie A, Vollenweider P, Waeber G, Heath S, Lathrop M, Muglia P, Barnes MR, Whittaker JC, Tozzi F, Holsboer F, Preisig M, Farmer AE, Breen G, Craig IW, McGuffin P (2010). Genome-wide association study of major recurrent depression in the U.K. population. American Journal of Psychiatry, 167(8):949-957.

Li Q, Holmes A, Ma L, Van de Kar LD, Garcia F, Murphy DL (2004). Medial hypothalamic 5-hydroxytryptamine (5-HT)1A receptors regulate neuroendocrine responses to stress and exploratory locomotor activity: application of recombinant adenovirus containing 5-HT1A sequences. The Journal of Neuroscience, 24:10868-10877.

Li Q, Wichems C, Heils A, Lesch KP, Murphy DL (2000). Reduction in the density and expression, but not G-protein coupling, of serotonin receptors (5-HT1A) in 5-HT transporter knock-out mice: gender and brain region differences. The Journal of Neuroscience, 20:7888-7895.

Li Q, Wichems C, Heils A, Van De Kar LD, Lesch KP, Murphy DL (1999). Reduction of 5-hydroxytryptamine (5-HT)(1A)-mediated temperature and neuroendocrine responses and 5-HT(1A) binding sites in 5-HT transporter knockout mice. The Journal of Pharmacology and Experimental Therapeutics, 291:999-1007.

Li Q, Wichems CH, Ma L, Van de Kar LD, Garcia F, Murphy DL (2003). Brain region-specific alterations of 5-HT2A and 5-HT2C receptors in serotonin transporter knockout mice. Journal of Neurochemistry84:1256-1265.

Lin Z, Walther D, Yu X-Y, Drgon T, Uhl GR (2004). The human serotonin 2B: coding region polymorphisms and association with vulnerability to illegal drug abuse. Pharmacogenetics, 14:805-811.

Lindauer RJ, Olff M, van Meijel EP, Carlier IV, Gersons BP (2006). Cortisol, learning, memory, and attention in relation to smaller hippocampal volume in police officers with posttraumatic stress disorder. Biological Psychiatry, 59 (2):171-177.

Lindauer RJ, Vlieger EJ, Jalink M, Olff M, Carlier IV, Majoie CB, den Heeten GJ, Gersons BP (2004). Smaller hippocampal volume in Dutch police officers with posttraumatic stress disorder Biological Psychiatry, 56 (5): 356-363.

Lindauer RJ, Vlieger EJ, Jalink M, Olff M, Carlier IV, Majoie CB, Den Heeten GJ, Gersons BP (2005). Effects of psychotherapy on hippocampal volume in out-patients with post-traumatic stress disorder: a MRI investigation. Psychological Medicine, 35 (10):1421-1431.

Lira A, Zhou M, Castanon N, Ansorge MS, Gordon JA, Francis JH, Bradley-Moore M, Lira J, Underwood MD, Arango V, Kung HF, Hofer MA, Hen R, Gingrich JA (2003). Altered depression-related behaviors and functional changes in the dorsal raphe nucleus of serotonin transporter-deficient mice. Biological Psychiatry, 54:960-971.

Lloyd AJ, Ferrier IN, Barber R, Gholkar A, Young AH, O'Brien JT (2004). Hippocampal volume change in depression: late- and early-onset illness compared The British Journal of Psychiatry, 184:488-495.

Lloyd KG, Farley IJ, Deck JHN, Hornykiewicz O (1974). Serotonin and 5-hydroxyindole acetic acid in discrete aread of the brainstem of suicide victims and control patients Advances in Biochemical Psychopharmacology, 11:387-

397.

Lopez JF, Chalmers DT, Little KY, Watson SJ (1998). Regulation of serotonin 1A, glucocorticoid and mineralocorticoid receptor in rat and human hippocampus: implications for the neurobiology of depression. Biological Psychiatry, 43:547-573.

Lopez-Leon S, Janssens ACJW, Gonzalez-Zuloeta Ladd AM, Del-Favero J, Claes SJ, Oostra BA, van Duijn CM (2008). Meta-analyses of genetic studies on major depressive disorder. Molecular Psychiatry, 13:722-785.

Lovenberg TW, Baron BM, de Lecea L, Miller JD, Prosser RA, Rea MA, Foye PE, Racke M, Slone AL, Siegel BW, et al. (1993). A novel adenylyl cyclase-activating serotonin receptor (5-HT7) implicated in the regulation of mammalian circadian rhythms. Neuron, 11(3):449-58.

Lynford GL, Yamagata K, Kaufmann WWE, Barnes CA, Sanders LK, Copeland NG, Gilbert DJ, Jenkins NA, Lanahan AA, Worley PF (1995). Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. Neuron, 14:433-445.

MacFall JR, Payne ME, Provenzale JE, Krishnan KR (2001). Medial orbital frontal lesions in late-onset depression. Biological Psychiatry, 49:803-806.

MacMaster FP, Kusumakar V (2004). Hippocampal volume in early onset depression BMC Medicine, 2:2

MacMaster FP, Mirza Y, Szeszko PR, Kmiecik LE, Easter PC, Taormina SP, Lynch M, Rose M, Moore GJ, Rosenberg DR (2008). Amygdala and hippocampal volumes in familial early onset major depressive disorder Biological Psychiatry, 63(4):385-90.

MacQueen GM, Campbell S, McEwen BS, Macdonald K, Amano S, Joffe RT, Nahmias C, Young LT (2003). Course of illness, hippocampal function, and hippocampal volume in major depression Proceedings of the National Academy of Sciences USA, 100:1387-1392.

Mamounas LA and Molliver ME (1988). Evidence for dual serotonergic projections to neocortex: axons from the dorsal and median raphe nuclei are differentially vulnerable to the neurotoxin p-chloroamphetamine (PCA). Experimental Neurology, 102:23-36.

Manolio TA, Brooks LD, Collins FS (2008). A HapMap harvest of insights into the genetics of common disease. The Journal of Clinical Investigation, 118:1590-1605.

Massana J, Mercader JM, Gómez B, Tobeña A, Salamero M (2003). Amygdalar atrophy in panic disorder patients detected by volumetric magnetic resonance imaging. Neuroimage, 19 (1):80-90.

Mathews TA, Fedele DE, Coppelli FM, Avila AM, Murphy DL, Andrews AM (2004). Gene dose-dependent alterations in extraneuronal serotonin but not dopamine in mice with reduced serotonin transporter expression. Journal of Neuroscience Methods, 140:169-181.

Mayberg HS, Lozano AM, Voon V, McNeely HE, Seminowicz D, Hamani C, Schwalb JM, Kennedy SH (2005). Deep-brain stimulation for treatment-resistant depression. Neuron, 45:651-660.

McGuffin P, Katz R, Watkins S, Rutherford J (1996). A hospital-based twin register of the heritability of DSM-IV unipolar depression Archives of General Psychiatry, 53:129-136.

McIntosh A, Cohen A, Turnbull N, Esmonde L, Dennis P, Eatock J, Feetam C, Hague J, Hughes I, Kelly J, Kosky N, Lear G, Owens L, Ratcliffe J, Salkovskis P

(2004). Guidelines and Evidence Review for Panic Disorder and Generalised Anxiety Disorder University of Sheffield/London: National Collaborating Centre for Primary Care, UK,

McKittrick CR, Blanchard DC, Blanchard RJ, McEwen BS, Sakai RR (1995). Serotonin receptor binding in a colony model of chronic stress Biological Psychiatry, 37:383-393.

McMahon FJ, Akula N, Schulze TG, Muglia P, Tozzi F, Detera-Wadleigh SD, Steele CJM, Breuer R, Strohmaier J, Wendland JR, Mattheisen M, Muehleisen TW, Maier W, Noethen MM, Cichon S, Farmer A, Vincent JB, Holsboer F, Preisig M, Rietschel M, the Bipolar Disorder Genome Study Consortium (2010). Meta-analysis of genome-wide association data identifies a risk locus for major mood disorders on 3p21.1 Nature Genetics, 42(2):128-131.

McMahon F, Buervenich S, Charney D, Lipsky R, Rush AJ, Wilson AF, Sorant AJM, Papanicolau GJ, Laje G, Fava M, Trivedi MH, Wisniewski SR, Manji H (2006). Variation in the gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. American Journal of Human Genetics, 78:804-814.

Meineke C, Tzvetkov MV, Bokelmann K, Oetjen E, Hirsch-Ernst K, Kaiser R, Brockmoeller J (2008). Functional characterization of a -100_-102delAAG deletion-insertion polymorphism in the promoter region of the HTR3B gene. Pharmacogenetics and Genomics, 18:219-230.

Melke J, Westberg L, Nillson S, Landen M, Soderstrom H, Baghaei F, Rosmond R, Holm G, Bjorntorp P, Nilsson LG, Adolffson R, Eriksson E (2003). A polymorphism in the serotonin receptor 3A (HTR3A) gene and its association with harm avoidance in women. Archives of General Psychiatry, 60:1017-1023.

Meltzer CC, Price JP, Mathis CA, Butters MA, Ziolko SK, Moses-Kolko E, Mazumdar S, Mulsant BH, Houck PR, Lopresti BJ, Weissfeld LA, Reynolds CF (2004). Serotonin 1A receptor binding and treatment response in late-life depression. Neuropsychopharmacology, 29:2258-2265.

Meltzer HY, Arora RC, Baber R, Tricou BJ (1981). Serotonin uptake in blood paltelets of psychiatric patients Archives of General Psychiatry, 38:1322-1326.

Mendelson SD, McEwen BS (1991). Autoradiographic analyses of restraint-induced stress on 5-HT1A, 5-HT1C and 5-HT2 receptors in the dorsal hippocampus of male and female rats. Neuroendocrinology, 54:454-461.

Mervaala E, Fohr J, Kononen M, Valkonen-Korhonen M, Vainio P, Partanen K, Tiihonen J, Viinamäki H, Karjalainen AK, Lehtonen J (2000). Quantitative MRI of the hippocampus and amygdala in severe depression Psychological Medicine, 30:117-125.

Messa C, Colombo C, Moresco RM, Gobbo C, Galli L, Lucignani G, Gilardi MC, Rizzo G, Smeraldi E, Zanardi R, Artigas F, Fazio F (2003). 5-HT(2A) receptor binding is reduced in drug-naïve and unchanged in SSRI-responder depressed patients compared to healthy controls: a PET study. Psychopharmacology (Berlin), 167: 72-78.

Meyer J, Saam W, Mossner R, Cangir O, Ortega GR, Tatschner T, Riederer P, Wienker TF, Lesch KP (2002). Evolutionary conserved microsatellites in the promoter region of the 5-hydroxytryptamine receptor 2C gene (HTR2C) are not associated with bipolar disorder in females. Journal of Neural Transmission, 109(5-6):939-946.

Meyer JH, Houle S, Sagrati S, Carella A, Hussey DF, Ginovart N, Goulding V, Kennedy J, Wilson AA (2004). Brain serotonin transporter binding potential measured with carbon 11-labeled DASB positron emission tomography: effects

of major depressive episodes and severity of dysfunctional attitudes. Archives of General Psychiatry, 61: 1271-1279.

Meyer JH, McMain S, Kennedy SH, Korman L, Brown GM, DaSilva JN, Wilson AA, Blak T, Eynan-Harvey R, Goulding VS, Houle S, Links P (2003). Dysfunctional attitudes and 5-HT2 receptors during depression and self-harm. American Journal of Psychiatry, 160: 90-99.

Meyerson LR, Wennogle LP, Abel MS, Coupet J, ALippa AS, Rauh CE, Beer B (1982). Human brain receptor alterations in suicide victims Pharmacology, Biochemistry and Behaviour, 17:159-163.

Mickey BJ, Ducci F, Hodgkinson CA, Langenecker SA, Goldman D, Zubieta JK (2008). Monoamine oxidase A genotype predicts human serotonin 1A receptor avilability in vivo. The Journal of Neuroscience, 28:11354-11359.

Milham MP, Nugent AC, Drevets WC, Dickstein DP, Leibenluft E, Ernst M, Charney D, Pine DS (2005). Selective reduction in amygdala volume in pediatric anxiety disorders: a voxel-based morphometry investigation. Biological Psychiatry, 57 (9): 961-966.

Miller JM, Oquendo MA, Ogden RT, Mann JJ, Parsey RV (2008). Serotonin transporter binding as a possible predictor of one-year remission in major depressive disorder. Journal of Psychiatric Research, 42: 1137-1142.

Mintun MA, Sheline YI, Moerlein SM, Vlassenko AG, Huang Y, Snyder AZ (2004). Decreased hippocampal 5-HT2A receptor binding in major depressive disorder: in vivo measurement with [18F]altanserin positron emission tomography. Biological Psychiatry, 55: 217-224.

Mitchell JB, Iny LJ, Meaney MJ (1990). The role of serotonin in the devlopment and environmental regulation of type II corticosteroid binding in rat

hippocampus. Brain Research, Developmental Brain Research, 55:231-235.

Mizuta N, Akiyoshi J, Sato A, Hanada H, Tanaka Y, Tsuru J, Matshushita H, Kodama K, Isogawa K (2008). Serotonin receptor 3A (HTR3A) gene is associated with personality traits, but not panic disorder. Psychiatric Genetics, 18:44.

Moffitt, T.E., Caspi, A., Rutter, M., 2005. Strategy for investigating interactions between measured genes and measured environments. Archives of General Psychiatry. 62, 473-481.

Molliver ME (1987). Serotonergic neuronal systems: what their anatomic organization tells us about function. Journal of Clinical Psychopharmacology. 7:3S-23S.

Montanez S, Owens WA, Gould GG, Murphy DL, Daws LC (2003). Exaggerated effect of fluvoxamine in heterozygote serotonin transporter knockout mice. Journal of Neurochemistry, 86:210-219.

Montgomery SA, Asberg M (1979). A new depression scale designed to be sensitive to change. The British Journal of Psychiatry, 134: 382–389.

Moret C, Briley M (2000). The possible role of 5-HT1B/1D receptors in psychiatric disorders and their potential target for therapy. European Journal of Pharmacology, 404:1-12.

Moses-Kolko EL, Price JC, Thase ME, Meltzer CC, Kupfer DJ, Mathis CA, Bogers WD, Berman SR, Houck PR, Schneider TN, Drevets WC (2007). Measurement of 5-HT1A receptor binding in depressed adults before and after antidepressant drug treatment using positron emission tomography and [11C]WAY-100635. Synapse, 61:523-53.

Moskvina V, Smith M, Ivanov D, Blackwood D, Stclair D, Hultman C, Toncheva D, Gill M, Corvin A, O'Dushlaine C, Morris DW, Wray NR, Sullivan P, Pato C, Pato MT, Sklar P, Purcell S, Holmans P, O'Donovan MC, Owen MJ, Kirov G (2010). Genetic Differences between Five European Populations. Human Heredity, 70(2):141-149.

Mueller TI, Leon AC (1996). Recovery, chronicity and levels of psychopathology in major depression The Psychiatric Clinics of North America, 19:85-102.

Muglia P, Tozzi F, Galwey NW, Francks C, Upmanyu R, Kong XQ, Antoniades A, Domenici E, Perry J, Rothen S, Vandeleur CL, Mooser V, Waeber G, Vollenweider P, Preisig M, Lucae S, Mueller-Myshok B, Holsboer F, Middleton LT, Roses AD (2010). Genome-wide association study of recurrent major depressive mood disorder in two European case-control cohorts. Molecular Psychiatry, (6):589-601.

Muller EE, Nistico G, Scapagnini U (1976). Neurotransmitters and Anterior Pituitary Function. Academic Press, New York, USA

Munafo MR, Clark TG, Roberts KH, Johnstone EC (2006). Neuroticism mediates the association of the serotonin transporter gene with lifetime major depression. Neuropsychobiology, 53:1-8.

Munafo MR, Flint J (2009). Replication and heterogenity in gene x environment interaction studies. International Journal of Neuropsychopharmacology, 12:727-729.

Muntaner C, Eaton WW, Miech R, O'Campo P (2004). Socioeconomic position and major mental disorders. Epidemiologic Reviews, 26:53-62.

Murphy DL, Fox MA, Timpano KR, Moya PR, Ren-Patterson R, Andrews AM, Holmes A, Lesch KP, Wendland JR (2008). How the serotonin story is being

rewritten by new gene-based discoveries principally related to SLC6A4, the serotonin transporter gene, which functions to influence all cellular serotonin systems. Neuropharmacology, 55:932-960.

Murphy DL, Lesch KP (2008). Targeting the murine serotonin transporter: insights into human neurobiology. Nature Reviews, Neuroscience, 9:85-96.

Murphy DL, Li Q, Engel S, Wichems C, Andrews A, Lesch KP, Uhl G (2001). Genetic perspectives on the serotonin transporter. Brain Research Bulletin, 56:487-494.

Murray CJ, Lopez AD (1996). Evidence-based health policy-lessons from the Global Burden of Disease Study Science, 274:740-743.

Nagai M (1992). The role of serotonergic system in body temperature regulation. Physiological Research, 41:65-69.

Nakamura M, Ueno S, Sano A, Tanabe H (2000). The human serotonin transporter gene linked polymorphsm (5-HTTLPR) shows ten novel allelic variants. Molecular Psychiatry, 5:32-38.

Nash JR, Sargent PA, Rabiner EA, Hood SD, Argyropoulos SV, Potokar JP, Grasby PM, Nutt DJ (2008). Serotonin 5-HT1A receptor binding in people with panic disorder: Positron emission tomography study. The British Journal of Psychiatry, 193: 229-234.

Nebigil CG, Choi D-S, Dietrich A, Hickel P, Le Meur M, Messaddeq N, Launay J-M, Maroteaux L (2000). Serotonin 2B receptor is required for heart development. Proceedings of the National Academy of Sciences USA, 97:9508-9513.

Neigh GN, Gillespie CF, Nemeroff CB (2009). The neurobiological tool of child

abuse and neglect. Trauma, Violence and Abuse, (10):389-410.

Neumeister A, Bain E, Nugent AC, Carson RE, Bonne O, Luckenbaugh DA, Eckelman W, Herscovitch P, Charney DS, Drevets WC (2004a). Reduced serotonin type 1A receptor binding in panic disorder. The Journal of Neuroscience, 3:589-591.

Neumeister A, Drevets WC, Belfer I, Luckenbaugh DA, Henry S, Bonne O, Herscovitch P, Goldman D, Charney DS (2006). Effects of alpha-2C-adrenoreceptor gene polymorphism on neural responses to facial expressions in depression. Neuropsychopharmacology, 31:1750-1756.

Neumeister A, Nugent AC, Waldeck T, Geraci M, Schwarz M, Bonne O, Bain EE, Luckenbaugh DA, Herscovitch P, Charney DS, Drevets WC (2004b). Neural and behavioral responses to tryptophan depletion in unmedicated patients with remitted major depressive disorder and controls. Archives of General Psychiatry, 61:765-773.

Niesler B, Flohr T, Nothen MM, Fischer C, Rietschel M, Franzek E, Albus M, Propping P, Rappold GA (2001). Association between the 5'UTR variant C178T of the serotonin receptor gene HTR3A and bipolar affective disorder. Pharmacogenetics, 11:471-475.

Nikolaus S, Antke, C, Mueller HW (2009). In vivo imaging of synaptic function in the central nervous system: II. Mental and affective disorders. Behavioural Brain Research, 204:32-66.

Niswender CM, Copeland SC, Herrick-Davis K, Emeson RB, Sanders-Bush E (1999). RNA editing of the human serotonin 5-Hydroxytryptamine 2C receptor silences constitutive activity. The Journal of Biological Chemistry, 274:9472-9478.

Niswender CM, Herrick-Davis K, Dilley GE, Meltzer HY, Overholser JC, Stockmeier CA, Emeson RB, Sanders-Bush E (2001). RNA ediring of the human serotonin 5-HT2C receptor: alterations in suicide and implications for serotonergic pharmacotherapy. Neuropsychopharmacology, 24:478-491.

Niswender CM, Sanders-Bush E, Emeson RB (1998). Identification and characterization of RNA editing events within the 5-HT2C receptor. Annals of the New York Acadademy of Sciences, 861:38-48.

Nobuhara K, Okugawa G, Sugimoto T, Minami T, Tamagaki C, Takase K, Saito Y, Sawada S, Kinoshita T (2006). Frontal white matter anisotropy and symptom severity of late-life depression: a magnetic resonance diffusion tensor imaging study. Journal of Neurology, Neurosurgery and Psychiatry, 77:120-122.

Nordquist N, Oreland L (2010). Serotonin, genetic variability, behaviour, and psychiatric disorders- a review. Upsala Journal of Medical Sciences, 115:2-10.

Norton N, Owen MJ (2005). HTR2A: association and expression studies in neuropsychiatric genetics. Annals of Medicine, 37:121-129.

O'Brien JT, Lloyd A, McKeith I, Gholkar A, Ferrier N (2004). A longitudinal study of hippocampal volume, cortisol levels, and cognition in older depressed subjects American Journal of Psychiatry, 161:2081-2090.

Ohtsuki T, Ishiguro H, Detera-Wadleigh SD, Toyota T, Shimizu H, Yamada K, Yoshitsugu K, Hattori E, Yoshikawa T, Arinami T (2002). Association between serotonin 4 receptor gene polymorphisms and bipolar disorder in Japanes case-control samples and the NIMH Genetics Initiative Bipolar Pedigrees. Molecular Psychiatry, 7:954-961.

Okada M, Northup JP, Ozaki N, Russell JT, Linnoila M, Goldman D (2004). Modification of human 5-HT(2C) receptor function by Cys23Ser, an abundant

naturally occuring amino-acid substitution. Molecular Psychiatry, 9:55-64.

Otano A, Frechilla D, Cobreros A, Cruz-Orive LM, Insausti A, Insausti R, Hamon M, Del Rio J (1999). Anxiogenic-like effects and reduced stereolgical counting of immunolabelled 5 hydroxytriptamine 6 receptors in rat nucelus accumbens by antisense nucleotides. Neuroscience, 92:1001-1009.

Ou XM, Lemonde S, Jafar-Nejad H, Brown CD, Goto A, Rogaeva A, Albert PR (2003). Freud-1: a neuronal calcium-regulated repressor of the HT1A receptor gene. The Journal of Neuroscience, 23:7415-7425.

Ozaki N, Goldman D, Kaye WH, Plotnicov K, Greenberg BD, Lappalainen J, Rudnick G, Murphy DL (2003). Serotonin transporter missense mutation associated with a complex neuropsychiatric phenotype. Molecular Psychiatry, 8:933-936.

Ozaki N, Manji H, Lubierman V, Lu SJ, Lappalainen J, Rosenthal NE, Goldman D (1997). A naturally occuring amoni acid substitution of the human serotonin 5-HT2A receptor influences amplitude and timing of intracellular calcium mobilisation. Journal of Neurochemistry, 68:3186-2193.

Ozsarac N, Santha E, Hoffman BJ (2002). Alternative non-coding exons support serotonin transporter mRNA expression in the brain and gut. Journal of Neurochemistry, 82:336-344.

Parashos IA, Tupler LA, Blitchington T, Krishnan KR (1998). Magnetic-resonance morphometry in patients with major depression Psychiatry Research, 84:7-15.

Pare CMB, Yeung DPH, Price K, Stacey RS (1969). 5-hydroxytryptamine, noradrenaline and dopamine in brainstem, hypothalamus and caudate nucleus of controls and of patients committing suicide by coal-gas poisoning. Lancet,

2:133-135.

Parsey RV, Hastings RS, Oquendo MA, Huang YY, Simpson N, Arcement J, HuangY, Ogden RT, vanHeertum RL, Arango V, Mann JJ (2006a). Lower serotonin transporter binding potential in the human brain duringmajor depressive episodes. The American Journal of Psychiatry, 163: 52-58.

Parsey RV, Oquendo MA, Ogden RT, Olvet DM, Simpson N, Huang YY, van Heertum RL, Arango V, Mann JJ (2006b). Altered serotonin serotonin 1A binding in major depression: a [carbonyl-C-11]WAY-100635 positron emission tomography study. Biological Psychiatry, 59:106-113.

Parsons MJ, D'Souza UM, Arranz M-J, Kerwin RW, Makoff AJ (2004). The -1438A/G (rs6311) polymorphism in the 5-hydroxytryptamine type 2A gene affects promoter activity. Biological Psychiatry, 56:406-410.

Paul SM, Rehavi M, Skolnick P, Ballenger JC, Goodwin FK (1981). Depressed patients have decreased binding of tritiated imipramine to platelet serotonin 'transporter' Archives of General Psychiatry, 38:1315-1317.

Pederson CL, Maurer SH, Kaminski PL, Zander KA, Peters CM, Stokes-Crowe LA, Osborn RE (2004). Hippocampal volume and memory performance in a community-based sample of women with posttraumatic stress disorder secondary to child abuse. Journal of Traumatic Stress, 17:37-40.

Pei Q, Lewis L, Sprakes ME, Jones EJ, Grahame-Smith DG, Zetterstroem TSC (2000). Serotonergic regulation of mRNA expression of Arc, an immediate early gene selectively localized at neuronal dendrites. Neuropharmacology, 39:463-470.

Perez XA, Andrews AM (2005). Chronoamperometry to determine differential reductions in uptake in brain synaptosomes from serotonin transporter knockout

mice. Analytical Chemistry, 77:818-826.

Peroutka SJ, Snyder SH (1980). Long-term antidepressant treatment decreased spiroperidol-labelled serotonin receptor binding Science, 210:88-90.

Perry EK, Marshall EF, Blessed G, Tomlinson BE, Perry RH (1983). Decreased imipramine binding in the brains of patients with depressive illness The British Journal of Psychiatry, 142:188-192.

Peters EJ, SL Slager, GD Jenkins, MS Reinalda, HA Garriock, SI Shyn JB Kraft, PJ McGrath, SP Hamilton (2009). Resequencing of serotonin-related genes and association of tagging SNPs to citalopram response. Pharmacogenetics and Genomics, 19:1-10.

Peters EJ, Slager SL, McGrath PJ, Knpwles JA, Hamilton SP (2004). Investigation of serotonin-related genes in antidepressant response Molecular Psychiatry, 9:879-889.

Pezawas L, Meyer-Lindenberg A, Drabant EM, Verchinski BA, Munoz KE,Kolachana BS, Egan MF, Mattay VS, Hariri AR, Weinberger DR (2005). 5-HTTLPR polymorphism impacts human cingulateamygdala interactions: a genetic susceptibility mechanism for depression. Nature Neuroscience, 8:828-834.

Philibert R, Madan A, Andersen A, Cadoret R, Packer H, Sandhu H (2007). Serotonin transporter mRNA levels are associated with the methylation of an upstream CpG island. American Journal of Medical Genetics, Part B, Neuropsychiatric Genetics, 144B:101-105.

Pletscher A, Shore PA, Brodie BB (1956). Serotonin as a mediator of reserpine action in the brain The Journal of Pharmacology and Experimental Therapeutics, 116:84-89.

Plomin R, Haworth CMA, Davis OSP (2009). Common disorders are quantitative traits. Nature Reviews, Genetics, 10(12):872-878.

Prasad HC, Zhu CB, McCauley JL, Samuvel DJ, Ramamoorthy S, Shelton RC, Hewlett WA, Sutcliffe JS, Blakely RD (2005). Human serotonin transporter variants display altered sensitivity to protein kinase G and p38 mitogen-activated protein kinase. Proceedings of the National Academy of Sciences USA, 102:11545-11550.

Praschak-Rieder N, Hussey D, Wilson AA, Carella A, Lee M, Dunn E, Willeit M, Bagby RM, Houle S, Meyer JH (2004). Tryptophan depletion and serotonin loss in selective serotonin reuptake inhibitor-treated depression: an [(18)F]MPPF positron emission tomography study. Biological Psychiatry, 56:587-591.

Psychiatric GWAS Consortium Coordinating Committee (2009a). Genomwide association studies: history, rationale and prospects for psychiatric disorders. American Journal of Psychiatry, 166:540-556.

Psychiatric GWAS Consortium Steering Committee (2009b). A framework for interpreting genom-wide association studies of psychiatric disorders. Molecular Psychiatry, 14:10-17.

Purves D, Augustine GJ, Fitzpatrick D, Katz LC, LaMantia AS, McNamara JO, Williams SM (2001). Neuroscience. Sunderland (MA): Sinauer Associates, Inc.

Quirk GJ, Armony JL, LeDoux JE (1997). Fear conditioning enhances different temporal components of tone-evoked spike trains in auditory cortex and lateral amygdala. Neuron, 19 (3):613-624.

Rabbit PMA, McInnes L, Diggle P, Holland F, Bent N, Abson V, Pendleton N, Horan M (2004). The University Of Manchester Longitudinal study of cognition in normal helathy old age. Aging Neuropsychology and Cognition, 11(2-3):245-279.

Raisman R, Briley M, Bouchami F, Sechter D, Zafirian E, Langer SZ (1982). 3H-imipramine binding and serotonin utake in platelets from untreated depressed patients and contol volunteers. Psychopharmacology, 77:332-335.

Rapport MM, Green AA, Page IH (1948) Serum vasoconstrictor (serotonin). IV. Isolation and characterisation. The Journal of Biological Chemistry, 176:1243-1251.

Rausch JL, Johnson ME, Corley KM, Hobby HM, Shendarkar N, Fei Y, Ganapathy V, Leibach FH (2003). Depressed patients have higher body temperature: 5-HT transporter long promoter region effects. Neuropsychobiology, 47:120-127.

Rees S, den Daas I, Foord S, Goodson S, Bull D, Kilpatrick G, Lee M (1994). Cloning and characterisation of the human 5-HT5A serotonin receptor. FEBS Letters, 355:242–246.

Reimold M, Batra A, Knobel A, Smolka MN, Zimmer A, Mann K, Solbach C, Reischl G, Schwarzler F, Grunder G, Machulla HJ, Bares R, Heinz A (2008). Anxiety is associated with reduced central serotonin transporter availability in unmedicated patients with unipolar major depression: a [11C]DASB PET study. Molecular Psychiatry, 13: 606-613.

Reivich M, Amsterdam JD, Brunswick DJ, Shiue CY (2004). PET brain imaging with [11C](+)McN5652 shows increased serotonin transporter availability in major depression. Journal of Affective Disorders, 82: 321-327.

Riccio O, Potter G, Walzer C, Vallet P, Szabo G, Vutskits L, Kiss JZ, Dayer AG (2009). Excess of serotonin affects embryonic interneuron migration through activation of the serotonin receptor 6. Molecular Psychiatry, 14:280-290.

Rijsdijk FV, Sham PC, Sterne A, Purcell S, McGuffin P, Farmer A, Goldberg D, Mann A, Cherny SS, Webster M, Ball D, Eley TC, Plomin R (2001). Life events and depression in a community sample of siblings. Psychological Medicine, 31:401-410.

Rioux A, Fabre V, Lesch KP, Moessner R, Murphy DL, Lanfumey L, Hamon M, Martres MP (1999). Adaptive changes of serotonin 5-HT2A receptors in mice lacking the serotonin transporter. Neuroscience Letters, 262:113-116.

Risch N, Herrell R, Lehner T, Liang K-Y, Eaves L, Hoh J, Griem A, Kovacs M, Ott J, Merikangas KR (2009). Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events and risk of depression. Journal of American Medical Association, 301(23):2462-2471.

Rocha BA, Ator R, Emmett-Oglesby MW, Hen R (1997). Intravenous cocaine self-administration in mice lacking 5-HT1B receptors. Pharmacology, Biochemistry and Behaviour, 57:407–412.

Rosenblum LA, Coplan JD, Friedman S, Basoff T, Gorman JM, Andrews MW (1994). Adverse early experinces affect noradrenergic and serotonergic functioning in adult primates. Biological Psychiatry, 35:221-227.

Ruhé HG, Mason NS, Schene AH (2007). Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. Molecular Psychiatry, 12(4):331-59.

Rusch BD, Abercrombie HC, Oakes TR, Schaefer SM, Davidson RJ (2001). Hippocampal morphometry in depressed patients and control subjects:

relations to anxiety symptoms Biological Psychiatry, 50:960-964.

Sakai K, Nakamura M, Ueno S, Sano A, Sakai N, Shirai Y, Saito N (2002). The silencer activity of the novel human serotonin transporter linked polymorphic regions. Neuroscience Letters, 327:13-16.

Sanders AR, Duan J, Gejman PV (2002). DNA variation and psychopharmacology of the human serotonin receptor 1B (HTR1B) gene. Pharmacogenomics, 6:745-762.

Sargent PA, Kjaer KH, Bench CJ, Rabiner EA, Messa C, Meyer J, Gunn RN, Grasby PM, Cowen PJ (2000). Brain serotonin 1A receptor binding measured by positron emission tomography with [11C]WAY-100635: effects of depression and antidepressant treatment. Archives of General Psychiatry, 57:174-180.

Sari Y (2004). Serotonin 1B receptors: from protein to psyhological function and behavior. Neuroscience and Biobehavioral Reviews, 28:565-582.

Saudou F, Amara DA, Dierich A, LeMeur M, Ramboz S, Segu L, Buhot MC, Hen R (1994). Enhanced aggressive behavior in mice lacking 5-HT1B receptor. Science, 265:1875–1878.

Savitz J, Drevets WC (2009a). Imaging phenotypes of major depressive disorder: genetic correlates. Neuroscience, 164:300-330.

Savitz J, Drevets WC (2009b). Bipolar and major depressive disorder: Neuroimaging the developmental-degenerative divide. Neuroscience and Biobehavioral Reviews, 33:699-771.

Savitz J, Lucki I, Drevets WC (2009). 5-HT1A receptor function in major

depressive disorder. Progress in Neurobiology, 88:17-31.

Scharinger C, Rabl U, Sitte HH, Pezawas L (2010). Imaging genetics of mood disorders. Neuroimage, 53(3):810-821.

Schlosser A, Kasper S (2009). The role of pharmacogenetics in the treatment of depression and anxiety disorders. International Clinical Psychopharmacology, 24:277-288.

Scruggs JL, Patel S, Bubser M, Deutch AY (2000). DOI-induced activation of the cortex; dependence on 5-HT2A heteroreceptors on thalamocortical glutamatergic neurons. The Journal of Neuroscience, 23:8846-8852.

Sellinger-Barnette MM, Mendels J, Frazer A (1980). The effect of psychoactive drugs on beta-adrenergic receptor binding sites in rat brain Neuropharmacology, 19:447-454.

Sen S, Burmeister M, Ghosh D (2004). Meta-analysis of the association between a serotonin transporter promoter polymorphism (5-HTTLPR) and anxiety-related personality traits. American Journal of Medical Genetics, Part B, Neuropsychiatric Genetics, 127:85-89.

Sette M, Raisman R, Briley M, Langer SZ (1981). Localisation of tricyclic antidepressant binding sites on serotonin nerve terminals Journal of Neurochemistry, 37:40-42.

Shaw DM, Camps FE, Eccleston EG (1967). 5-hydroxytryptamine in the hind-brain of depressive suicides The British Journal of Psychiatry, 113:1407-1411.

Sheline YI, Barch DM, Donnelly JM, Ollinger JM, Snyder AZ, Mintun MA (2001). Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study

Biological Psychiatry, 50:651-658.

Sheline YI, Gado MH, Price JL (1998). Amygdala core nuclei volumes are decreased in recurrent major depresion. Neuroreport, 9:2023-2028.

Sheline YI, Mintun MA, Barch DM, Wilkins C, Snyder AZ, Moerlein SM (2004). Decreased hippocampal 5-HT(2A) receptor binding in older depressed patients using [18F]altanserin positron emission tomography. Neuropsychopharmacology, 29: 2235-2241.

Sherman AD, Petty F (1984). Learned helplessness decreased (3H) imipramine binding in rat cortex Journal of Affective Disorders, 6:25-32.

Shi J, Potash JB, Knowles JA, Weissman MM, Coryell W, Scheftner WA, Lawson WB, DePaulo JR Jr, Gejman PV, Sanders AR, Johnson JK, Adams P, Chaudhury S, Jancic D, Evgrafov O, Zvinyatskovskiy A, Ertman N, Gladis M, Neimanas K, Goodell M, Hale N, Ney N, Verma R, Mirel D, Holmans P, Levinson DF (2010). Genome-wide associaton study of recurrent early-onset major depressive disorder. Molecular Psychiatry, Feb 2. E-pub, ahead of print

Shifman S, Bhomra A, Smiley S, Wray NR, James MR, Martin NG, Hettema JM, An SS, Neale MC, van den Oord EJCG, Kendler KS, Chen X, Boomsma DI, Middeldorp CM, Hottenga JJ, Slagboom PE, Filnt J (2008). A whole genome association study of neuroticism using DNA pooling. Molecular Psychiatry, 13:302-312.

Shih JC, Zhu Q, Chen K (1996). Determination of transcription initiation sites and promoter activity of the human 5-HT2A receptor gene. Behavioural Brain Research, 73:59-62.

Shimron-Abarbanell D, Erdmann J, Vogt IR Bryant SP, Spurr NK, Knapp M, Propping P, Noethen MM (1997). Human 5-HT5A receptor gene: systematic

screening for DNA sequence variation and linkage mapping on chromosome 7q34-q36 using a polymorphism in the 5' untranslated region. Biochemical and Biophysical Research Communications, 283:6-9.

Shin LM, Liberzon I (2010). The neurocircuitry of fear, stress and anxiety disorders. Neuropsychopharmacology, 35:169-191.

Shively CA, Friedman DP, Gage HD, Bounds MC, Brown-Proctor C, Blair JB, Henderson JA, Smith MA, Buchheimer N (2006). Behaviroal depression and positron emission tomography-determined serotonin 1A receptor binding potential in cynomolgus monkeys. Archives of General Psychiatry, 63:396-403.

Shyn SI, Shi J, Kraft JB, Potash JB, Knowles JA, Weissman MM, Garriock HA, Yokoyama JS, McGrath PJ, Peters EJ, Scheftner WA, Coryell W, Lawson WB, Jancic D, Gejman PV, Sanders AR, Holmans P, Slager SL, Levinson DF, Hamilton SP (2009). Novel loci for major depression identified by genome-wide association study of Sequenced Treatment Alternatives to Relieve Depression and meta-analysis of three studies. Molecular Psychiatry, E-pub, ahead of print

Siegel GJ, Albers RW, Brady ST, Price DL (2006). Basic Biochemistry Academic Press, New York, USA Seventh edititon, pages 227-248.

Siegle GJ, Steinhauer SR, Thase ME, Stenger VA, Carter CS (2002). Can’t shake that feeling: event-related fMRI assessment of sustained amygdala activity in response to emotional information in depressed individuals Biological Psychiatry, 51:693-707.

Sirviö J, Riekkinen P Jr, Jäkälä P, Riekkinen PJ (1994). Experimental studies on the role of serotonin in cognition. Progress in Neurobiology, 43:363-79.

Smeraldi E, Zanardi R, Benedetti F, Di Bella D, Perez J, Catalano M (1998).

Polymorphism within the promoter of the serotonin transporter gene and antidepressant efficacy of fluvoxamine. Molecular Psychiatry, 3(6):508-11.

Smith AK, Dimulescu I, Falkenberg VR, Narasimhan S, Heim C, Vernon SD, Rajeevan MS (2008). Genetic evaluation of the serotonergic system in chronic fatigue syndrome. Psychoneuroendocrinology, 33:188-197.

Snaith RP, Baugh SJ, Clayden AD, Husain A, Sipple MA (1982). The Clinical Anxiety Scale: an instrument derived from the Hamilton Anxiety Scale. The British Journal of Psychiatry, 141: 518–523.

Spielberger CD (1970). Manual for the State-Trait Anxiety Inventory. Consulting Psychologists Press, Palo Alto, CA, USA

Spindelegger C, Lanzenberger R, Wadsak W, Mien LK, Stein P, Mitterhauser M, Moser U, Holik A, Pezawas L, Kletter K, Kasper S (2009). Influence of escitalopram treatment on 5-HT(1A) receptor binding in limbic regions in patients with anxiety disorders. Molecular Psychiatry, 14(11):1040-50.

Spurlock G, Heils A, Holmans P, Williams J, D'Souza UM, Cardno A, Murphy KC, Jones L, Buckland PR, McGuffin P, Lesch KP, Owen MJ (1998). A family based association study of T102C polymorphism in 5HT2A and schizophrenia plus identification of new polymorphisms in the promoter. Molecular Psychiatry, 3:42-49.

Srivastava S, John OP, Gosling SD, Potter J (2003). Development of personality in early and middle adulthood: set like plaster or persistent change? Journal of Personality and Social Psychology, 84(5):1041-53.

Stanley M, Mann JJ (1983). Increased serotonin-2 binding sites in frontal cortex of suicide victims Lancet, 1:214-216.

Stanley M, Virgilio J, Gershon S (1984). Tritiated imipramine binding sites are decreased in the frontal cortex of suicides Science, 1337-1339.

Steffens DC, Byrum CE, McQuoid DR, Greenberg DL, Payne ME, Blitchington TF., MacFall JR, Krishnan KR (2000). Hippocampal volume in geriatric depression Biological Psychiatry, 48:301-309.

Steffens DC, McQuoid DR, Welsh-Bohmer KA, Krishnan KR (2003). Left orbital frontal cortex volume and performance on the benton visual retention test in older depressives and controls. Neuropsychopharmacology, 28:2179-2183.

Steinbusch HW and Nieuwenhuys R (1981). Localization of serotonin-like immunoreactivity in the central nervous system and pituitary of the rat, with special references to the innervation of the hypothalamus. Advances in Experimental Medicine and Biology, 133:7-35.

Strobel A, Gutknecht L, Rothe C, Reif A, Mossner R, Zheng Y et al. (2003). Allelic variation in 5-HT1A receptor expression is associated with anxiety and depression-related personality traits. Journal of Neural Transmission, 110:1445-1453.

Stutzmann GE, LeDoux JE (1999). GABAergic antagonists block the inhibitory effects of serotonin in the lateral amygdala: a mechanism for modulation of sensory inputs related to fear conditioning. The Journal of Neuroscience, 19(11):RC8.

Stutzmann GE, McEwen BS, LeDoux JE (1998). Serotonin modulation of sensory inputs to the lateral amygdala: dependency on corticosterone. The Journal of Neuroscience, 18(22):9529-38.

Sugden K, Tichopad Al, Khan N, Craig IW, D'Souza UM (2009). Genes within

the serotonergic system are differently expressed in human brain. BMC Neuroscience, 10:50.

Sullivan GM, Oquendo MA, Simpson N, van Heertum RL, Mann JJ, Parsey RV (2005). Brain serotonin 1A receptor binding in major depression is related to psychic and somatic anxiety. Biological Psychiatry, 58:947-954.

Sullivan PF, de Geus EJ, Willemsen G, James MR, Smit JH, Zandbelt T, Arolt V, Baune BT, Blackwood D, Cichon S, Coventry WL, Domschke K, Farmer A, Fava M, Gordon SD, He Q, Heath AC, Heutink P, Holsboer F, Hoogendijk WJ, Hottenga JJ, Hu Y, Kohli M, Lin D, Lucae S, Macintyre DJ, Maier W, McGhee KA, McGuffin P, Montgomery GW, Muir WJ, Nolen WA, Nöthen MM, Perlis RH, Pirlo K, Posthuma D, Rietschel M, Rizzu P, Schosser A, Smit AB, Smoller JW, Tzeng JY, van Dyck R, Verhage M, Zitman FG, Martin NG, Wray NR, Boomsma DI, Penninx BW (2009). Genome-wide association for major depressive disorder: a possible role for the presynaptic protein piccolo. Molecular Psychiatry, 14(4):359-75.

Sullivan PF, Neale MC, Kendler KS (2000). Genetic epidemilogy of major depression American Journal of Psychiatry, 157(10):1552-62.

Sulser F, Vetulani J, Mobley PL (516). Mode of acting of antidepressant drugs Biochemical Pharmacology, 27:257-261.

Sutcliffe JS, Delahanty RJ, Prasad HC, McCauley JL, Han Q, Jiang L, Li C, Folstein SE, Blakely RD (2005). Allelic heterogeneity at the serotonin transporter locus (SLC6A4) confers susceptibility to autism and rigid-compulsive behaviors. American Journal of Human Genetics, 77:265-279.

Svenningsson P, Chergui K, Rachleff I, Flajolet M, Zhang X, El Yacoubi M, Vaugeois, JM, Nomikos GG, Greengard P (2006). Alterations in 5-HT1B receptor function by p11 in depression-like states. Science, 311:77-80.

Szewczyk B, Albert PR, Burns AM, Czesak M, Overholser JC, Jurjus GJ, Meltzer HY, Konick LC, Dieter L, Herbst N, May W, Rajkowska G, Stockmeier CA, Austin MC (2009). Gender-specific decrease in NUDR and 5-HT1A receptor proteins in the prefrontal cortex of subjects with major depressive disorder. The International Journal of Neuropsychopharmacology, 12(2):155-68.

Tae WS, Kim SS, Lee KU, Nam EC, Kim KW (2008). Validation of hippocampal volumes measured using a manual method and two automated methods (FreeSurfer and IBASPM) in chronic major depressive disorder Neuroradiology, 50:569-581.

Tecott LH, Sun LM, Akana SF, Lowenstein DH, Dallmann MF, Julius D (1995). Eating disorder and epilepsy in mice lacking 5-HT2C serotonin receptors. Nature, 374:542-546.

Teicher MH, Dumont NL, Ito Y, Vaituzis C, Giedd JN, Andersen SL (2004). Childhood neglect is associated with reduced corpus callosum area. Biological Psychiatry, 56(2):80-85.

Terracciano A, Sanna S, Uda M, Deiana B, Usala G, Busonero F, Maschio A, Scally M, Patriciu N, Chen W-M, Distel MA, Slagboom EP, Boomsma DI, Villafuerte S, Sliwerska E, Burmeister M, Amin N, Janssens ACJW, van Duijn CM, Schlessinger D, Abecasisi GR, Costa PT Jr. (2010). Genome-wide association scan for five major dimensions of personality. Molecular Psychiatry, 15(6):647-56.

The International HapMap Consortium (2005). A haplotype map of the human genome. Nature, 437(7063):1299-1320.

Theodore WH, Hasler G, Giovacchini G, Kelley K, Reeves-Tyer P, Herscovitch P, Drevets WC (2007). Reduced hippocampal 5HT1A PET receptor binding

and depression in temporal lobe epilepsy. Epilepsia, 48:1526-1530.

Twarog BM, Page IH (1953). Serotonin content of some mammalian tissues and urine and a method for its determination. American Journal of Physiology, 175:157-61.

Uher R, McGuffin P (2008). The moderation by the serotonin transporter gene of environmental adversity in the aetiology of mental illness: review and methodological analysis. Molecular Psychiatry, 13(2):131-46.

Uher R, Huezo-Diaz P, Perroud N, Smith R, Rietschel M, Mors O, Hauser J, Maier W, Kozel D, Henigsberg N, Barreto M, Placentino A, Dernovsek MZ, Schulze TG, Kalember P, Zobel A, Czerski PM, Larsen ER, Souery D, Giovannini C, Gray JM, Lewis CM, Farmer A, Aitchison KJ, McGuffin P, Craig I (2009). Genetic predictors of response to antidepressants in the GENDEP project. The Pharmacogenomics Journal, (4):225-233.

Uher R, Perroud N, Ng MY, Hauser J, Henigsberg N, Maier W, Mors O, Placentino A, Rietschel M, Souery D, Zagar T, Czerski PM, Jerman B, Larsen ER, Schulze TG, Zobel A, Cohen-Woods S, Pirlo K, Butler AW, Muglia P, Barnes MR, Lathrop M, Farmer A, Breen G, Aitchison KJ, Craig I, Lewis CM, McGuffin P (2010). Genome-wide pharmacogenetics of antidepressant response in the GENDEP project. American Journal of Psychiatry, 167(5):555-564.

Vakili K, Pillay SS, Lafer B, Fava M, Renshaw PF, Bonello-Cintron CM, Yurgelun-Todd DA (2008). Hippocampal volume in primary unipolar major depression: a magnetic resonance imaging study Biological Psychiatry, 47:1087-1090.

Vallender EJ, Priddy CM, Hakim S, Yang H, Chen G-L, Miller GM (2008).

Functional variation in the 3' untranslated region of the serotonin transporter in human and rhesus macaque. Genes Brain and Behavior, 7:690-697.

van den Oord EJCG, Kuo P-H, Hartmann AM, Webb BT, Moeller H-J, Hettema JM, Giegling I, Bukszar J, Rujescu D (2008). Genomewide association analysis followed by a replication study implicates a novel candidate gene for neuroticism. Archives of General Psychiatry, 65(9):1062-1071.

van Praag HM (2004). Can stress cause depression? Progress in Neuro-Psychopharmacology and Biological Psychiatry, 28:891-907.

Varnas K, Hall H, Bonaventure P, Sedvall G (2001). Autoradiographic mapping of 5-HT(1B) and 5-HT(1D) receptors in the post mortem human brain using [(3)H]GR 125743. Brain Research, 915:47–57.

Vermetten E, Vythilingam M, Southwick SM, Charney DS, Bremner JD (2003). Long-term treatment with paroxetine increases verbal declarative memory and hippocampal volume in posttraumatic stress disorder. Biological Psychiatry, 54 (7): 693-702.

Videbech P, Ravnkilde B, Pedersen AR, Egander A, Landbo B, Rasmussen NA, Andersen F, Stødkilde-Jørgensen H, Gjedde A, Rosenberg R (2001). The Danish PET/depression project: PET findings in patients with major depression Psychological Medicine, 31:1147-1158.

Voineskos AN, Wilson AA, Boovariwala A, Sagrati S, Houle S, Rusjan P, Sokolov S, Spencer EP, Ginovart N, Meyer JH (2007). Serotonin transporter occupancy of high-dose selective serotonin reuptake inhibitors during major depressive disorder measured with [11C]DASB positron emission tomography. Psychopharmacology (Berlin), 193: 539-545.

von Gunten A, Fox NC, Cipolotti L, Ron MA (2000). A volumetric study of hippocampus and amygdala in depressed patients with subjective memory problems The Journal of Neuropsychiatry and Clinical Neurosciences, 12:493-498.

Wahlstedt H, Daniel C, Ensteroe M, Oehman M (2009). Large-scale mRNA sequencing determines global regulation of RNA editing during brain development. Genome Research, 19:978-986.

Walitza S, Wewetzer C, Warnke A, Gerlach M, Geller F, Gerber G, Görg T, Herpertz-Dahlmann B, Schulz E, Remschmidt H, Hebebrand J, Hinney A (2002). 5-HT2A promoter polymorphism -1438G/A in children and adolescnets with obsessive-compulsive disorder. Molecular Psychiatry, 7:1054-1057.

Walstab J, Hammer C, Boenisch H, Rappold G, Niesler B (2008). Naturally occuring variants in the HTR3B gene significantly alter properties of human heteromeric 5-hydroxytryptamine-3A/B receptors. Pharmacogenetics and Genomics, 18:793-802.

Walstab J, Rappold G, Niesler B (2010). 5-HT3 receptors: role in disease and target of drugs. Pharmacology and Therapeutics, Jul 16. E-pub, ahead of print

Watkins PC, Mathews A, Williamson DA, Fuller RD (1992). Mood-congruent memory in depression: emotional priming or elaboration? Journal of Abnormal Psychology, 101:581-586.

Weich S, Patterson J, Shaw R, Stewart-Brown S (2009). Family relationships in childhood and common psychiatric disorders in later life: systematic review of prospective studies. The British Journal of Psychiatry, 194(5):392-398.

Weisstaub NV, Zhou M, Lira A, Lambe E, Gonzalez-Maeso J, Hornung JP, Sibille E, Underwood M, Itohara S, Dauer WT, Ansorge MS, Morelli E, Mann JJ, Toth M, Aghajanian G, Sealfon SC, Hen R, Gingrich JA (2006). Cortical 5-

HT2A receptor signaling modulates anxiety-like behaviors in mice. Science, 313:536–540.

Wellman CL, Izquierdo A, Garrett JE, Martin KP, Carroll J, Millstein R, Lesch KP, Murphy DL, Holmes A (2007). Impaired stress-coping and fear extinction and abnormal corticolimbic morphology in serotonin transporter knock-out mice. The Journal of Neuroscience, 27(3):684-91.

Wendland JR, DeGuzman TB, McMahon F, Rudnick G, Detera-Wadleigh SD, Murphy DL (2008a). SERT Ileu425Val in autism, Asperger syndrome and obsessive–compulsive disorder. Psychiatric Genetics, 18:31-39.

Wendland JR, Kruse MR, Cromer KC, Murphy DL (2007). A large case–control study of common functional SLC6A4 and BDNF variants in obsessive–compulsive disorder. Neuropsychopharmacology, 32:2543-2551.

Wendland JR, Moya PR, Kruse MR, Ren-Patterson RF, Jensen CL, Timpano KR, Murphy DL (2008b). A novel, putative gain-of-function haplotype at SLC6A4 associates with obsessive–compulsive disorder. Human Molecular Genetics, 17:717-723.

Wilkie MJW, Smith G, Day RK, Matthews K, Smith D, Blackwood D, Reid IC, Wolf CR (2009). Polymorphisms in the SLC6A4 and HTR2A genes influence treatment outcome following antidepressant therapy. Pharmacogenomics Journal, 9:61-70.

Winter H, Irle E (2004). Hippocampal volume in adult burn patients with and without posttraumatic stress disorder. American Journal of Psychiatry, 161 (12):2194-2200.

Wisor JP, Wurts SW, Hall FS, Lesch KP, Murphy DL, Uhl GR, Edgar DM (2003). Altered rapid eye movement sleep timing in serotonin transporter knockout mice. Neuroreport, 14:233-238.

Wohlpart KL, Molinoff PB (1998). Regulation of levels of 5-HT2A receptor mRNA. Annals of the New York Acadademy of Sciences, 861:128-35.

Wolfe PD, Lee L (1977). Effect of serotonin precursor tryptophan on pituitary hormone secretion. The Journal of Clinical Endocrinology and Metabolism, 45:123-133.

Woolley DW, Shaw E (1954). A biological and pharmacological suggestion about certain mental disorders Proceedings of the National Academy of Sciences USA, 40(4):228-3.

World Health Organization (1992). The ICD-10 Classification of Mental and Behavioural Disorders: Clinical descriptions and diagnostic guidelines. World Health Organization, Geneva, Switzerland, Geneva

Wu J, Buchsbaum MS, Gillin JC, Tang C, Cadwell S, Wiegand M, Najafi A, Klein E, Hazen K, Bunney W E Jr, Fallon JH, Keator D (1999). Prediction of antidepressant effects of sleep deprivation by metabolic rates in the ventral anterior cingulate and medial prefrontal cortex. American Journal of Psychiatry, 156:1149-1158.

Wu S, Comings DE (1999). A common C-1018G polymorphism in the human 5-HT1A receptor gene. Psychiatric Genetics, 9:105-106.

Yamada K, Hattori E, Iwayama Y, Ohnishi T, Ohba H, Toyota T, Takao H, Minabe Y, Nakatani N, Higuchi T, Detera-Wadleigh SD, Yoshikava T (2006). Distinguishable haplotype blocks in the HTR3A and HTR3B region in japanese reveal evidence of association of HTR3B with female major depression. Biological Psychiatry, 60:192-201.

Yesavage JA, Bruik TL, Rose TL (1983). Development and validation of a

geriatric depression screening scale: A preliminary report. Journal of Psychiatric Research, 17:37-49.

Yu YW, Tsai SJ, Chen TJ, Lin CH, Hong CJ (2002). Association study of the serotonin transporter promoter polymorphism and symptomatology and antidepressant response in major depressive disorders, Molecular Psychiatry, 7(10):1115-1119.

Zhao S, Edwards J, Carroll J, Wiedholz L, Millstein RA, Jaing C, Murphy DL, Lanthorn TH, Holmes A (2006). Insertion mutation at the C-terminus of the serotonin transporter disrupts brain serotonin function and emotion-related behaviors in mice. Neuroscience, 140:321-334.

Zhou FC, Lesch KP, Murphy DL (2002). Serotonin uptake into dopamine neurons via dopamine transporters: a compensatory alternative. Brain Research, 942:109-119.

Zhu Q, Chen K, Shih JC (1995). Characterization of the human 5HT2A receptor gene promoter. The Journal of Neuroscience, 15:4885-4895.

Zhuang X, Gross C, Santarelli L, Compan V, Trillat AC, Hen R (1999). Altered emotional states in knockout mice lacking 5-HT1A or 5-HT1B receptors. Neuropsychopharmacology, 21:52-60.

Zung WWK (1965). A self-rating depression scale. Archives of General Psychiatry, 12:63–70.

10. Appendix

10.1 Imaging studies on depression and anxiety

10.1.1 5-HT neurochemistry and depression; imaging with Magnetic Resonance Imaging

The MRI technique gives a detailed map of the brain. In the most common form of MRI the hydrogen atoms are quantified. This technique is very valuable for detecting structural changes in the living brain, such as lesions, tumours and volume loss (Bear *et al.* 2007).

Recent technological advances have allowed neuroscientists to observe the activity of the nervous system in living subjects. The functional MRI (fMRI) method takes advantage of the fact that the oxygenated form of haemoglobin (oxyhaemoglobin) has a different magnetic resonance than the deoxygenated form. FMRI detects the locations of increased neuronal activity by measuring changes in blood oxygenation dependent (BOLD) signal (Bear *et al.* 2007). The BOLD signal is not measured in absolute units and so cannot be used to compare neural activity at rest between groups. Rather, BOLD signal is sensitive to changes evoked by mental activity. During fMRI sessions participants are given various tasks and the task induced blood oxygen level dependent (BOLD) signal change is measured in different brain areas (Hariri and Weinberger 2003).

Absolute quantification of regional metabolic activity and flow can be achieved using positron emission tomography (PET). The technique relies on the administration of trace amounts of positron emitting isotopes of oxygen or carbon. Emitted positrons are immediately annihilated by electrons to produce two photons that fly apart at 180 degrees. These are detected by a ring of photomultiplyer coincidence detectors around the head (the PET camera) that work out the line of flight and thus the location of the isotope in brain. The rate of clearance of a pulse of O15 labelled water is used to measure flow. [C^{15}] flourodeoxyglucose (FDG) uptake is used as a measure of metabolism. This relies on the property of deoxyglucose that it cannot be fully metabolised and so becomes trapped in cells in proportion to recent metabolic activity.

The retention of C15 labelled drugs detected by PET has been extensively used to measure the density of drug binding sites including receptors and uptake sites. In the serotonin system various radioligands have been used to estimate in 5HT uptake sites and 5-HT1A and 5-HT2A receptor binding sites in depression and the studies are summarised in Table 10.1 below.

Although the anatomical and physiological basis of depression is still the subject of extensive investigation, MDD most likely involves the limbic structures (amygdala and hippocampus), basal ganglia and some cortical regions some of which exert top-down regulatory control of limbic reponsiveness.

10.1.1.1 Functional changes in the amygdala in depression and anxiety disorders

The amygdala is found in the medial temporal lobe and is part of the limbic system. Its nuclei receive and projects to different brain areas with a major connection to the hypothalamus (Drago and Serretti 2008). The amygdala is thought to mediate the emotional aspects of life events and appears to play a primary role in colouring perceptual stimuli with emotion by emphasizing fear, anger and sadness (Gloor *et al.* 1982, Drago and Serretti 2008).

The finding of increased amygdala reactivity to negative stimuli is one of the most consistent findings in the literature both in depression and in various forms of anxiety disorder (PTSD, panic disorder, phobias, OCD) (for review see Savitz and Drevets 2009b, Etkin and Wagner 2007, Shin and Liberzon 2010, Damsa *et al.* 2008 Review). In addition, it seems that amygdala responses to negative words are detectable for longer periods of time in depressed patients than in healthy controls (Siegle *et al.* 2002). Similarly, patients with MDD reportedly remember negative better than positive words (Watkins *et al.* 1992), a finding that correlates with increased BOLD activity of the right amygdala (Hamilton and Gotlib 2008). In several studies the amygdala activation was positively correlated with depression and anxiety symptom severity (Savitz and Drevets 2009b, Shin and Liberzon 2010). Finally, an fMRI study involving 14 women found that greater amygdala activation to negative stimuli was associated with neuroticism, measured on the Big Five Inventory (Canli 2004). This is particularly interesting, as neuroticism is considered as a trait rather

than an illness. The apparent efficacy of antidepressant medications and cognitive-behaviour treatment in attenuating amygdala hyper-activity has been supported by other studies in depression (Sheline *et al.* 2001, Fu *et al.* 2004, Chen *et al.* 2008) and in anxiety disorders (Shin and Liberzon 2010, Damsa et al. 2008). Despite the majority of literature reports on increased activity of amygdala in depression and psychiatric disorders, there are some studies that have reported no changes or even decreased activity (Savitz and Drevets 2009b, Shin and Liberzon 2010, Damsa *et al*. 2008).

10.1.1.2 Morphological changes in the amygdala in depression and anxiety disorders

One study has found reduced connectivity between the left and right amygdala in MDD (Irwin *et al*. 2004). There is inconsistency in the volumetric MRI literature with studies reporting smaller left amygdala volumes in depressed patients (von Gunten *et al.* 2000, Caetano *et al.* 2007), no volume differences (Frodl *et al.* 2004, Frodl *et al.* 2007, MacMaster *et al.* 2008) and an increased volume in women (Lange and Irle 2004). In the anxiety literature finding varies between decreased amygdala volumes to no change (Shin and Liberzon 2010, Massana *et al.* 2003). This inconsistency may indicate that the volume itself is less important than the activity.

10.1.1.3 Functional changes in the hippocampus in depression and anxiety disorders

The hippocampus is a key structure for the encoding of emotionally relevant data into memory and interacts with the amygdala to provide input regarding the context in which stimuli occur (LaBar and Cabeza 2006). A functional study has found increased activity of right hippocampus in MDD (Videbech *et al*. 2001). Recovery from MDD was associated with either decreased or increased hippocampal metabolism, depending on whether the therapy was drug based (Kennedy *et al*. 2001) or cognitive behaviour based (Goldapple *et al.* 2004).

The anxiety literature is contradictory on functional changes in the hippocampus (Shin and Liberzon 2010, Damsa *et al*. 2008).

10.1.1.4 Morphological changes in the hippocampus in depression and anxiety disorders

Hippocampal volume reduction has been widely reported in the literature (Caetano *et al.* 2004, Lange and Irle 2004, MacQueen *et al.* 2003,O'Brien *et al.* 2004) particularly in the basolateral region (Steffens *et al.* 2000, Lloyd *et al.* 2004, MacMaster and Kusumakar 2004, Frodl *et al*. 2006), the left (Bremner *et al.* 2000, Tae *et al.* 2008, Mervaala *et al.* 2000) and the right regions (Bell-McGinty *et al.* 2002) indicating that hippocampal volume loss may be characteristic in depression. However, other studies did not find volume differences between depressed and control groups (von Gunten *et al.* 2000, Axelson *et al*. 1993, Ashtari *et al.* 1999, Vakili *et al.* 2008, Rusch *et al.* 2001, Hastings *et al.* 2004, Keller *et al.* 2008). Similarly, in anxiety hippocampal volume loss is supported by several studies, with most reporting symptom severity being inversely associated with the hippocampal volume which appears to increase after treatment with serotonin reuptake inhibitors (Shin and Liberzon 2010, Bremner *et al.* 2003, Lindauer et al. 2004, Lindauer *et al.* 2005, Lindauer *et al.* 2006, Winter and Irle 2004, Vermetten *et al*. 2003, Carmona *et al*. 2007).

10.1.1.5 Functional changes in the basal ganglia in depression

The basal ganglia are made up of the caudate nucleus, putamen, and globus pallidus and regulate voluntary movement, posture and muscle tone (Klein 2000).

Studies of the basal ganglia in depression seem more consistent than other regions. Most of the fMRI studies have found decreased activity in the caudate (Baxter *et al.* 1985, Drevets *et al.* 1992, Mayberg *et al.* 2005), striatum (Dunn *et al.* 2002, Wu *et al.* 1999) and the putamen (Kegeles *et al.* 2003). However, some studies did not find a reduced activity in these areas (Baxter *et al.* 1987, Videbech *et al.* 2001). In depressed patients exposure to sad faces caused either an exaggerated response in the left ventral striatum and caudate which improved after antidepressant treatment (Fu *et al.* 2004) or reduced regional cerebral blood flow in the ventral striatum in remitted patients (Neumeister *et al.* 2006). Other studies have observed an increase in activity in the caudate (Brody *et al.* 2001, Chen *et al.* 2007) of MDD

patients. Tryptophan depletion has also been shown to trigger increased activity of the ventral striatum (Neumeister *et al.* 2004b).

10.1.1.6 Morphological changes in the basal ganglia in depression

Several early studies reported basal ganglia volume reductions in MDD, such as smaller putamen (Husain *et al.* 1991), bilateral reduction in caudate nucleus (Krishnan *et al.* 1992), reduced volume of both caudate and putamen (Parashos *et al.* 1998) and greater left caudate atrophy in late-onset compared to early-onset MDD (Greenwald *et al.* 1997). Later studies using depressed, euthymic and remitted patients found no significant associations with any basal ganglia area volumes (Sheline *et al.* 1998, Kim *et al.* 1999, Lenze *et al.* 1999, Bremner *et al.* 2000, Hannestad *et al.* 2006).

10.1.1.7 Functional changes in cortical regions in depression and anxiety disorders

Three cortical regions have been widely reported in the imaging literature in connection with depression and these comprise the orbital frontal cortex, the ventro-medial prefrontal cortex and the dorso-lateral prefrontal cortex.

The orbitofrontal cortex integrates limbic data with sensory input thus providing a first pass analysis before feeding into higher level processing circuits such as the medial-prefrontal cortex. Both these regions are under the control of the dorsal-prefrontal cortex. The medial-prefrontal cortex acts together with the orbital frontal cortex to guide behaviour. According to a current model in depression and anxiety disorders; a deficit in the top-down inhibitory control of the dorso-lateral prefrontal cortex over the amygdala and limbic structures may result in chronic amygdala and limbic over-activity and negative emotions (Davidson 2002).

- Orbital frontal cortex

PET studies rather unanimously report increased metabolism and flow in OFC in depression. (Drevets *et al.* 2007, Biver *et al.* 1994, Drevets *et al*. 2002), which normalises after recovery on paroxetine antidepressant (Brody *et al.* 1999), after

deep brain stimulation (Mayberg *et al.* 2005) or cognitive behaviour therapy (Goldapple *et al.* 2004). One study investigating the activity of the OFC in anxiety reported increased activity in this region (Shin and Liberzon 2010 Review).

- Medial-prefrontal cortex

Studies have provided evidence of increased MDD-associated activity in the medial-prefrontal cortex (Drevets *et al*. 2007, Mayberg *et al.* 2005, Clark *et al.* 2006). It is possible that this elevated activity is characteristic of the acute depressed phase of the illness, as it was not present in the remitted patients (Drevets *et al.* 2007) or decreased after deep brain stimulation therapy (Mayberg *et al.* 2005) and sleep deprivation (Clark *et al.* 2006). Consistent with this data, an fMRI study reported a decreased response to sad facial stimuli in the right subgenual anterior cingulate cortex (sgACC) after antidepressant treatment (Keedwell *et al.* 2009).

The literature is inconsistent regarding activity changes in anxiety. The ventral regions showed mostly increased but sometimes decreased activity in various anxiety disorders (Shin and Liberzon 2010). The dorsal areas (dACC) responded with increased activity to various stimuli, which were decreased after antidepressant or cognitive-behaviour treatment (Shin and Liberzon 2010).

- Dorsolateral-prefrontal cortex

Hypometabolism of the dorsal PFC is one of the most robust findings in MDD (Hurwitz *et al.* 1990, Cohen *et al.* 1989, Biver *et al*. 1994, Bench *et al.* 1995, Dunn *et al.* 2002, Davidson *et al.* 2003, Chen *et al*. 2007) and in some studies appears to normalize with successful treatment (Hurwitz *et al.* 1990, Cohen *et al.* 1989, Mayberg *et al.* 2005, Kennedy *et al.* 2001, Fales *et al.* 2009).

This area in anxiety has not been extensively investigated. Two studies found evidence of decreased activity which increased following antidepressant treatment (Shin and Liberzon 2010).

10.1.1.8 Morphological changes in the cortical regions in depression and anxiety disorders

- The orbital frontal cortex

Morphometric OFC volume loss in depression is widely reported in the literature. Bilateral reduction of OFC in MDD has been reported in three studies (Lai *et al.* 2000, Steffens *et al.* 2003, Lavretsky *et al.* 2004), a 20-30% reduction in medial OFC (gyrus rectus) has been found in two studies (Ballmaier *et al.* 2004, Bremner *et al.* 2002) and an increased lesion of medial OFC has been correlated with the severity of depression in another study (MacFall *et al.* 2001). Not only the volume, but the integrity of the white matter tracts in the OFC is important. This was demonstrated by one study which found a negative association between integrity and the severity of depression (Nobuhara *et al.* 2006). Not every study found evidence of association between volume loss and depression (Parashos *et al.* 1998, Hastings *et al.* 2004, Janssen *et al*. 2004).

In certain anxiety disorders there are reports of reduction bilaterally (Atmaca *et al.* 2007, Atmaca *et al*. 2006) and in the left side (Kang *et al*. 2004, Choi *et al.* 2004).

- Medial-prefrontal cortex

The first imaging study of the medial prefrontal cortex in MDD patients with a family history of affective illness (Drevets *et al*. 1997) showed left hemisphere grey matter loss in the sgACC. Further, medication-free patients displayed reduced metabolism of the left sgACC as demonstrated by positron emission tomography. Since then other studies have also provided evidence for MDD-associated grey matter loss in the sgACC (Boteron *et al.* 2002, Hastings *et al.* 2004, Boes *et al.* 2008). A recent meta-analysis (Hajek *et al.* 2008) confirms that on average both left and right sgACC volumes are decreased in mood disorders, which especially effect the left hemisphere and that this appears to be driven by cases with a family history of illness.

Grey matter volume and density appeared to be smaller in anxiety disorders (Shin and Liberzon 2010), with one study suggesting that the grey matter density decrease may be an acquired condition rather than a familiar risk factor (Kasai *et*

al. 2008). In the rostral anterior cingulate cortex (rACC) increased white matter integrity has also been reported in anxiety (Shin and Liberzon 2010).

- Dorsolateral-prefrontal cortex

Severity of depression has been negatively correlated with grey matter volume of dorsolateral-prefrontal cortex (DLPFC) (Chen *et al.* 2007). Once again the literature is conflicting on this matter (Brody *et al.* 1999, Brody *et al*. 2001, Drevets *et al.* 2002). This may be due to the fact that the vast area of the cortex is subsumed under the term of DLPFC and this makes the data difficult to interpret.

In conclusion, there is strong evidence in the literature of increased amygdala reactivity to negative stimuli in depression and anxiety disorders, which can be reversed by antidepressant treatment. On the other hand, hippocampal volume loss also seems to be characteristic to these disorders. The early studies on volume loss in the basal ganglia were not confirmed by later studies, but decreased activity seems to be well established. As for the cortical regions, there is evidence for increased metabolism in the orbitofrontal cortex in depression (and perhaps in anxiety) which returns to normal after antidepressant treatment, psychotherapy or deep brain stimulation. Grey matter loss and reduced metabolism of the subgenual anterior cingulate cortex is characteristic to depression and anxiety. This metabolism increases in the acute phase of depression, and can be reverted by successful antidepressant treatment. Finally, the same is true for the dorsolateral-prefrontal cortex hypometabolism, which can be normalised by antidepressant treatment.

Table 10.1 Receptor binding studies in depression

Receptor	Radioligand	Subjects, Diagnostics and Medication status	Finding	Reference
HT1A	[^{11}C]WAY-100635	15 unmedicated MDD 20 medicated MDD 18 HC	Decreased BP in OFC, dACC, VLPFC, DLPFC in both MDD groups.	Sargent *et al.* 2000
HT1A	[^{11}C]WAY-100635	14 recovered depressed (SCID) medication free men 18 HC men	17% less BP of [^{11}C]WAY-100635 in recovered depressed in the in the cerebral cortex incl. amygdala and hippocampus, no difference in raphe nuclei.	Bhagwagar *et al.* 2004
HT1A	[^{11}C]WAY-100635	17 elderly depressed (SCID) medication free 17 HC elderly	37% less BP [^{11}C]WAY-100635 in dorsal raphe in depressed elderly patients but no difference in hippocampus.	Meltzer *et al.* 2004
HT1A	[^{11}C]WAY-100635	28 depressed (DSM-IV) medication free for at least 2 weeks	BP of HT1A receptor is positively correlated psychic anxiety and negatively with somatic anxiety in the anterior cingulate cortex, OPF cortex. No relationship between anxiety and BP of HT1A receptor in amygdala, hippocampus and raphe.	Sullivan *et al.* 2005
HT1A	[^{11}C]WAY-100635	28 depressed MDD mediation free 48 HC	No difference in BP of HT1A receptor between depressed and healthy subjects. But antidepressant naïve patients have higher BP of HT1A receptor in hippocampus and DLPFC than antidepressant experienced patients and healthy controls.	Parsey *et al*. 2006b
HT1A	[^{11}C]WAY-100635	21 depressed (Hamilton scale) medication and psychotherapy free 15HC	9-25% (average 19%) less binding of [^{11}C]WAY-100635 in amygdala, hippocampus and mPFC. No correlation between the BP of [^{11}C]WAY-100635 and the depressed patients' clinical condition.	Hirvonen *et al.* 2008
HT1A	[^{11}C]WAY-100635	16 depressed (DSM-IV) medication free 8 HC	43% less BP of [^{11}C]WAY-100635 in raphe, on average 26% lower in amygdala and hippocampus in depressed. No correlation	Drevets *et al.* 2007

			between the BP of [^{11}C]WAY-100635 and the depressed patients' clinical condition.	
HT1A	[^{11}C]WAY-100635	14 MDD (SCID) medication free 17 HC	No difference in BP of HT1A receptor either pre- or postsynaptical regions.	Mickey *et al.* 2008
HT1A	[^{11}C]WAY-100635	15 depressed (SCID) before and after antidepressant treatment (citalopram, venlafaxine or both)	No reliable effect of antidepressant drug on BP of HT1A receptor in amygdala, hippocampus and cerebral cortex.	Moses-Kolko *et al.* 2007
HT1A	[^{18}F]FCWAY	45 temporal lobe epileptic depressed patients (Beck depression inventory) medication free 10 HC	18% less binding of [^{18}F]FCWAY in depressed epileptics in the hippocampal region compared to healthy subjects.	Theodore *et al.* 2007
HT1A	[^{18}F]MPPF	8 remitted depressive (SCID) medicated with citalopram	Tryptophan depletion caused depression symptoms to reappear in most of the patients, but it had no effect on the BP of [^{18}F]MPPF in frontal, temporal and cingulate cortex and hippocampus.	Prashak-Rieder *et al.* 2004
HT1A	[^{18}F]FCWAY	9 PD and 7PD+MDD (SCID) medication free 15HC	Reduced HT1A receptor binding in PD patients' anterior and posterior cingulate cortex and raphe. No difference between patients with or without MDD.	Neumeister *et al.* 2004
HT1A	[^{11}C]WAY-100635	9 PD, unmedicated 7 recovered after SSRI medication 19 HC	Reduced HT1A receptor binding in patients with PD in the raphe, amygdala and OFC, pre- and postsynaptically. In recovered patients only presynaptic binding was reduced.	Nash *et al.* 2008
HT1A	[^{11}C]WAY-100635	12 males, SAD, unmedicated 18 HC	Lower HT1A receptor BP in the hippocampus, anterior cingulate cortex, amygdala, raphe, OFC.	Lanzenberger et al. 2007
HT1A	[^{11}C]WAY-100635	12 patients with anxiety disorders	Significant reduction in HT1A receptor binding after 12 weeks of escitalopram in patients with anxiety disorders.	Spindelegger *et al*. 2009

SERT	[^{11}C]McNeil-5652	19 depressed unmedicated (SCID) 41HC	27% less binding in the amygdala, midbrain and cingulate of non-remitted depressed patients than in HC.	Miller *et al.* 2008
SERT	[^{11}C]McNeil-5652	25 patients with current MDD unmedicated (DSM-IV) 41 HC	22% less binding in the amygdala and in the raphe. Reduced SERT BP was most pronounced in patient who never received AD medication. The SERT BP was not correlated with the severity of depression.	Parsey *et al.* 2006a
SERT	[^{11}C]McNeil-5652	4 depressed (SCID) 4 HC	15-20% more binding in the left frontal and the right cingulate cortex of depressed.	Reivich *et al*. 2004
SERT	[^{11}C]DASB	20 patients with current MDD (SCID) unmedicated 20 HC	The BP of [^{11}C]DASB was not different in brain areas (including prefrontal and cingulate cortex, putamen and midbrain) in depressed and in HC.	Meyer *et al.* 2004
SERT	[^{11}C]DASB	24 recovered depressed males, unmedicated 20 HC	No difference in binding in the cerebral cortex.	Milham *et al.* 2005
SERT	[^{11}C]DASB	18 depressed (SCID) 34 HC	On average 20% more binding in the cingulate cortex and raphe in depressed, but the severity of depression correlated negatively with the BP of [^{11}C]DASB in the cingular cortex (paradox)	Cannon *et al.* 2007
SERT	[^{11}C]DASB	7 depressed with Parkinson disease, unmedicated with AD 7 HC	Parkinson patients showed increased binding of [^{11}C]DASB in the prefrontal cortical regions and the severity of depression correlated with the [^{11}C]DASB in the OFC.	Boileau *et al.* 2008
SERT	[^{11}C]DASB	10 depressed (DSM-IV) unmedicated 19 HC	No difference in the BP of [^{11}C]DASB in amygdala and midbrain between groups. Less binding of [^{11}C]DASB in anterior cingulate in depressed.	Reimold *et al*. 2008
SERT	[^{11}C]DASB	12 depressed medicated with high dose of venlafaxine, citalopram or sertraline 12 HC	High dose AD produced 84-98% of serotonin transporters in midbrain and cerebral cortex of depressed.	Voineskos et al. 2007

HTR2A	[^{18}F]Altanserin	46 depressed (DSM-IV) medication free 29 HC	29% less binding in hippocampus of depressed, no difference in cerebral cortex.	Mintun *et al.* 2004
HTR2A	[^{18}F]Altanserin	16 depressed (DSM-IV) medication free 9 HC	39% less binding in hippocampus of depressed, no difference in cerebral cortex.	Sheline *et al.* 2004
HTR2A	[^{11}C]MDL100, 907	20 recovered depressive (SCID) medication free 20 HC	More binding in certain cortical regions of recovered depressed (among them the frontal cortex).	Bhagwagar *et al*. 2006
HTR2	[^{18}F]Setoperone	22 depressed (SCID) medication free 22 HC	21-29% more binding in the cerebral cortex in depressed with high dysfunctional attitude. No correlation to the duration of illness and past use of antidepressant drugs.	Meyer *et al*. 2003
HTR2	[^{18}F]FESP	19 drug-naïve depressed (DSM-IV) 15 paroxetine treated remitted 20 HC	20-26% less binding in the cerebral cortex of antidepressant naïve depressed compared to controls.	Messa *et al.* 2003

Abbreviations

BP: binding potential
MDD: major depressive disorder
PD: panic disorder
SAD: social anxiety disorder
SCID:
DSM-IV
HC: healthy controls
SSRI: selective serotonin reuptake inhibitor
AD: antidepressant

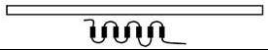
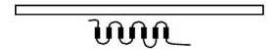
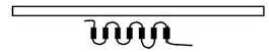
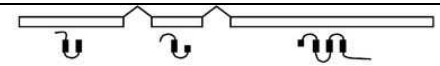
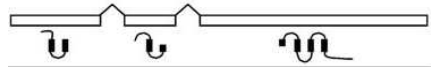
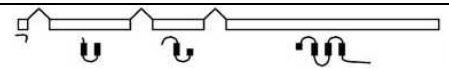
Tracers:
[^{11}C]WAY-100635: *N*-[2-[4-(2-Methoxyphenyl)-1-piperazynil] ethyl]-*N*-2-pyridinyl-cyclohexylcarboxamide
[^{18}F]FCWAY: *N*-[2-[4-(2-Methoxyphenyl)-1-piperazynil] ethyl]-*N*-2-pyridinyl-*trans*-4-fluorocyclohexylcarboxamide
[^{18}F]MPPF: 4-Fluoro-*N*-[2-[4-(2-Methoxyphenyl)-1-piperazynil] ethyl]-*N*-2-pyridinylbenzamide

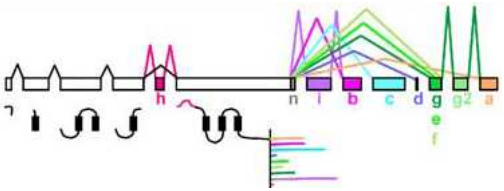
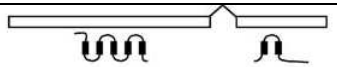
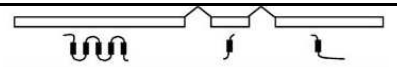
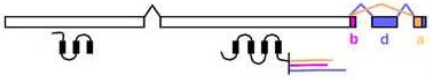
[^{11}C]McNeil-5652: (6S, 10bR)-1,2,3,5,6,10b-Hexahydro-6-[4(methylthio)phenyl]-pyrrolo[2,1-a] isoqunoline
[^{11}C]DASB: 3-Amino-4-[[2-[(dimethylamino) methyl] phenyl] thio] benzonitrile
[^{18}F]Altanserin: 3-(2-(4-(4-Fluorobenzoyl)-1-piperidinyl)ethyl)-2,3-dihydro-2-thioxo-4(1*H*)-quinazolinone
[^{11}C]MDL100,907: (*R*)-1-[2-(4-Fluorophenyl)ethyl]-(4-(2,3-dimethoxyphenyl)-4-piperidinemethano
[^{18}F]Setoperone: 6-[2-[4-(4-Fluorobenzoyl)-1-piperidinyl]ethyl]-2,3-dihydro-7-methyl-5*H*-thiazolo[3,2a]pyrimidin-5-one
[^{18}F]FESP: 3-(2-Fluoroethyl)-8-[4-(4-fluorophenyl)-4-oxobutyl]-1-phenyl-1,3,8-triazospiro[4.5]decan-4-one

Brain regions:
OFC: orbitofrontal cortex
dACC: dorsal anterior cingulate cortex
VLPFC: ventrolateral prefrontal cortex
DLPFC: dorsolateral prefrontal cortex

10.2 Genetics of the serotonergic system

Table 10.2. Genome position of genes investigated in this study

Gene	Chr	Position (hg19)	Size (bp)	Strand	Exon (coding)	Gene structure	Amino acid	Remark
HTR1A	5	63,256,279-63,257,546	1,268	Rev	1 (1)		422	
HTR1B	6	78,171,948-78,173,120	1,173	Rev	1 (1)		390	
HTR1D	1	23,518,389-23,521,222	2,834	Rev	1 (1)		377	
HTR1E	6	87,647,024-87,726,390	79,367	Fwd	2 (1)		365	
HTR1F	3	88,031,726-88,042,919	11,194	Fwd	2 (1)		366	
HTR2A	13	47,407,513-46,470,369	62,857	Rev	4 (3)		471	
HTR2B	2	231,972,952-231,989,824	16,873	Rev	4 (3)		481	
HTR2C	X	113,818,551 - 114,144,624	326,074	Fwd	7 (4)		458	
HTR3A	11	113,845,797-113,861,032	15,236	Fwd	9 (9)		478	
HTR3B	11	113,775,589-113,817,282	41,694	Fwd	9 (9)		441	
HTR3C	3	183,770,835 - 183,778,459	7,625	Fwd	9 (9)		447	5-HT receptor 3 subunit C
HTR3D	3	183,750,619-183,757,156	6,538	Fwd	6 (4)		233	5-HT receptor 3 subunit D
HTR3E	3	183,817,967 - 183,824,782	6,816	Fwd	9 (9)		456	5-HT receptor 3 subunit E

HTR4	5	147,861,120-148,033,741	172,622	Rev	8 (7)		387	Several isoforms
HTR5A	7	154,862,546-154,877,459	14,914	Fwd	2 (2)		357	
HTR6	1	19,991,780-20,006,054	14,275	Fwd	3 (3)		440	
HTR7	10	92,500,578-92,617,671	117,094	Rev	4 (4)		445	3 isoforms
SLC6A4	17	28,523,380-28,562,954	39,575	Rev	15 (13)		630	

Abbreviations:
Genes
HTR1A: 5-hydroxytryptamine receptor 1A
HTR1B: 5-hydroxytryptamine receptor 1B
HTR1D: 5-hydroxytryptamine receptor 1D
HTR1E: 5-hydroxytryptamine receptor 1E
HTR1F: 5-hydroxytryptamine receptor 1F
HTR2A: 5-hydroxytryptamine receptor 2A
HTR2B: 5-hydroxytryptamine receptor 2B
HTR2C: 5-hydroxytryptamine receptor 2C
HTR3A: 5-hydroxytryptamine receptor 3A
HTR3B: 5-hydroxytryptamine receptor 3B
HTR3C: 5-hydroxytryptamine receptor 3C
HTR3D: 5-hydroxytryptamine receptor 3D
HTR3E: 5-hydroxytryptamine receptor 3E
HTR4: 5-hydroxytryptamine receptor 4
HTR5: 5-hydroxytryptamine receptor 5
HTR6: 5-hydroxytryptamine receptor 6
HTR7: 5-hydroxytryptamine receptor 7
SLC6A4: 5-hydroxytryptamine transporter

Fwd: forward direction, (+) strand

Rev: Reverse direction, (-) strand
Chr: Chromosome
Source: UCSC Genome Browser (http://www.genome.ucsc.edu/).

10.3 Questionnaires used in this study

10.3.1 Beck Depression Inventory

- A (Mood)
  0 I do not feel sad
  1 I feel blue or sad
  2.a I am blue or sad all the time and I can't snap out of it
  2.b I am so sad or unhappy, that it is very painful
  3 I am so sad or unhappy, that I can't stand it
- B. (Pessimism)
  0 I am not particularly pessimistic or discouraged about the future
  1.a I feel discouraged about the future
  2.a I feel I have nothing to look forward to
  2.b I feel that I won't get over my troubles
  3 I feel that the future is hopeless and things cannot improve
- C (Sense of Failure)
  0 I do not feel like a failure
  1 I feel I have failed more than the average person
  2.a I feel I have accomplished very little that is worthwhile or that means anything
  2.b As I look back on my life all I can see is a lot of failures
  3 I feel I am a complete failure as a person (parent, husband, wife)
- D (Lack of Satisfaction)
  0 I am not particularly dissatisfied
  1.a I feel bored most of the time
  1.b I don't enjoy things the way I used to
  2 I don't get satisfaction out of anything any more
  3 I am dissatisfied with everything
- E (Guilty Feeling)
  0 I don't feel particularly guilty
  1 I feel bad or unworthy a good part of the time
  2.a I feel quite guilty
  2.a I feel bad or unworthy practically all the time now
  3 I feel as though I am very bad or worthless
- F (Sense of Punishment)
  0 I don't feel I am being punished
  1 I have a feeling that something bad may happen to me
  2 I feel I am being punished or will be punished
  3.a I feel I deserve to be punished
  3.b 1 want to be punished
- G (Self Hate)
  0 I don't feel disappointed in myself
  1.a I am disappointed in myself
  1.b I don't like myself
  2 I am disgusted with myself
  3 I hate myself
- H (Self Accusations)

0 I don't feel I am any worse than anybody else
1 I am very critical of myself for my weaknesses or mistakes
2.a I blame myself for everything that goes wrong
2.b I feel I have many bad faults

- I (Self-punitive Wishes)
  0 I don't have any thoughts of harming myself
  1 I have thoughts of harming myself but I would not carry them out
  2.a I feel I would be better off dead
  2.b I have definite plans about committing suicide
  2.c I feel my family would be better off if I were dead
  3 I would kill myself if I could
- J (Crying Spells)
  0 I don't cry any more than usual
  1 I cry more now then I used to
  2 I cry all the time now. I can't stop it
  3 I used to be able to cry but now I can't cry at all even though I want to
- K (Irritability)
  0 I am no more irritated now than I ever am
  1 I get annoyed or irritated more easily than I used to
  2 I feel irritated all the time
  3 I don't get irritated at all at the things that used to irritate me
- L (Social Withdrawal)
  0 I have not lost interest in other people
  1 I am less interested in other people now than I used to be
  2 I have lost most of my interest in other people and have little feeling for them
  3 I have lost all my interest in other people and don't care about them at all
- M (Indecisiveness)
  0 I make decisions about as well as ever
  1 I am less sure of myself now and try to put off making decisions
  2 I can't make decisions any more without help
  3 I can't make any decisions at all any more
- N (Body Image)
  0 I don't feel I look any worse than I used to
  1 I am worried that I am looking old or unattractive
  2 I feel that there are permanent changes in my appearance and they make me look unattractive
  3 I feel that I am ugly or repulsive looking
- O (Work Inhibition)
  0 I can work about as well as before
  1.a It takes extra effort to get started at doing something
  1.b I don't work as well as I used to
  2 I have to push myself very hard to do anything
  3 I can't do any work at all
- P (Sleep Disturbance)
  0 I can sleep as well as usual
  1 I wake up more tired in the morning than I used to
  2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep
  3 I wake up early every day and can't get more than 5 hours sleep

- Q (Fatigability)
  0 I don't get any more tired than usual
  1 I get tired more easily than I used to
  2 I get tired from doing anything
  3 I get too tired to do anything
- R (Loss of Appetite)
  0 My appetite is no worse than usual
  1 My appetite is not as good as it used to be
  2 My appetite is much worse now
  3 I have no appetite at all any more
- S (Weight Loss)
  0 I haven't lost much weight, if any, lately
  1 I have lost more than 5 pounds
  2 I have lost more than 10 pounds
  3 I have lost more than 15 pounds
- T (Somatic Preoccupation)
  0 I am no more concerned about my health than usual
  1 I am concerned about aches and pains *or* upset stomach *or* constipation *or* other unpleasant feelings in my body
  2 I am so concerned with how I feel or what I feel that it's hard to think of much else
  3 I am completely absorbed in what I feel
- U (Loss of Libido)
  0 I have not noticed any recent change in my interest in sex
  1 I am less interested in sex than used to be
  2 I am much less interested in sex now
  3 I have lost interest in sex completely

Participants should circle one statement/question.

10.3.2 Eynseck Personality Questionnaire

(Neuroticism questions are underlined)

- Do you have many different hobbies?
- Do you stop to think things over before doing anything?
- Does your mood often go up and down?
- Have you ever taken the praise for something you knew someone else had really done?
- Are you a talkative person?
- Would being in debt worry you?
- Do you ever feel just miserable for no reason?
- Were you ever greedy by helping yourself to more than your share of anything?
- Do you lock up the house carefully at night?
- Are you rather lively?
- Would it upset you to see a child or animal suffer?
- Do you often worry about things you should have done or said?
- If you say you will do something, do you always keep your promise no matter how inconvenient it might be?

- Can you usually let yourself go and enjoy yourself at a lively party?
- Are you an irritable person?
- Have you ever blamed someone for doing something you knew was really your fault?
- Do you enjoy meeting new people?
- Do you believe insurance schemes are a good idea?
- Are your feelings easily hurt?
- Are all your habits good and desirable ones?
- Do you tend to keep in the background on social occasions?
- Would you take drugs, which may have strange or dangerous effects?
- Do you often feel fed-up?
- Have you ever taken anything (even a pin or a button) that belonged to someone else?
- Do you like going out a lot?
- Do you enjoy hurting people you love?
- Are you often troubled about feelings of guilt?
- Do you sometimes talk about things you know nothing about?
- Do you prefer reading to meeting people?
- Do you have enemies that want to harm you?
- Would you call yourself a nervous person?
- Do you have many friends?
- Do you enjoy practical jokes that can sometimes really hurt people?
- Are you a worrier?
- As a child did you do as you were told immediately and without grumbling?
- Would you call yourself happy-go-lucky?
- Do good manners and cleanliness matter much to you?
- Do you worry about awful things that might happen?
- Have you ever broken or lost something belonging to someone else?
- Do you usually take the initiative in making new friends?
- Would you call yourself tense or highly-strung?
- Are you mostly quiet when you are with other people?
- Do you think marriage is old-fashioned and should be done away with?
- Do you sometimes boast a little?
- Can you easily get some life into a rather dull party?
- Do people who drive carefully annoy you?
- Do you worry about your health?
- Have you ever said anything bad or nasty about anyone?
- Do you like telling jokes and funny stories to your friends?
- Do most things taste the same to you?
- As a child were you ever cheeky to your parents?
- Do you like mixing with people?
- Does it worry you if you know there are mistakes in your work?
- Do you suffer from sleeplessness?
- Do you always wash before a meal?
- Do you nearly always have a ready answer when people talk to you?
- Do you like to arrive at appointments in plenty of time?
- Have you often felt listless or tired for no reason?
- Have you ever cheated at a game?
- Do you like doing things in which you have to act quickly?

- Is (or was) your mother a good woman?
- Do you often feel life is very dull?
- Have you ever taken advantage of someone?
- Do you often take on more activities than you have time for?
- Are there several people who keep trying to avoid you?
- Do you worry a lot about your looks?
- Do you think people spend too much time safeguarding their future with savings and insurances?
- Have you ever wished that you were dead?
- Would you ever dodge paying taxes if you were sure you could never be found out?
- Can you get a party going?
- Do you try not to be rude to people?
- Do you worry too long after an embarrassing experience?
- Have you ever insisted on having your own way?
- When you catch a train do you often arrive at the last minute?
- Do you suffer from nerves?
- Do your friendships break up easily without it being your fault?
- Do you often feel lonely?
- Do you always practice what you preach?
- Do you like sometimes teasing animals?
- Are you easily hurt when people find fault with you or the work you do?
- Have you ever been late for an appointment or work?
- Do you like plenty of bustle and excitement around you?
- Would you like other people to be afraid of you?
- Are you sometimes bubbling over with energy and sometimes very sluggish?
- Do you sometimes put off until tomorrow what you ought to do today?
- Do other people think of you as being very lively?
- Do people tell you a lot of lies?
- Are you touchy about some things?
- Are you always willing to admit it when you have made a mistake?
- Would you feel very sorry for an animal caught in a trap?

Possible answers: yes or no to each question.

10.3.3 Cornell Medical Index

10.3.3.1 Personality

- Do you sweat or tremble a lot during examinations or questioning?
- Do you get nervous and shaky when approached by a superior?
- Does your work fall to pieces when someone is watching you?
- Does your thinking get completely mixed up when you have to do things quickly?
- Must you do things very slowly in order to do them without mistakes?
- Do you always get directions and orders wrong?
- Do strange people or places make you afraid?
- Are you scared to be alone when there are no friends near you?
- Is it always hard for you to make up your mind?

- Do you wish you always had someone at your side to advise you?
- Are you considered a clumsy person?
- Does it bother you to eat anywhere except in your own home?
- Do you feel alone and sad at a party?
- Do you usually feel unhappy and depressed?
- Do you often cry?
- Are you always miserable and blue?
- Does life look entirely hopeless?
- Do you often wish you were dead and away from it all?
- Does worrying continually get you down?
- Does worrying run in your family?
- Does every little thing get on your nerves and wear you out?
- Are you considered a nervous person?
- Does nervousness run in your family?
- Did you ever have a nervous breakdown?
- Did anyone in your family ever have a nervous breakdown?
- Were you ever a patient in a mental hospital (for your nerves)?
- Was anyone in your family ever a patient in a mental hospital (for their nerves)?
- Are you extremely shy or sensitive?
- Do you come from a shy or sensitive family?
- Are your feelings easily hurt?
- Does criticism always upset you?
- Are you considered a touchy person?
- Do people usually misunderstand you?
- Do you have to be on your guard, even with friends?
- Do you always do things on sudden impulse?
- Are you easily upset or irritated?
- Do you go to pieces if you don't constantly control yourself?
- Do little annoyances get on your nerves and make you angry?
- Does it make you angry to have anyone tell you what to do?
- Do people often annoy and irritate you?
- Do you flare up in anger if you can't have what you want right away?
- Do you often get into a violent rage?
- Do you often shake or tremble?
- Are you constantly keyed up and jittery?
- Do sudden noises make you jump or shake badly?
- Do you tremble or feel weak whenever someone shouts at you?
- Do you become scared at sudden movements or noises at night?
- Are you often awakened out of your sleep by frightening dreams?
- Do frightening thoughts keep coming back in your mind?
- Do you often become suddenly scared for no good reason?
- Do you often break out in a cold sweat?

Possible answers: yes or no to each question.

10.3.3.2 CMI Cardiovascular system

- Has a doctor ever said that your blood pressure was too high?
- Has a doctor ever said that your blood pressure was too low?
- Do you have pains in the heart or chest?
- Are you often bothered by thumping of the heart?
- Does your heart often race like mad?
- Have you ever had a heart attack?
- Do you often have difficulty breathing?
- Do you get out of breath long before anyone else?
- Do you sometimes get out of breath just sitting still?
- Are your ankles often badly swollen?
- Do cold hands or feet trouble you even in hot weather?
- Do you suffer from frequent cramps in your legs?
- Has your doctor ever said you have heart trouble?
- Does heart trouble run in your family?
- Do you suffer from any circulatory problems?

Possible answers: yes or no to each question.

10.3.3.3 CMI Fatigue

- Do you often get spells of complete exhaustion or fatigue?
- Does working tire you out completely?
- Do you usually get up tired and exhausted in the morning?
- Does every little effort wear you out?
- Are you constantly too tired and exhausted even to eat?
- Do you suffer from severe nervous exhaustion?
- Does nervous exhaustion run in your family?
- Are you frequently ill?
- Are you frequently confined to bed by illness?
- Are you always in poor health?
- Are you considered a sickly person?
- Do you come from a sickly family?
- Do severe aches and pains make it impossible for you to do your work?
- Do you wear yourself out worrying about your health?
- Are you always ill and unhappy?

Possible answers: yes or no to each question.

10.3.3.4 CMI Sleep disorders and Addiction

- Do you usually have great difficulty in falling asleep or staying asleep?
- Do you find it impossible to take a regular rest period each day?
- Do you find it impossible to take regular daily exercise?
- Do you smoke?
- Do you smoke more than 20 cigarettes a day?
- Do you drink more than six cups of coffee or tea a day?
- Do you usually take two or more alcoholic drinks a day?

Possible answers: yes or no to each question.

10.3.4 Yesavage Geriatric Depression Inventory

- Are you basically satisfied with your life?
- Have you dropped many of your activities and interests?
- Do you feel that your life is empty?
- Do you often get bored?
- Are you hopeful about the future?
- Are you bothered by thoughts you can't get out of your head?
- Are you in good spirits most of the time?
- Are you afraid that something bad is going to happen to you?
- Do you feel happy most of the time?
- Do you often feel helpless?
- Do you often get restless and fidgety?
- Do you prefer to stay at home, rather than going out and doing new things?
- Do you frequently worry about the future?
- Do you feel you have more problems with memory than most?
- Do you think it is wonderful to be alive now?
- Do you often feel downhearted and blue?
- Do you feel pretty worthless the way you are now?
- Do you worry a lot about the past?
- Do you find life very exciting?
- Is it hard for you to get started on new projects?
- Do you feel full of energy?
- Do you feel that your situation is hopeless?
- Do you think that most people are better off than you are?
- Do you frequently get upset over little things?
- Do you frequently feel like crying?
- Do you have trouble concentrating?
- Do you enjoy getting up in the morning?
- Do you prefer to avoid social gatherings?
- Is it easy for you to make decisions?
- Is your mind as clear as it used to be?

Possible answers are: yes or no to each question.

10.3.5 Big Five Inventory (BFI-44)

I see myself as someone who:

- Is talkative
- Tends to find fault with others
- Does a thorough job
- Is depressed, blue
- Is original, comes up with new ideas
- Is reserved
- Is helpful and unselfish with others

- Can be somewhat careless
- Is relaxed, handles stress well
- Is curious about many different things
- Is full of energy
- Starts quarrels with others
- Is a reliable worker
- Can be tense
- Is ingenious, a deep thinker
- Generates a lot of enthusiasm
- Has a forgiving nature
- Tends to be disorganized
- Worries a lot
- Has an active imagination
- Tends to be quiet
- Is generally trusting
- Tends to be lazy
- Is emotionally stable, not easily upset
- Is inventive
- Has an assertive personality
- Can be cool and aloof
- Perseveres until the task is finished
- Can be moody
- Values artistic, aesthetic experiences
- Is sometimes shy, inhibited
- Is considerate and kind to almost everyone
- Does thing efficiently
- Remains calm in tense situations
- Prefers work that is routine
- Is outgoing, sociable
- Is sometimes rude to others
- Makes plans and follows through them
- Gets nervous easily
- Likes to reflect, play with ideas
- Has few artistic interest
- Likes to cooperate with others
- Is easily distracted
- Is sophisticated in art, music and literature

Participants are asked to mark how much they agree with each statement (disagree strongly, disagree a little, neither agree nor disagree, agree a little, agree strongly). Neuroticism items are underlined.

10.3.6 Brief Symptom Inventory

How much were you distressed by:

- *Nervousness or shakiness inside*
- Trouble remembering things
- Thought of ending your life
- Poor appetite

- *Suddenly scared for no reason*
- Feeling blocked in getting things done
- Feeling lonely
- Feeling blue
- Feeling no interest in things
- *Feeling fearful*
- Your feeling easily hurt
- Feeling that people are unfriendly or dislike you
- Feeling inferior to others
- Trouble falling asleep
- Having to check and double-check what you do
- Difficulty making decisions
- Your mind going blank
- Feeling hopeless about the future
- Trouble concentrating
- *Feeling tense or keyed up*
- Thoughts of death or dying
- Feeling very self-conscious with others
- *Spells of terror or panic*
- *Feeling so restless you couldn't sit still*
- Feeling of worthlessness
- Feeling of guilt

Participants are asked to mark how much each problem has distressed them or bothered them during the past week. Possibilities are: not at all, a little bit, moderately, quite a bit, extremely. Depression items are underlined, anxiety items are in italics.

10.3.7 Life Events Questionnaire

Event:

- You yourself suffered a serious illness, injury or an assault
- A serious illness, injury or assault happened to a close relative
- Your parent, child, spouse died
- A close family friend or another relative (aunt, cousin, grandparent) died
- You had a separation due to marital difficulties
- You broke off a steady relationship
- You had a serious problem with a close friend, neighbour or relative
- You became unemployed or you were seeking work unsuccessfully for more than one month
- You were sacked from your job
- You had a major financial crisis
- You had problems with the police and a court appearance
- Something you valued was lost or stolen.
- Additional question; In the last year has anything else serious happened? (Please, specify.)

Participants were asked to indicate whether or not each event has happened to them in the past. Possibities: it did not happen, it happened in the last 2 months, it happened in the last year, it happened in more than a year. Then they were asked to

decide, how much each event still affects them. Possibilities: still strongly affects me, doesn't affect me strongly.

10.3.8 Childhood Trauma Questionnaire

When I was growing up:

- I was happy
- I believe that I was abused or neglected
- People in my family looked out for each other
- My parents/guardians weren't able to take care of me

Participants were asked to decide how much each statement is true for them. Possibilities: never true, rarely true, sometimes true, often true, very often true.

Additional questions about lost parents
When I was growing up:

- I lost my mother
- I lost my father

Possible answers; yes or no.

10.4 Primers and probes used in the New Mood study

10.4.1 Primers

	Oligo Name	Oligo Sequence
HTR1A	rs6295_FWD_PRIM	ACG TTG GAT GCG AGA ACG GAG GTA GCT TTT
HTR1A	rs6295_REV_PRIM	ACG TTG GAT GGT CAG TCT CCC AAT TAT TGC
HTR1A	rs749098_FWD_PRIM	ACG TTG GAT GGA TTG CAA AAA TTG CCA GTG
HTR1A	rs749098_REV_PRIM	ACG TTG GAT GTT CAA CTG ACA GAG CTG TCC
HTR1A	rs878567_FWD_PRIM	ACG TTG GAT GCC ACC GCA AAG ATT TAG GAG
HTR1A	rs878567_REV_PRIM	ACG TTG GAT GAG CGA CAT TGG CTC AGA CTT
HTR1B	rs11568817_FWD_PRIM	ACG TTG GAT GTT CCA GAG CGC CTA GCT AAG
HTR1B	rs11568817_REV_PRIM	ACG TTG GAT GGT GGG CCA GCT CTT AGC AA
HTR1B	rs130058_FWD_PRIM	ACG TTG GAT GCA GGT TCA CAG CTG AAA CTA
HTR1B	rs130058_REV_PRIM	ACG TTG GAT GTT AGC TAG GCG CTC TGG AAG
HTR1B	RS6296_FWD_PRIM	ACG TTG GAT GTC GGA GAC TCG CAC TTT GAC
HTR1B	RS6296_REV_PRIM	ACG TTG GAT GCT CTA TTA ACT CGC GGG TTC
HTR1B	RS6297_FWD_PRIM	ACG TTG GAT GGC ATT CCA TAA ACT GAT ACG
HTR1B	RS6297_REV_PRIM	ACG TTG GAT GAA CTT GGT CCC CAA AGG TCG
HTR2A	rs1928040_FWD_PRIM	ACG TTG GAT GAT CAC TCA TAA CTG AAG ATC
HTR2A	rs1928040_REV_PRIM	ACG TTG GAT GTG TTT CCT GAC TTT GCC TAG
HTR2A	rs2296972_FWD_PRIM	ACG TTG GAT GCT GGA GTT GAC AGG AGG ATC
HTR2A	rs2296972_REV_PRIM	ACG TTG GAT GCT GAG GAA CAC CTA AGC TTG
HTR2A	rs2770296_FWD_PRIM	ACG TTG GAT GTC TGC AGA GCT ATC CTG TAC
HTR2A	rs2770296_REV_PRIM	ACG TTG GAT GCA CCA TTT ACA TTC CTC AGC
HTR2A	rs3125_FWD_PRIM	ACG TTG GAT GTT TGT AGC ATT GAA CCC CGC
HTR2A	rs3125_REV_PRIM	ACG TTG GAT GCA AGT CTA GTG GAA CCA ACG
HTR2A	rs518147_FWD_PRIM	ACG TTG GAT GAA GAG CGT GGT GCA GAT TCA
HTR2A	rs518147_REV_PRIM	ACG TTG GAT GAG GCA GTA TCG GGA AGA TCG
HTR2A	rs6310_FWD_PRIM	ACG TTG GAT GTT AGC ATA GAG GTT GCA GGG

HTR2A	rs6310_REV_PRIM	ACG TTG GAT GGT TAA ACA AGA GTC CCA GTA G
HTR2A	rs6311_FWD_PRIM	ACG TTG GAT GTG GAC ACA AAC ACT GTT GGC
HTR2A	rs6311_REV_PRIM	ACG TTG GAT GGG CTA GAA AAC AGT ATG TCC
HTR2A	rs6314_FWD_PRIM	ACG TTG GAT GTG CTC AAT GGT TGC TCT AGG
HTR2A	rs6314_REV_PRIM	ACG TTG GAT GCT TTT CAT TCA CTC CGT CGC
HTR2A	rs731779_FWD_PRIM	ACG TTG GAT GAA AGT AGA AGG CAG CTA GGC
HTR2A	rs731779_REV_PRIM	ACG TTG GAT GTC ATG TCA TTT CAC TCC CAC
HTR2A	rs9316232_FWD_PRIM	ACG TTG GAT GGG GAC AAA CGA TGA GCT ATG
HTR2A	rs9316232_REV_PRIM	ACG TTG GAT GTG CCG CTC CTC ATA TCA TAG
HTR2A	rs985934_FWD_PRIM	ACG TTG GAT GAA ACC AAA GGA GAC TTG GAC
HTR2A	rs985934_REV_PRIM	ACG TTG GAT GAC TTC CAC TTC TGG GAG ATC
HTR2C	rs10875536_FWD_PRIM	ACG TTG GAT GTT TGA CAC CCC TTG GTA GTG
HTR2C	rs10875536_REV_PRIM	ACG TTG GAT GAT GAG AAC TGG CTT CAC ACC
HTR2C	rs3813928_FWD_PRIM	ACG TTG GAT GGG AGG GGT ATG CTA TGA ATC
HTR2C	rs3813928_REV_PRIM	ACG TTG GAT GAT GGC ACC GGA TAG AGA GAG
HTR2C	rs3813929_FWD_PRIM	ACG TTG GAT GGA ATC TGC ACC ACG CTC TTG
HTR2C	rs3813929_REV_PRIM	ACG TTG GAT GCA CGT AAT GCT GAG TGC TG
HTR2C	rs498207_FWD_PRIM	ACG TTG GAT GCT TTG CGC ACT CAC CAA ATG
HTR2C	rs498207_REV_PRIM	ACG TTG GAT GCT TCA AGG GTC TCT GTT CCG
HTR2C	rs6318_FWD_PRIM	ACG TTG GAT GTC AGT GTG CAC CTA ATT GGC
HTR2C	rs6318_REV_PRIM	ACG TTG GAT GTA CTA TAG CTG CTA CTG GGC
SLC6A4	RS1042173_FWD_PRIM	ACG TTG GAT GGA ACA GGG ATG CTA TCT CGC
SLC6A4	RS1042173_REV_PRIM	ACG TTG GAT GAG GTT CTA GTA GAT TCC AGC
SLC6A4	rs140700_FWD_PRIM	ACG TTG GAT GGT GTG ACT CCA AGG GTT GTG
SLC6A4	rs140700_REV_PRIM	ACG TTG GAT GGG TGA ATG GAT GTC AGT GTC
SLC6A4	rs2020934+_FWD_PRIM	ACG TTG GAT GCA AAC CTC ATA AGA ACC TGC
SLC6A4	rs2020934+_REV_PRIM	ACG TTG GAT GTT TTC CTG CCA CGC ACT CTG
SLC6A4	rs2020942_FWD_PRIM	ACG TTG GAT GGA AGG CCA TCA CGA GAA CAC
SLC6A4	rs2020942_REV_PRIM	ACG TTG GAT GAC CTG AGG TCT GTG CAA ATC
SLC6A4	rs3794808_FWD_PRIM	ACG TTG GAT GTG AAC GTA GAA GTG GAA GAC
SLC6A4	rs3794808_REV_PRIM	ACG TTG GAT GAT GTT TGC CAT ACT CAC CCC
SLC6A4	rs6354_FWD_PRIM	ACG TTG GAT GGT GGC TAA GCC CCT TGT TAT
SLC6A4	rs6354_REV_PRIM	ACG TTG GAT GAC ACT CTT CTC CCT AGG TC

10.4.2 Probes

	Oligo Name	Oligo Sequence
HTR1A	rs6295_PROBE_A	AGA CCG AGT GTG TCT TC
HTR1A	rs749098_PROBE_A	TCG TCC TTT CAC TTT CTT AAT TAT AAA
HTR1A	rs878567_PROBE_A	TCA TCA GTT TTG ATC CCA G
HTR1B	rs11568817_PROBE_A	GCC CGC TCT TAG CAA CCC AGG
HTR1B	rs130058_PROBE_A	CCC CTT GCC TTG GCT GCC GCA CCC
HTR1B	RS6296_PROBE_A	GAG TCC GGA TCT CCT GTG TAT GT
HTR1B	RS6297_PROBE_A	GCG ACC CCA CTG CAA A
HTR2A	rs1928040_PROBE_A	AAC AAG AGA CAA ATT CTC ATT CAA A
HTR2A	rs2296972_PROBE_A	GGA TTT GTA ATG CTG CTT ATT AGA
HTR2A	rs2770296_PROBE_A	AGC TGC TCT CTC CCC T
HTR2A	rs3125_PROBE_A	AGT GGA ACC AAC GAT CAT ATC T
HTR2A	rs518147_PROBE_A	GGA GTA GGA AGG AAG CGT CCT C
HTR2A	rs6310_PROBE_A	CCC GGA CAT TTA TCT TCC CGA
HTR2A	rs6311_PROBE_A	GAG TGC TGT GAG TGT C
HTR2A	rs6314_PROBE_A	GGG ATT TAG AAG CCT CTT CAG AAT
HTR2A	rs731779_PROBE_A	ATG TCA TTT CAC TCC CAC ACT TTC A
HTR2A	rs9316232_PROBE_A	CCG CTC CTC ATA TCA TAG AAC AAT TA
HTR2A	rs985934_PROBE_A	CCG GTA GCA TCT ACC AGA ATA CAA A
HTR2C	rs10875536_PROBE_A	AAC ACA CCA CTA GAA TGG CTA AAA
HTR2C	rs3813928_PROBE_A	AAG GCT TTC TCA AGA ATG TA
HTR2C	rs3813929_PROBE_A	GCT CCT CCC CTC ATC C
HTR2C	rs498207_PROBE_A	GAC GAC TCT TGC CCA TTC
HTR2C	rs6318_PROBE_A	TGG GCT CAC AGA AAT ATC A
SLC6A4	RS1042173_PROBE_A	AGT AGA TTC CAG CAA TAA AAT T
SLC6A4	rs140700_PROBE_A	TGA CCT TGA GAA AGG AGG G
SLC6A4	rs2020934+_PROBE_A	GCA GTG ACC GTT CCA A
SLC6A4	rs2020942_PROBE_A	AAG TTA CAG TCA CAC TGG GTA AAC C
SLC6A4	rs3794808_PROBE_A	GGC CTA GTG CCT GAG AGA
SLC6A4	rs6354_PROBE_A	CGA CCT TGC TTG CCC TCT

10.4.3 Other primers and probes

I have genotyped these SNPs, but they were excluded from the analysis as they were out of the Hardy-Weinberg equilibrium.

SLC6A4	rs1042173x_FWD_PRIM	ACG TTG GAT GAG GTT CTA GTA GAT TCC AGC
SLC6A4	rs1042173x_PROBE_A	CCA TAT ATT TTC TGA GTA GCA TAT A
SLC6A4	rs1042173x_REV_PRIM	ACG TTG GAT GAG AAC AGG GAT GCT ATC TCG
SLC6A4	rs2020932_FWD_PRIM	ACG TTG GAT GAA CAC AAG CGC GAG CAA CCA
SLC6A4	rs2020932_PROBE_A	CCA CCC CAT CTC TAG AAA AAA AA
SLC6A4	rs2020932_REV_PRIM	ACG TTG GAT GGC AAG ACC CCA TCT CTA GAA
SLC6A4	rs2020933+_FWD_PRIM	ACG TTG GAT GGA TGA GAG TTA GCT AGC AGG
SLC6A4	rs2020933+_PROBE_A	TGT CCA GAA AAG TGA ACC
SLC6A4	rs2020933+_REV_PRIM	ACG TTG GAT GTT ACC ATC AGT TTT GTC CAG
SLC6A4	rs2228673_FWD_PRIM	ACG TTG GAT GGC GCT ATA CTA CCT CAT CTC
SLC6A4	rs2228673_PROBE_A	GGG GCC AGT GTT CCA GGA GTT
SLC6A4	rs2228673_REV_PRIM	ACG TTG GAT GCG GAG AAG TAA TTG GTG CAG

SLC6A4	rs25533_FWD_PRIM	ACG TTG GAT GCT CCC GCT GGA TGG GGT TG
SLC6A4	rs25533_PROBE_A	TTG TAG CGC GGC CCC TCC C
SLC6A4	rs25533_REV_PRIM	ACG TTG GAT GTC CCT CCC CTC CTG GCT CT

10.5 List of Illumina SNPs of Dyne Steel cohort

10.5.1 HTR2A gene

Chr	SNP	Position
13	rs7333412	46301361
13	rs3803189	46306571
13	rs6314	46307035
13	rs977003	46313002
13	rs1923884	46319837
13	rs1923885	46321087
13	rs1923886	46321292
13	rs1745837	46322813
13	rs622337	46325627
13	rs655854	46326201
13	rs2296972	46326472
13	rs4942578	46330611
13	rs1928042	46335217
13	rs2760345	46336575
13	rs2770296	46338561
13	rs1328674	46339708
13	rs9316235	46343704
13	rs582385	46343995
13	rs2770298	46344848
13	rs972979	46347165
13	rs731779	46350039
13	rs2770304	46353366
13	rs985933	46353864
13	rs927544	46354052
13	rs17288723	46355694
13	rs9534505	46358745
13	rs9534507	46359735
13	rs4942587	46360801
13	rs4941573	46362858
13	rs12584920	46363038
13	rs1328684	46364231
13	rs2296973	46364782
13	rs2070037	46365071
13	rs9534511	46366581
13	rs6313	46367941
13	rs6312	46368825
13	rs6306	46369462

10.5.2 SLC6A4 gene

Chr	SNP	Position
17	rs1906451	25539605
17	rs1042173	25549137
17	rs12449783	25551779
17	rs3794808	25555919
17	rs4583306	25562841
17	rs140700	25567515
17	rs6354	25574024
17	rs2020936	25574940
17	rs2066713	25575791
17	rs4251417	25575984
17	rs8071667	25576899
17	rs1487971	25596879

10.5.3 HTR6 gene

Chr	SNP	Position
1	rs6693503	19859881
1	rs4912138	19866072
1	rs3790756	19868819
1	rs6699866	19872232

10.5.4 HTR1D gene

Chr	SNP	Position
1	rs2806566	23379759
1	rs641032	23386567
1	rs627304	23410142

10.5.5 HTR2B gene

Chr	SNP	Position
2	rs1011502	217516448
2	rs11694724	231655711
2	rs13394402	231677164
2	rs17586405	231687502
2	rs10194776	231688263
2	rs4973377	231690236
2	rs6437002	231733528

10.5.6 HTR1F gene

Chr	SNP	Position
3	rs7648805	88086502
3	rs7653582	88103530
3	rs7652406	88135928
3	rs9310061	88146455

10.5.7 HTR3 gene, C,D, E subunits

Chr	SNP	Position
3	rs7613237	185223828
3	rs939335	185228357
3	rs939334	185232425
3	rs4912518	185234721
3	rs12493550	185235467
3	rs6792482	185236723
3	rs1467257	185238225
3	rs7621975	185241200
3	rs10937160	185241479
3	rs9819507	185249395
3	rs6808122	185255515
3	rs6766410	185257456
3	rs9869582	185269372
3	rs6443940	185277130
3	rs4912521	185280657
3	rs7648737	185283157
3	rs6443942	185285291
3	rs9784375	185289100
3	rs7627615	185301110
3	rs7432211	185301849
3	rs11718245	185313087
3	rs9815292	185317184

10.5 8 HTR4 gene

Chr	SNP	Position
5	rs4274968	147805783
5	rs9325098	147807631
5	rs7727161	147812679
5	rs17639006	147816439
5	rs3995090	147826008
5	rs4597955	147827466
5	rs7702840	147846075
5	rs7723153	147857917
5	rs1368383	147870317
5	rs1368384	147875141
5	rs1368386	147875331
5	rs1368387	147876501
5	rs7725785	147882896
5	rs2277049	147883281
5	rs12152801	147888538
5	rs13359903	147908092
5	rs2278392	147908418
5	rs1345697	147922431
5	rs17720733	147930671
5	rs4599527	147932732

5	rs17108435	147933440
5	rs4336354	147939379
5	rs6580557	147940321
5	rs867522	147946439
5	rs1833710	147947939
5	rs4280857	147952812
5	rs2964276	147959171
5	rs7711800	147963048
5	rs1011427	147968170
5	rs13182913	147975798
5	rs2068190	147987206
5	rs17706942	147987905
5	rs13157587	147988614
5	rs9686886	147991106
5	rs5028114	147991932
5	rs6865654	147992149
5	rs1972644	147999741
5	rs1833704	148004894
5	rs7713886	148005351
5	rs888961	148019090
5	rs1820076	148025475

10.5 9 HTR1E gene

Chr	SNP	Position
6	rs9450576	87672689
6	rs12181765	87673857
6	rs9450587	87680287
6	rs16877971	87680749
6	rs16877989	87683168
6	rs16877997	87685278
6	rs11966339	87686726
6	rs9450594	87695058
6	rs1819144	87699703
6	rs828361	87705190
6	rs10944288	87708461
6	rs1886333	87721328
6	rs2875512	87731570
6	rs942472	87733426
6	rs6922679	87738844
6	rs7751022	87741519
6	rs1041058	87754721
6	rs1324433	87755477
6	rs6926547	87760844
6	rs11961178	87786587
6	rs16878151	87787574
6	rs7740936	87794412
6	rs10806386	87796903
6	rs7739124	87806534

10.5.10 HTR5A gene

Chr	SNP	Position
7	rs6597451	154479788
7	rs1440459	154480786
7	rs1561603	154486412
7	rs1561602	154486574
7	rs2919435	154489085
7	rs2241859	154494400
7	rs732050	154504328

10.5.11 HTR7 gene

Chr	SNP	Position
10	rs1107688	92485316
10	rs17526697	92490499
10	rs1418213	92491630
10	rs11812708	92495245
10	rs10881830	92503229
10	rs11186300	92504719
10	rs7072526	92519250
10	rs7086484	92523709
10	rs11599921	92523921
10	rs7920627	92534625
10	rs10881832	92538743
10	rs7916403	92549888
10	rs10881838	92580213
10	rs2901127	92585046
10	rs10785973	92588151
10	rs11596991	92588438
10	rs11186333	92595099
10	rs7074715	92599286
10	rs7916720	92616547
10	rs4282910	92621224

10.5.12 HTR3 gene, AB subunits

Chr	SNP	Position
11	rs869451	113258222
11	rs2097078	113258726
11	rs2011249	113273848
11	rs1176758	113275565
11	rs3891484	113277799
11	rs11606194	113286191
11	rs1176744	113308238
11	rs2276307	113309097
11	rs3782025	113312817
11	rs1672717	113317943
11	rs7129190	113323962
11	rs720396	113327955

11	rs7942029	113329075
11	rs1176754	113332860
11	rs1150229	113338362
11	rs10789980	113346213
11	rs1176752	113348687
11	rs1150226	113350751
11	rs1150225	113351898
11	rs1985242	113353483
11	rs2276302	113355350
11	rs10891611	113356623
11	rs1176719	113357397
11	rs909411	113359492
11	rs10160548	113361891
11	rs1176713	113365635
11	rs1182457	113366959
11	rs17543669	113367667
11	rs11214800	113368140
11	rs7115470	113368341
11	rs897685	113369319
11	rs1379170	113371761
11	rs11214806	113377837
11	rs10891615	113381063
11	rs4938066	113390622
11	rs10790208	117149721
11	rs10488782	117179038

10.6. Supplementary material for Study 1

Supplementary Table 1 Summary of association studies of HTR1A single nucleotide polymorphisms and depression/antidepressant treatment

Study	Sample size	SNPs investigated	Population	Assessment	Finding
Association with depression					
Haenisch (2009)	426 cases 643 controls	rs1800044 (R219L)	German, mean age of cases 49 years, mean age of controls 44 years	DSM-IV	Association observed with rs1800044 and MDD.
Brezo (2009)	1255 French speaking Canadians from Quebec	rs878567	mean age: 27.3 years	DSM-III-R	Homozygosity for rs878567_T allele was associated with lower risk for MDD in childhood physical abuse victims.
Zhang (2009)	401 cases 391 controls	rs6295 (C-1019G)	Chinese, mean age of cases 33 years, mean age of controls 33 years	DSM-IV	Three-way interaction observed between rs6295, HTTLPR and negative life events. G allele frequency significantly increased in patients
Neff (2009)	419 Utah cases 182 Ashkenazi cases 244 Utah controls 85 Ashkenazi controls	rs6295 (C-1019G)	Utah and Ashkenazi	DSM-IV	Possible interaction between HTR1A and LHPP gene which is associated with MDD. G allele frequency significantly increased in patients. No differences between sexes
Wu (2008)	400 cases 400 controls	rs6295 (C-1019G)	Northern Han Chinese	DSM-IV	G allele frequency significantly increased in patients especially for late-onset depression and women
Kraus (2007)	139 hepatitis C-infected outpatients	rs6295 (C-1019G)	German, mean age 43 years	HADS	Association with interferon-induced depression. GG genotype frequency significantly increased in patients
Anttila (2007)	119 cases 392 controls	rs6295 (C-1019G)	Finnish, mean age of cases 58 years, mean age of controls 44 years	DSM-IV	HTR1A GG and BDNF Met alleles interact to increase risk of MDD
Association with treatment					
Kato (2009)	137 cases	rs6295 (C-1019G) rs10042486 rs1364043	Japanese	DSM-IV	Improved response to antidepressant in rs10042486 C/C, rs6295 G/G and rs1364043 T/T patients
Hong (2006)	224 cases, 81 responder, 143 non-responders	rs6295 (C-1019G)	Southern Taiwan Chinese, mean age 44 years	DSM-IV and HAM-D	CC carriers exhibited a better therapeutic response to fluoxetine
Parsey (2006a)	22 cases	rs6295 (C-	American, mean age of	DSM-IV	GG carriers exhibited a poorer therapeutic

	43 controls	1019G)	cases 41 years, mean age of controls 38 years	HAM-D	response to antidepressant treatment
Suzuki (2006)	65 cases	Gly272Asp	Japanese, mean age 41 years	DSM-IV HAM-D	Presence of Asp allele associated with improved response to fluvoxamine
Yu (2006)	222 cases	Gly272Asp rs6295 (C-1019G)	Taiwanese ethnic Han Chinese, mean age 43 years	HAM-D	No association between Gly272Asp and fluoxetine response. Association between improved fluoxetine response and rs6295 C/C genotype in women patients
Lemonde (2004)	118 cases	rs6295 (C-1019G)	Caucasian, mean age 47 years	HAM-D	GG carriers exhibited a poorer therapeutic response to antidepressant treatment
No association with depression or treatment					
Illi (2009)	106 cases 395 controls	rs6295 (C-1019G)	Finnish, mean age of cases 41 years, mean age of controls 44 years	MADRS	No association with MDD or treatment response
Yoon (2009)	181 suicidal cases 143 non-suicidal cases 176 controls	rs6295 (C-1019G)	Korean, mean age of suicidal cases 40 years, mean age of non-suicidal cases 41 years, mean age of controls 40 years	DSM-IV	No association with MDD
Hettema (2008)	589 cases 539 controls	rs878567 and rs6295 (C-1019G)	Caucasians	DSM-III-R	No association with MDD
Huang (2004)	326 cases 107 controls	rs6295 (C-1019G)	Various ethnic backgrounds, mean age of cases 38 years, mean age of controls 39 years	DSM-IV	No association with MDD
Arias (2002)	249 cases 170 controls	rs6295 (C-1019G) rs1799921 (Ile28Val) Asp272Gly rs1800041 (Pro16Leu)	Spanish, mean age of cases 47 years, mean age of controls 38 years	DSM-III-R	No association with MDD

Other investigations

Serretti (2009)	111psychiatric patient group of suicide attempters 289 controls	rs878567 and rs6295 (C-1019G)	German, mean age of cases 39.2 years, mean age of controls 45.2 years	Temperament and Character Inventory	No association was observed to personality traits.
Serretti (2007)	167 German suicide attempters, 92 Caucasian suicide completers, 312 German controls, 152 Italian suicide attempters, 131 Italian controls	rs878567 and rs6295 (C-1019G)		DSM-IV, State-Trait Anger Expression Inventory	No association with suicide-, anger-or aggression-related behaviour.
Wasserman (2006)	272 family trios of suicide attempters	rs6295 (C-1019G)	Caucasians	DSM-IV, Life Event Inventory	Overtransmission of G allele in a subset of subjects with stressful life events prior to suicide attempt.
Parsey (2006b)	28 cases 43 controls	rs6295 (C-1019G)	18-65 years of age, antidepressant naïve cases	DSM-IV	The GG genotype was more common in MDD and correlated with higher binding potential of the receptor.
Huang (2003)	208 cases (MDD) and 183 controls, 83 cases (substance abuse) and 311 controls	rs6296 (G861C)	African-Americans, Caucasians, Hispanics, Asians and Others	DSM-III R	C allele is associated with MDD and substance abuse disorder.

Diagnostic and Statistical Manual of Mental Disorders version 3 revised (DSM-III-R) and version 4 (DSM-IV)
Hospital Anxiety and Depression Scale (HADS)
Montgomery–Asberg Depression Rating Scale (MADRS)
Hamilton Depression Rating Scale (HAM-D)
Phospholysine phosphohistidine inorganic pyrophosphate phosphatise (LHPP)
Major Depressive Disorder (MDD)
Serotonin transporter promoter polymorphism (HTTLPR).

10.7 Manuscripts in preparation –Further results

- I investigated the role of HTR2A and SLC6A4 in depression, trait anxiety and neuroticism in interaction with recent life events and childhood adversity in the New Mood cohort. When childhood adversity was included in the model, two of the HTR2A polymoprphisms showed nominally significant values (rs6314_T with BSI anxiety scores, p=0.015, β=0.043, rs3125_C to BigFive Neuroticism scores, p=0.02, β=0.034, none of them surviving correction for multiple testing. SLC6A4 did not yield any significant results. Neither HTR2A nor SLC6A4 showed significant association to the psychiatric phenotypes in interaction with recent life events.
- HTR2C was not associated with any psychiatric phenotypes in the New Mood cohort (Tables 13a and 13b shows the results).
- Epistatic interaction has been observed in the Dyne Steel cohort between the subunits of the HTR3 gene. In depression, this interaction exists between the HTR3D subunit promoter region and the HTR3A subunit while in anxiety and neuroticism an interaction was observed between the HTR3D subunit promoter and the HTR3E subunit.

Table 13a. Results of association between HTR2C and psychiatric phenotypes in the New Mood cohort (Homozygote females)

Homozygote females			BSI Depression		BSI Anxiety		BiG5 Neuroticism	
CHR	SNP	BP	β	P-value	β	P-value	β	P-value
23	HTR2C rs498207 **C**	113724372	-0.143	0.387	-0.101	0.489	-0.047	0.709
23	HTR2C rs3813928 **A**	113724538	-0.455	0.064	-0.383	0.080	-0.299	0.109
23	HTR2C_3813929 **T**	113724776	-0.462	0.060	-0.380	0.082	-0.304	0.106
23	HTR2C_518147 **C**	113724838	-0.356	0.064	-0.197	0.245	-0.156	0.285
23	HTR2C_6318 **C**	113871991	0.140	0.501	0.064	0.730	0.177	0.261
23	HTR2C_6643913 **T**	114034831	0.269	0.227	0.184	0.356	0.199	0.240

Table 13b. Results of association between HTR2C and psychiatric phenotypes in the New Mood cohort (Males)

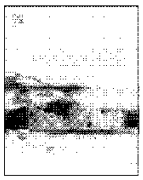
Males			BSI Depression		BSI Anxiety		BiG5 Neuroticism	
CHR	SNP	BP	β	P-value	β	P-value	β	P-value
23	HTR2C_498207 **C**	113724372	0.169	0.103	0.039	0.661	0.068	0.485
23	HTR2C_3813928 **A**	113724538	0.108	0.479	-0.012	0.925	0.118	0.401
23	HTR2C_3813929 **T**	113724776	0.112	0.454	-0.008	0.948	0.121	0.386
23	HTR2C_518147 **C**	113724838	0.103	0.447	-0.011	0.921	-0.011	0.929
23	HTR2C_6318 **C**	113871991	0.095	0.47	0.054	0.631	-0.045	0.717
23	HTR2C_6643913 **T**	114034831	0.175	0.199	0.092	0.429	0.019	0.885

Mathematical models were additive. Covariate: age.
Phenotypes are identical as described in the Methods section.

10.8 Published version of Study 1

NEUPSY-10296; No of Pages 11
European Neuropsychopharmacology (2010) xx, xxx–xxx

www.elsevier.com/locate/euroneuro



The HTR1A and HTR1B receptor genes influence stress-related information processing

Krisztina Mekli [a,b,c], Antony Payton [b], Fabio Miyajima [b], Hazel Platt [b], Emma Thomas [a], Darragh Downey [e], Kathryn Lloyd-Williams [a], Diana Chase [a], Zoltan G. Toth [f], Rebecca Elliott [a], William E. Ollier [b], Ian M. Anderson [a], J.F. William Deakin [a], Gyorgy Bagdy [c,d], Gabriella Juhasz [a,*]

[a] Neuroscience and Psychiatry Unit, School of Community Based Medicine, Faculty of Medical and Human Sciences, The University of Manchester, UK
[b] Centre for Integrated Genomic Medical Research, The University of Manchester, Manchester, UK
[c] Department of Pharmacodynamics, Semmelweis University, Budapest, Hungary
[d] Group of Neurochemistry, Hungarian Academy of Science and Semmelweis University, Budapest, Hungary
[e] Imaging Science and Biomedical Engineering, The University of Manchester, Manchester, UK
[f] Faculty of Life Sciences, The University of Manchester, Manchester, UK

Received 8 March 2010; received in revised form 17 June 2010; accepted 23 June 2010

KEYWORDS
Stress;
HTR1A;
HTR1B;
Depression;
Anxiety

Abstract

The serotonergic system has been widely implicated in stress related psychiatric disorders such as depression and anxiety. We investigated the possible association between depression and anxiety scores and SNPs within the HTR1A and HTR1B genes in a population sample (n=1387). There was no direct SNP-phenotype association, but in interaction with recent stressful life events rs6295 G, rs878567 T alleles and rs6296 C alleles were associated with significantly higher symptom scores. A subset of control subjects (n 101) took part in a computerised face emotion processing task. Healthy rs6295 GG carriers did not show an affective bias to perceive more negative emotions but reacted more quickly to fearful faces. Thus we conclude that the serotonin-1A receptor conveys vulnerability to these psychiatric disorders by modulating threat-related information processing. Our results extend previous findings of an interaction between stressful life events and the serotonin transporter gene to two other genes in the serotonergic pathway and emphasise the possible role of increased threat-related information processing as an intermediate phenotype.

* Corresponding author. Neuroscience and Psychiatry Unit, School of Community Based Medicine, Faculty of Medical and Human Sciences, The University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, UK. Tel.: +44 161 275 6915; fax: +44 161 275 7429.
E-mail address: gabriella.juhasz@manchester.ac.uk (G. Juhasz).

0924-977X/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.euroneuro.2010.06.013

1. Introduction

Depression has a strong genetic basis with twin studies reporting heritability estimates of 40–50% (Levinson, 2006). Investigations that have attempted to identify the biochemical and genetic background of depression and the highly correlated trait of anxiety have been extensive (Mineka et al., 1998). Genes within the serotonergic system, particularly the serotonergic transporter gene (SLC6A4) have received much attention, since the proteins they are encode are the most common targets for the treatment of anxiety disorders and depression. Interaction analysis, both epistatic and environmental, has provided important insights into the development of behavioural traits. In 2003 Caspi and colleagues demonstrated that stressful life events in the presence of the short allele, resides within the SLC6A4 gene promoter (5-HTTLPR), predisposed to develop depression (Caspi et al., 2003). Since then several studies have aimed to replicate this finding (e.g. Cervilla et al., 2006; Contreras et al., 2009; Drachmann et al., 2009; Lazary et al., 2008; Wray et al., 2009; Zalsman et al., 2006). However, a meta-analysis, which included 14 studies, has not found evidence that 5-HTTLPR is associated with depression when analysed in interaction with stressful life events (Risch et al., 2009). Another more extensive recent meta-analysis of 34 studies found that 17 studies replicated the original Caspi-finding, 8 were partial replications and 9 were non-replications (Uher and McGuffin, 2010). Success of replication heavily depended on the method used for evaluation of phenotypes and environmental factors (Caspi et al., 2010; Rutter et al., 2009; Uher and McGuffin, 2010). As most of the evidence supports the 5-HTTLPR findings, it can be hypothesised that other genes throughout the serotonergic pathway that modulate serotonergic activity, such as the serotonin receptor genes 1A and 1B (HTR1A and HTR1B), are potential candidates to mediate the risk of stressful life events on behaviour.

5-HT_{1A} receptors are present in high density in serotonergic cell body areas, particularly in the dorsal and median raphe nuclei, where they function as somatodendritic autoreceptors. Activation of these autoreceptors has been shown to decrease the rate of firing of serotonergic neurons and cause a reduction in the release of serotonin (Bohmaker et al., 1993; Kennett et al., 1987; Savitz et al., 2009). 5-HT_{1A} receptors are also postsynaptic receptors with high density in the hippocampus, septum, amygdala, hypothalamus and neocortex (Hall et al., 1997; Pazos and Palacios, 1985) Many of the serotonergic terminal areas are located in the limbic system which has been shown to be involved in the modulation of emotions, raising the possibility that these receptors may regulate emotional states (Drevets et al., 2008; Fisher et al., 2006). 5-HT_{1B} receptors are also located both pre- and postsynaptically and appear to regulate the release of 5-HT from the dorsal raphe nucleus (Lanfumey and Hamon, 2004). In addition, 5-HT_{1B} receptors also modulate the release of other neurotransmitters, such as acetylcholine in the hippocampus, dopamine in the prefrontal cortex and γ-aminobutiric-acid (GABA)-throughout the forebrain (Barnes and Sharp, 1999; Drago et al., 2010). In summary, there is evidence that the activity of serotonergic neurons, and also other neurotransmitter pathways, depends on 5-HT_{1A} and probably 5-HT_{1B} receptor function (Lanfumey and Hamon, 2004; Savitz et al., 2009).

Mice lacking the 5-HT_{1A} receptors show enhanced anxiety, increased response to stress (Parks et al., 1998) and changes of anxiety related behaviours, such as avoidance of open spaces and novel objects, increased cover seeking and reduced exploratory behaviour (Heisler et al., 1998). In contrast, mice without 5-HT_{1B} receptors exhibited an increase in aggression which is consistent with reduced anxiety and with increased impulsivity (Brunner and Hen, 1997; Ramboz et al., 1998). Other observations have shown that HTR1B knockout mice are more susceptible to the behavioural and reinforcing effects of cocaine (Crabbe et al., 1996; Rocha et al., 1998), suggesting increased sensitivity to reward. However, it is important to note that in knockout animals the relevant genes are completely inactivated while in cases of human polymorphisms the physiological pattern of gene expression is altered but not totally disrupted in most instances (Plomin and Crabbe, 2000).

Studies investigating the role of HTR1A genetic polymorphisms in human subjects with depression have produced mixed results (Supplementary material Table 1). Whilst a number of different single nucleotide polymorphisms (SNPs) have been studied, the majority of groups have looked at a functional promoter polymorphism (rs6295) which has been shown to alter binding potential in antidepressant naive major depressive disorder (MDD) patients (Parsey et al., 2006b). All the studies that observed an association with this polymorphism found significant interaction effects, either with other genes (Anttila et al., 2007; Neff et al., 2009; Zhang et al., 2009) with genes and negative life events (Zhang et al., 2009), with interferon-induced depression (Kraus et al., 2007) or with sex specific effects (Wu et al., 2008). Interestingly, all these studies found that the presence of the rs6295 G allele was associated with an increased susceptibility to depression. In support of these findings human drug studies have reported that carriers of the G allele respond less well to antidepressant treatment (Hong et al., 2006; Lemonde et al., 2004; Parsey et al., 2006a; Yu et al., 2006) although one study has found that carriers of the GG genotype respond better (Kato et al., 2009). The G allele also appears to play a role in anxiety-related disorders. In one study the G allele showed significant association to higher neuroticism scores on the NEO personality inventory (NEO-PI-R), which was mainly due to associations with the Neuroticism facets Anxiety and Depression. G allele carriers also showed higher Tridimensional Personality Questionnaire (TPQ) Harm Avoidance scores (Strobel et al., 2003). There was also significant association between this gene and panic disorder with agoraphobia (Rothe et al., 2004). In addition, GG homozygous panic disorder patients showed poorer response to selective serotonin reuptake inhibitor (SSRI) treatment (Yevtushenko et al., 2010).

Investigation of the HTR1B gene is much less extensive with one report describing an association between depression and the synonymous SNP rs6296 (Huang et al., 2003) and another report finding no association with two promoter SNPs rs130058 and rs11568817 (Zhang et al., 2009).

Depression has been associated with a general perceptual deficit for face emotions with a mood-congruent response bias towards negative emotions (Gur et al., 1992; Surguladze

Please cite this article as: Mekli, K., et al., The HTR1A and HTR1B receptor genes influence stress-related information processing. Eur. Neuropsychopharmacol. (2010), doi:10.1016/j.euroneuro.2010.06.013

et al., 2004) and anxiety with an increased threat-related emotion processing (Mogg and Bradley, 2002). Negative and threat-related biases in perception, attention and memory are believed to play a key role in maintaining symptoms of depression and anxiety (Harmer et al., 2004; Harmer, 2008). The serotonergic system is involved in the face emotion recognition (Merens et al., 2007). In control subjects acute tryptophan depletion has been shown to impair emotional processing similar to that seen in acute depression, while acute administration of SSRIs showed the opposite effect (Harmer, 2008). Differences in emotional processing were apparent without measurable mood changes suggesting that face emotion recognition tasks are more sensitive to serotonergic changes than self-reported mood (Harmer et al., 2003; Harmer, 2008). Furthermore it has been demonstrated that the functional rs6295 polymorphism of the HTR1A gene influences threat-related face emotion processing and through modification of amygdala reactivity may drive trait anxiety (Fakra et al., 2009; Le Francois et al., 2008).

In our study we used a Caucasian cohort from the UK consisting of 1387 individuals who had provided information on depression, anxiety and recent negative life events. HTR1A/1B genotypes derived from haplotype tagging (htSNPs) and functional SNPs were analysed independently against depression and anxiety scores and for interaction with stressful life events. Gender specific effects that have been previously reported were also investigated. In addition the effect of the known functional rs6296 polymorphism (HTR1A) was investigated on mood- and threat-related emotional face processing in a subset of control subjects. This intermediate phenotype is correlated with depression and anxiety as we mentioned above, and may be an indication of altered serotonergic function well before symptoms occur. We hypothesised that carriers of the rs6296 G allele would show more depressive and anxiety symptoms especially in relation to life stresses and that they would be biased towards threat-related information. We also hypothesized that other polymorphisms in the HTR1A and HTR1B gene would show similar associations with depression and anxiety as rs6296.

2. Experimental procedures

2.1. Participants

This study was part of the EU funded NewMood (New Molecules for Mood Disorders) Integrated Program that aims to identify new molecular mechanisms of vulnerability to depression. In the first phase (Level1) we recruited and obtained DNA and completed questionnaires from 1387 unrelated Caucasian participants aged between 18 and 60 years from Greater Manchester, UK through general practices, and a web-site (http://www.newmood.co.uk). In the second phase (Level2) a subgroup of Level1 participants supplemented by new recruits took part in face-to-face interviews and computerized tasks. We report here on results from Level2 healthy control volunteers (55 from Level1 and 46 supplemental recruits). Details of our recruitment strategy and responses are described elsewhere (Juhasz et al., 2009). The study was approved by the local Ethics Committees (North Manchester Local Research Ethics Committee (LREC) (ref. 05/Q1406/26), the University of Manchester's Ethics Committee (ref. 05056)) and was carried out in accordance with the Declaration of Helsinki. All participants provided written informed consent.

2.2. Phenotype assessments

The Level1 assessments consisted of brief or adapted versions of standard questionnaires that were designed for participants to complete and return to us by post. To properly test gene × environment interaction we followed the method suggested by Moffitt et al. (2005). We used the same background questionnaire as we used in our previous studies (Juhasz et al., 2009; Lazary et al., 2008). This structured self-rating questionnaire consists of 22 items and collects information about medical history including psychiatric history (with special interest on depression), family psychiatric history, and socioeconomic background. The Brief Symptom Inventory (BSI) questionnaire (Derogatis, 1993) was used for depressive symptoms (six items) and for anxiety symptoms (six items). A continuous weighted dimension score was calculated for both Depression and Anxiety symptoms, which were used in the analysis. The questionnaire data on depression and anxiety scores have been validated at a second level of the study, described in detail elsewhere (Juhasz et al., 2009). Our Recent Negative Life Events questionnaire was based on the validated List of Life Threatening Experiences (LTE) (Brugha et al., 1985; Rijsdijk et al., 2001) and was previously used to replicate a 5-HTTLPR x stressful life events interaction (Lazary et al., 2008) and demonstrate cannabinoid receptor 1 gene (CNR1) x stressful life events interaction (Juhasz et al., 2009). This questionnaire provided the total number of negative life events in the previous 12 months period and this sum was used in the analysis. For figures and to exclude scaling effect we grouped the participants into three groups based on our previous studies: 0–1 life event, 2 life events and 3-more life events. Questions based on the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 1994) were used to determine Childhood Adversity as a Likert score (ranging from never true to very often true) related to emotional and physical abuse, and emotional and physical neglect. An additional question asked about parental loss during childhood. The sum of the Likert score plus parental loss was used in the analysis. These questionnaire data were validated with the original CTQ (Pearson $R=0.75$, $p<0.001$, $n=142$) at a second level of the study, and were used to replicate significant interaction between rs6265 (BDNF Val66Met) and childhood adversity on depression (ACNP, Hollywood, Florida, USA, 6–10 December 2009).

Level2 participants provided background questionnaire, BSI data and took part in a face-to-face diagnostic interview and a computerised emotion recognition task. The participants in this study were free from any current or previous axis I psychiatric disorders based on Structured Clinical Interview for DSM-IV (SCID), (First et al., 2002).

2.2.1. Face emotion recognition task

The task contains six varieties of emotions (anger, disgust, fear, happiness, sadness and surprise) plus neutral (Ekman and Friesen, 1976). These emotions are represented by faces, acted out by four actors on three different levels (30%, 50% and 70%). During the practice session participants were shown 21 faces (each emotion three times) at 100% intensity. In the main task each of the six expressions was shown at each intensity four times, one presentation of each actor. A neutral expression of each individual was shown once in the task, giving a total of four neutral images. Therefore in the main task there were 76 stimuli presentations = 6 expressions (anger, disgust, fear, happiness, sadness surprise) × four actors × three intensities (30, 50 and 70%) plus 4 neutral. Each face was displayed for 1000 ms on the screen and was followed by a 4500 ms interstimulus interval. Participants were asked to identify the emotion by pressing the appropriate computer key from the seven possible choices. We recorded the percentage of the correctly identified emotions and we calculated an average reaction time for

Please cite this article as: Mekli, K., et al., The HTR1A and HTR1B receptor genes influence stress-related information processing, Eur. Neuropsychopharmacol. (2010), doi:10.1016/j.euroneuro.2010.06.013

each correctly recognised emotion for each participants and these values were used for analysis. Because our hypothesis focused on intermediate phenotypes to depression and anxiety, for genetic analysis we only used data for happiness, sad, and fear emotions, which activate specific neural networks that are important for mood and threat-related information processing (Fusar-Poli et al., 2009; Harmer, 2008).

A summary of demographic and phenotypic data is given in Table 1.

2.3. DNA extraction and genotyping

Participants received a cytology brush (Cytobrush plus C0012, Durbin PLC) and a 15-mL plastic tube containing 2.0 ml of collection buffer. Buccal mucosa cells were collected and genomic DNA was extracted according to a protocol previously described (Freeman et al., 2003). HaploView software (www.broad.mit.edu/personal/jcbarret/haploview/) was employed to identify htSNPs using the confidence interval method (Barrett et al., 2005; Gabriel et al., 2002). The tagging was based on the CEPH population genotype data that were available at the International HapMap Project Phase I, June 2005 release (www.hapmap.org). We also examined possibly functional htSNPs previously identified (Duan et al., 2003; Lemonde et al., 2003).

SNPs were genotyped using the Sequenom® MassARRAY technology (Sequenom®, San Diego, http://www.sequenom.com) using 25 ng of DNA. Primer sequences are available upon request. Genotyping was performed blind with regard to phenotype. All laboratory work was performed under the ISO 9001:2000 quality management requirements. In Level1 we genotyped six SNPs (See Table 2.); in Level2 we only investigated rs6295 on HTR1A, as only this polymorphism of our candidates has a well established function (Lemonde et al., 2003) with suggested role in face emotion recognition (Fakra et al., 2009; Le Francois et al., 2008).

Table 1 Demographic and phenotypic information of Level1 and Level2 participants.

	Level1	Level2
Number of volunteers		
Women	968	58
Men	419	43
Total	1387	101
Age in years	33.97 (0.27)	30.47 (0.99)
Symptom scores		
BSI depression	1.13 (0.03)	0.28 (0.05)
BSI anxiety	1.02 (0.03)	0.19 (0.03)
Adversities		
Recent negative life events	1.32 (0.037)	
Childhood adversity	3.70 (0.097)	
Emotion recognition task		
Happiness correctly identified %		68.03 (1.78)
Happiness identification RT		1950.40 (40.51)
Sadness correctly identified %,		49.44 (1.89)
Sadness identification RT		2234.35 (51.48)
Fear correctly identified %,		51.20 (1.92)
Fear identification RT		2114.85 (43.12)

Values are presented as mean (SEM).
SEM: Standard error of the mean.
BSI: Brief Symptom Inventory.
RT: reaction time for correctly identified emotions in msec.

2.4. Statistical analysis

Allelic association (using linear regression analysis with 1000 permutations), Hardy–Weinberg equilibrium and linkage disequilibrium (LD) calculations were performed using HelixTree™ 6.4.3 (Golden Helix, USA). PLINK v1.06 (http://pngu.mgh.harvard.edu/purcell/plink/) was used for testing association of different genetic models (dominant, recessive and additive; linear regression model) in both cohorts. Interactions were calculated with recent life events and childhood adversity. All association analyses were adjusted for age and sex. Correlation calculations and repeated measure ANOVA (emotion as within-subjects factor, genotype as between-subjects factor) were performed by using SPSS 15.0 for Windows software (SPSS Inc. Chicago, Illinois, USA). For PLINK analysis we used false discovery rate calculation at level of 5% (FDR; Qvalue: http://genomics.princeton.edu.storeylab/qvalue/) to adjust p values according to the number of hypotheses tested (Storey and Tibshirani, 2003). We report q value which is a measure of significance of each test of many tests performed simultaneously.

3. Results

All HTR1A/1B SNPs were in Hardy–Weinberg equilibrium in both Level1 and Level2 participants. Stong LD was observed within both the HTR1A and HTR1B genes ($R^2>0.4$), which is consistent with the HapMap data.

The Pearson correlation value between depression scores and anxiety scores was $R=0.778$, ($p<0.001$). The recent life events were in moderate correlation with depression ($R=0.251$, $p<0.001$) and with anxiety ($R=0.229$, $p<0.001$) scores. Childhood adversity showed stronger but still moderate correlation with depression ($R=0.366$, $p<0.001$) and anxiety ($R=0.313$, $p<0.001$) scores.

3.1. Allelic association test

None of the alleles showed significant association to either BSI depression scores or BSI anxiety scores using allelic association test for Level1 (data not shown). In Level2 significant association was observed between rs6295 G allele and decreased recognition time of identification of fearful faces ($\beta=-343.67$, permuted $p=0.014$), but not to the other emotions. Rs6295 G allele carriers also recognised less sad faces correctly compared to C allele carriers ($\beta=-14.61$, permuted $p=0.044$) (data not shown).

3.2. Genotypic association test

In the Level1 participant group none of the genotypes showed association with depression or anxiety scores using genotypic

Please cite this article as: Mekli, K., et al., The HTR1A and HTR1B receptor genes influence stress related information processing, Eur. Neuropsychopharmacol. (2010), doi:10.1016/j.euroneuro.2010.06.013

Table 2 Significant interaction results between SNPs and recent negative life events (RLE) on BSI depression and anxiety symptoms.

	BSI depression score						BSI anxiety score					
Model:	Additive		Dominant		Recessive		Additive		Dominant		Recessive	
Interaction:	RLE		RLE		RLE		RLE		RLE		RLE	
	β	P	β	P	β	P	β	P	β	P	β	P
HTR1A_rs6295 G	0.078	**0.009****	0.127	**0.009****	0.085	*0.088*	0.062	**0.018***	0.087	**0.042***	0.081	*0.056*
HTR1A_rs749098 C	−0.048	0.167	−0.047	0.278	−0.105	0.210	−0.062	**0.044***	−0.060	0.121	−0.140	*0.061*
HTR1A_rs878567 T	0.090	**0.012****	0.133	**0.021***	0.104	*0.076*	0.096	**0.002****	0.113	**0.026***	0.142	**0.006****
HTR1B_rs6296 C	0.108	**0.005****	0.133	**0.011****	0.178	**0.039***	0.091	**0.007****	0.096	**0.036***	0.192	**0.010****
HTR1B_rs130058 T	−0.009	0.807	−0.009	0.850	−0.023	0.786	0.001	0.982	−0.001	0.976	0.003	0.972
HTR1B_rs11568817 G	−0.023	0.502	−0.051	0.341	−0.007	0.916	−0.011	0.716	−0.058	0.224	0.040	0.462

Minor alleles are in bold after the rs numbers.
Italic – trend in interaction ($0.05 < p < 0.1$).
Bold – significant interaction ($p < 0.05$).
* False discovery rate correction for multiple testing $0.05 < q < 0.1$.
** False discovery rate correction for multiple testing $q < 0.05$.

association test, additive, dominant or recessive analytical models (data no shown).

When recent negative life events were included as an interacting factor, three (rs6295, rs878567 and rs6296) out of the six investigated SNPs showed significant interaction on current depression state in the additive and dominant model even after correction for multiple testing (Table 2). The same SNPs plus HTR1A rs749098 C also showed significant interaction on the current anxiety state in the additive model but only rs878567 and rs6296 effects remained significant after correction for multiple testing. These two SNPs also showed significant interaction with RLE on anxiety in the recessive model (Table 2 and Fig. 1 a–d).

Significant gene × RLE interactions were not due to association between RLE and SNPs as a preliminary analysis revealed no significant association between the HTR1A/1B SNPs and RLE scores, or childhood adversity scores, in any mathematical models (data not shown). This observation supports the role of epigenetic effects of negative life events on the HTR1A and HTR1B genes.

No significant interactions were observed between childhood adversity scores and genotype for any analytical model. No sex × SNP interactions were observed (data not shown).

In the Level2 study, rs6295 GG carriers had reduced accuracy in identifying emotions independently of the valence of emotion ($F=4.749$, $df=2,96$ $p=0.011$; Fig. 2a); for emotions considered separately this effect was only significant for sad faces (Table 3a). In general, rs6295 GG carriers were not significantly quicker to recognise emotions ($F=2.283$, $df=2,93$ $p=0.107$) but within-subjects contrasts showed significant interaction between happy and fear ($F=3.113$, $df=2,93$ $p=0.049$; Fig. 2b). Thus, omitting happiness from the model rs6295 GG carriers showed significantly shorter reaction times for negative emotions ($F=3.915$, $df=2,94$ $p=0.023$). As for separate emotions, this effect was significant for fear and a trend for sad (Table 3b, Fig. 2b).

4. Discussion

Our results emphasise that genetic variations not only in the HTR1A but also in the HTR1B genes confer susceptibility to depression and anxiety in adults in the presence of exposure to stressful life events. We could not find a direct association between either the HTR1A or HTR1B SNPs and depressive or anxiety symptoms. However, interaction analysis with recent stressful life events showed significant associations between two HTR1A SNPs (rs878567 T and rs6295 G) and one HTR1B SNPs (rs6296 C) and both phenotypes. The number of life events was not significantly different according to genotypes which suggests that the main contributor to these phenotypes is the epigenetic effect of negative life events on the HTR1A and HTR1B genes. Furthermore, we demonstrated that the effect of HTR1A rs6295 G allele is correlated with enhanced threat-related information processing based on a face emotion recognition study. Thus our results extend previous findings of 5-HTTLPR and stressful life events interaction for two other genes in the serotonergic pathway and emphasise the possible role of increased threat-related information processing as an intermediate phenotype.

The association between these genes with both depression and anxiety also supports previous findings from twin studies, namely that these two traits share common genetic factors (Kendler, 1996; Mosing et al., 2009; Roy et al., 1995). These shared genetic factors partially mediated their effects through neuroticism but in the present study this was not directly associated with the investigated SNPs (data not shown), although previously Strobel et al reported significant association with neuroticism and HTR1A rs6295 (Strobel et al., 2003). However, our findings suggest the relevance of other non-neuroticism related shared mechanisms (Hettema, 2008; Kendler et al., 2007). Mixed presentation of anxiety and depressive symptoms is usual in population samples and categorisation into major depressive disorder, anxiety disorder or mixed anxiety-depressive disorder mainly reflects the severity and predominance of these dimensions (Das-Munshi et al., 2008). Frequent transitions between these syndromes also support common pathophysiology (Hettema, 2008).

In our study the rs6295 G allele was not only a risk factor for anxiety and depression, but the healthy control GG carriers required less time to correctly identify fearful faces. They also responded more quickly to sad faces, although this difference did not reached significance, but there was no effect on reaction time to happy faces. Thus the increased tendency to

Please cite this article as: Mekli, K., et al., The HTR1A and HTR1B receptor genes influence stress-related information processing, Eur. Neuropsychopharmacol. (2010), doi:10.1016/j.euroneuro.2010.06.013

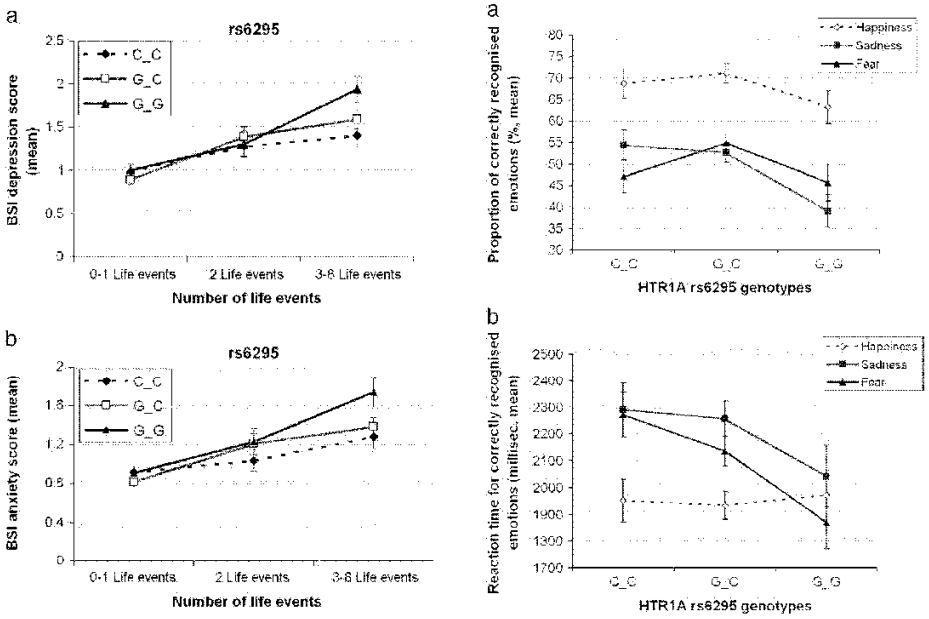


Figure 2 Correctly identified emotions (a, % mean) and reaction times for these (b, msec mean) according to HTR1A rs6295 genotypes (CC n=24; CG n=57; GG n=20). Rs6295 GG genotype carriers were less likely to correctly identify emotions but showed quicker response to negative emotions, such as sad and fear.

stress-induced anxiety and depression in GG carriers may involve speeded psychomotor responses to the perception of threat at an intermediate level. This is not due to an affective bias to perceive negative emotions that has been described in anxiety and depression (Harmer, 2008; Mogg et al., 2007) because the GG carriers were less accurate in detecting face

Figure 1 Interaction of recent negative life events with HTR1A rs6295 and HTR1B rs6296 polymorphisms on depression and anxiety in Level1. Individuals were grouped into 3 groups according to the number of stressful life events experienced in the past year, shown on the X-axis. 888 people (64%) experienced 0–1 stressful life event, 261 individuals (19%) 2 stressful life events and 238 individuals (17%) 3–8 life events. Mean values of BSI depression and anxiety scores were calculated for each group, shown on the Y axis.

BSI: Brief Symptom Inventory

a. HTR1A rs6295 and BSI depression score.
b. HTR1A rs6295 BSI anxiety score.
c. HTR1B rs6296 and BSI depression score.
d. HTR1B rs6296 and BSI anxiety score.

Please cite this article as: Mekli, K., et al., The HTR1A and HTR1B receptor genes influence stress-related information processing, Eur. Neuropsychopharmacol. (2010), doi:10.1016/j.euroneuro.2010.06.013

Table 3 The effect of rs6295 on correctly identified face emotions (%, table a) and on reaction time (RT) for correctly identified face emotions (table b).

	Additive		Dominant		Recessive	
	β	P	β	P	β	P
a						
Happiness_%	−2.437	0.348	0.247	0.951	−7.151	*0.099*
Sadness_%	−7.307	**0.005** **	−5.409	0.193	−14.17	**0.001** **
Fear_%	0.117	0.968	5.361	0.233	−6.667	0.172
b						
Happiness_RT	8.174	0.891	7.224	0.937	32.14	0.750
Sadness_RT	116.3	0.127	88.95	0.445	225.3	*0.082*
Fear_RT	171.8	**0.008** **	195.4	*0.053*	253.2	**0.020** *

Italic = trend in interaction ($0.05 < p < 0.1$).
Bold = significant interaction ($p < 0.05$).
* False discovery rate correction for multiple testing $0.05 < q < 0.1$.
** False discovery rate correction for multiple testing $q < 0.05$.

emotions including happy. A potentially related finding is the reported association of the rs6295 G allele with increased questionnaire measures of impulsivity (Benko et al., 2010). Further studies are needed to replicate our findings and to understand the nature of speeded responses to threat. One possible mechanism that can mediate this genetic effect is the activation of amygdala (Fisher et al., 2006), although contradictory results have been reported about the rs6295 G allele effect on amygdala activation in different imaging studies (Domschke et al., 2006; Fakra et al., 2009; Le Francois et al., 2008). This may be related to the less accurate perception of the three face emotions in the GG carriers, which is suggested by our results.

HTR1A and rs6295 have previously been investigated in depression studies but many could not find a direct association with diagnosis or symptoms (Illi et al., 2009; Serretti et al., 2009) as in our study (see, Supplementary Table 1). However, other studies, taking into consideration the stressful life events, have found associations. One study reported on 272 suicide attempter families and observed an over-transmission of the G allele in a subset where prior to the suicide attempt a high level of previous traumatic and/or stressful life events had occurred (Wasserman et al., 2006). There is no functional study available for the HTR1A 3′UTR polymorphism, rs878567, but some studies have investigated its role in mental disorders and suicide. Three studies found no association between this SNP and susceptibility to human internalizing phenotypes, including major depression, a range of anxiety disorders and neuroticism (Hettema et al., 2008) and suicidal behaviour (Serretti et al., 2007; Serretti et al., 2009). However, including childhood physical abuse, the TT homozygote was associated with a lower risk of major depression (Brezo et al., 2009), a finding that we did not replicate. This contradiction to our findings may be due to the limited number of cases involved in the Brezo study. Of the other studies that have investigated an association between HTR1B and depression; one found associations between the C allele of rs6296 and MDD (Huang et al., 2003). Another investigated rs130058 and rs11568817 in depression and did not find an association; despite negative life events being included in the model (Zhang et al., 2009). In our study also these two SNPs do not show any associative or interactive influences on depression.

Animal studies support our findings as HTR1A or HTR1B knockout animals develop normally without any obvious behavioural disturbances but stressful paradigms provoke specific emotional phenotypes (Zhuang et al., 1999) suggesting that these receptors have major roles in coping with stress. Furthermore, these genetic variants may well influence the development of non-5-HT neural systems concerned with emotion processing. That 5-HT genes may act via development was indeed suggested by the finding in 5-HT knock out mice that impaired 5-HT_{1A} function during development was necessary for expression of the adult anxious phenotype whereas inducing impaired 5-HT_{1A} function in adulthood did not affect anxiety (Gross et al., 2002). Exposure to childhood adversity is another developmental risk factor associated with greater sensitivity to later life events and the onset of depression. However, in our study none of the HTR1A and HTR1B SNPs showed an association with depression or anxiety when interaction with childhood adversity was tested. The interaction with recent life events rather than childhood adversity indicates that HTR1A/1B genes may act on a different pathway from the effect of childhood adversity to increase sensitivity to stressful events. However, since they act both pre- and postsynaptically, as auto- and heteroreceptors, it is difficult to make direct inferences about the mediating role of altered 5-HT function (Drago et al., 2010; Le Francois et al., 2008; Sari, 2004; Savitz et al., 2009).

The HTR1A rs6295 G allele has been shown to interrupt a nuclear deformed epidermal autoregulatory factor (Deaf-1/NUDR) binding site in the promoter region of HTR1A gene in a cell specific manner. Deaf-1/NUDR is a repressor transcription factor in raphe cells but acts as an enhancer in postsynaptic cells expressing 5-HT_{1A} receptors. Abrogation of its function leads to both the enhanced expression of HTR1A at the raphe and therefore reduced serotonergic tone, and to decreased postsynaptic HTR1A expression. Furthermore, the HTR1A rs6295 G allele, by interrupting the Hes5 (hairy and enhancer of split 5) transcription factor binding site, decreases HTR1A expression uniformly in raphe and cortical cell cultures (Le Francois et al., 2008; Savitz et al., 2009). Two PET studies in humans found increased raphe and hippocampal 5-HT_{1A} receptor binding in those with the rs6295 G allele but G alleles were over-represented in the depressed group and this is a

Please cite this article as: Mekli, K., et al., The HTR1A and HTR1B receptor genes influence stress-related information processing. Eur. Neuropsychopharmacol. (2010), doi:10.1016/j.euroneuro.2010.06.013

potential confound as the depressed sample overall had greater 5-HT_{1A} binding (David et al., 2005; Parsey et al., 2006b). Further studies are needed to determine the influence of the HTR1A rs6295 polymorphism on the expression and function of the receptor in depression.

The C allele of HTR1B rs6296 also showed association with depression and anxiety in interaction with recent life events in our study. Whether or not this synonymous SNP is functional, remains unknown. However, based on previous studies the C allele is in linkage disequilibrium with variants that code transcriptionally less active versions of the gene (Conner et al., 2010; Duan et al., 2003). Animal studies suggest that the decreased expression of the HTR1B is linked with aggressiveness and impulsivity but not anxiety (Clark et al., 2004; Drago et al., 2010). Moreover, one animal study found that 24 h after exposure to inescapable stress, 5-HT1B receptor was overexpressed in the dorsal raphe nuclei (DRN) and the rats' anxiety behaviour was elevated. Meanwhile 5-HT_{1B} overexpression in the DRN, in the absence of stress, increased exploratory behaviour (Clark et al., 2002) This finding indicates that the impact of 5-HT_{1B} in DRN is dependent on context i.e. stress and shows the complex role of 5-HT_{1B} in anxiety behaviour. However, the human phenotype appears to be more complex with men who possess the lower expression haplotypes reporting significantly more hostility but also significantly more anxiety and sadness (Conner et al., 2010).

The strength of our study is that we had relatively large number of individuals genotyped for these polymorphisms and we used continuous traits rather than diagnostic dichotomy. When using an additive linear model we had a 96% power in our population (N=1387) to detect a polymorphism (assuming a minor allele frequency of 25%) with an effect size of 1% at the 5% two-tailed significance level. To detect a gene x environment interaction with an effect size of 3%, in the same model as above, we had 97% power. Furthermore, we tested the rs6295 genetic effect in a face emotion task that is sensitive to serotonergic changes. The potential limitations of our study are that we have not investigated all the polymorphisms in our Level 2 face emotion recognition study, the lack of independent replication sample and the unknown functional relevance some of the genotyped SNPs.

In conclusion our results support previous studies suggesting that HTR1A and HTR1B polymorphisms are associated with stress-related neuropsychiatric conditions, such as depression and anxiety in the presence of environmental stress factors. In addition, our results propose that threat-related emotional processing might be an intermediate phenotype to these disorders. If so, $5HT_{1A}$ and $5HT_{1B}$ receptors may be targets for preventive treatment strategies of stress-related neuropsychiatric disorders, especially in at-risk individuals. Further studies are needed to investigate this hypothesis, and to clarify the effect of HTR1A and the HTR1B genes in different brain neuronal networks and their role in human anxiety, depression and impulsivity.

Role of the funding source

This study was supported by the Sixth Framework Program of the EU. NewMood, LSHM-CT-2004-503474, ETT 318-0412009 and TAMOP-4.2.1.B-09/1/KMR-2010-0001. They had no further role in study design; in the collection, analysis and interpretation of the data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

GJ, DC, ET, DD, ZGT, RE IMA, GB and JFWD participated in the design of the study, KM drafted the manuscript. GJ, DC, ET, DD, KLW, and AP contributed substantially to the data acquisition and data handling and revised the manuscript. KM, AP, HP, FM and WEO carried out the molecular genetic studies and helped with the interpretation of the data. KM, GJ performed the statistical analysis. All authors read and approved the final manuscript.

Conflict of interest

Prof Deakin has carried out consultancy and speaking engagements for Bristol Myers Squibb, AstraZeneca, Eli Lilly, Schering Plough, Janssen-Cilag, and Servier. All fees are paid to the University of Manchester to reimburse them for the time taken. He has share options in P1vital. Prof Anderson has received grant support from AstraZeneca, consultancy fees/honoraria for speaking/support to attend conferences from Wyeth, Servier, Eli Lilly, Lundbeck, Cephalon and Bristol Myers Squibb. Drs Juhasz, Thomas, Chase, Elliott, Downey, Miyajima and Payton, Prof Ollier, Mrs Lloyd-Williams, Ms Mekli, Ms Platt and Mr Toth report no relevant financial interest.

Acknowledgements

Authors are grateful to all the participants who are taking part in this study and to Heaton Mersey Medical Practice and Cheadle Medical Practice for their assistance in the recruitment.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2010.06.013.

References

Anttila, S., Huuhka, K., Huuhka, M., Rontu, R., Hurme, M., Leinonen, E., et al., 2007. Interaction between 5-HT1A and BDNF genotypes increases the risk of treatment-resistant depression. J. Neural Transm. 114, 1065–1068.

Barnes, N.M., Sharp, T., 1999. A review of central 5-HT receptors and their function. Neuropharmacology 38, 1083–1152.

Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21, 263–265.

Benko, A., Lazary, J., Molnar, E., Gonda, X., Tothfalusi, L., Pap, D., et al., 2010. Significant association between the C(-1019) G functional polymorphism of the HTR(1A) gene and impulsivity. Am. J. Med. Genet. B Neuropsychiatr. Genet. 153B, 592–599.

Bernstein, D.P., Fink, L., Handelsman, L., Foote, J., Lovejoy, M., Wenzel, K., et al., 1994. Initial reliability and validity of a new retrospective measure of child abuse and neglect. Am. J. Psychiatry 151, 1132–1136.

Bohmaker, K., Eison, A.S., Yocca, F.D., Meller, E., 1993. Comparative effects of chronic 8-OH-DPAT, gepirone and ipsapirone treatment on the sensitivity of somatodendritic 5-HT1A autoreceptors. Neuropharmacology 32, 527–534.

Please cite this article as: Mekli, K., et al., The HTR1A and HTR1B receptor genes influence stress-related information processing. Eur. Neuropsychopharmacol. (2010), doi:10.1016/j.euroneuro.2010.06.013

Brezo, J., Bureau, A., Merette, C., Jomphe, V., Barker, E.D., Vitaro, F., et al., 2009. Differences and similarities in the serotonergic diathesis for suicide attempts and mood disorders: a 22-year longitudinal gene-environment study. Mol. Psychiatry Electronic publication ahead of print.

Brugha, T., Bebbington, P., Tennant, C., Hurry, J., 1985. The List of Threatening Experiences: a subset of 12 life event categories with considerable long-term contextual threat. Psychol. Med. 15, 189–194.

Brunner, D., Hen, R., 1997. Insights into the neurobiology of impulsive behavior from serotonin receptor knockout mice. Ann. NY Acad. Sci. 836, 81–105.

Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., et al., 2003. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301, 386–389.

Caspi, A., Hariri, A.R., Holmes, A., Uher, R., Moffitt, T.E., 2010. Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. Am. J. Psychiatry 167, 509–527.

Cervilla, J.A., Rivera, M., Molina, E., Torres-Gonzalez, F., Bellon, J.A., Moreno, B., et al., 2006. The 5-HTTLPR s/s genotype at the serotonin transporter gene (SLC6A4) increases the risk for depression in a large cohort of primary care attendees: the PREDICT-gene study. Am. J. Med. Genet. B Neuropsychiatr. Genet. 141B, 912–917.

Clark, M.S., Sexton, T.J., McClain, M., Root, D., Kohen, R., Neumaier, J.F., 2002. Overexpression of 5-HT1B receptor in dorsal raphe nucleus using Herpes Simplex Virus gene transfer increases anxiety behavior after inescapable stress. J. Neurosci. 22, 4550–4562.

Clark, M.S., Vincow, E.S., Sexton, T.J., Neumaier, J.F., 2004. Increased expression of 5-HT1B receptor in dorsal raphe nucleus decreases fear-potentiated startle in a stress dependent manner. Brain Res. 1007, 86–97.

Conner, T.S., Jensen, K.P., Tennen, H., Furneaux, H.M., Kranzler, H.R., Covault, J., 2010. Functional polymorphisms in the serotonin 1B receptor gene (HTR1B) predict self-reported anger and hostility among young men. Am. J. Med. Genet. B Neuropsychiatr. Genet. 153B, 67–78.

Contreras, J., Hare, L., Camarena, B., Glahn, D., Dassori, A., Medina, R., et al., 2009. The serotonin transporter 5-HTTPR polymorphism is associated with current and lifetime depression in persons with chronic psychotic disorders. Acta Psychiatr. Scand. 119, 117–127.

Crabbe, J.C., Phillips, T.J., Feller, D.J., Hen, R., Wenger, C.D., Lessov, C.N., et al., 1996. Elevated alcohol consumption in null mutant mice lacking 5-HT1B serotonin receptors. Nat. Genet. 14, 98 101.

Das-Munshi, J., Goldberg, D., Bebbington, P.E., Bhugra, D.K., Brugha, T.S., Dewey, M.E., et al., 2008. Public health significance of mixed anxiety and depression: beyond current classification. Br. J. Psychiatry 192, 171–177.

David, S.P., Murthy, N.V., Rabiner, E.A., Munafo, M.R., Johnstone, E.C., Jacob, R., et al., 2005. A functional genetic variation of the serotonin (5-HT) transporter affects 5-HT1A receptor binding in humans. J. Neurosci. 25, 2586 2590.

Derogatis, L., 1993. BSI: Brief Symptom Inventory: Administration, Scoring and Procedures Manual. National Computer Systems, Pearson Inc, Minneapolis.

Domschke, K., Braun, M., Ohrmann, P., Suslow, T., Kugel, H., Bauer, J., et al., 2006. Association of the functional -1019C/G 5-HT1A polymorphism with prefrontal cortex and amygdala activation measured with 3 T fMRI in panic disorder. Int. J. Neuropsychopharmacol. 9, 349–355.

Drachmann, B.J., Bock, C., Vinberg, M., Werge, T., Gether, U., Vedel, K.L., 2009. Interaction between genetic polymorphisms and stressful life events in first episode depression. J. Affect. Disord. 119, 107–115.

Drago, A., Alboni, S., Nicoletta, B., De Ronchi, D., Serretti, A., 2010. HTR1B as a risk profile maker in psychiatric disorders: a review through motivation and memory. Eur. J. Clin. Pharmacol. 66, 5–27.

Drevets, W.C., Price, J.L., Furey, M.L., 2008. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. Brain Struct. Funct. 213, 93 118.

Duan, J., Sanders, A.R., Molen, J.E., Martinolich, L., Mowry, B.J., Levinson, D.F., et al., 2003. Polymorphisms in the 5′-untranslated region of the human serotonin receptor 1B (HTR1B) gene affect gene expression. Mol. Psychiatry 8, 901–910.

Ekman, P., Friesen, W.V., 1976. Pictures of Facial Affect. Consulting Psychologists Press, Palo Alto, California.

Fakra, E., Hyde, L.W., Gorka, A., Fisher, P.M., Munoz, K.E., Kimak, M., et al., 2009. Effects of HTR1A C(-1019)G on amygdala reactivity and trait anxiety. Arch. Gen. Psychiatry 66, 33–40.

First, M.B., Spitzer, R.L., Gibbon, M., Williams, J.B.W., 2002. Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version. New York State Psychiatric Institute, New York.

Fisher, P.M., Meltzer, C.C., Ziolko, S.K., Price, J.C., Moses-Kolko, E.L., Berga, S.L., et al., 2006. Capacity for 5-HT1A-mediated autoregulation predicts amygdala reactivity. Nat. Neurosci. 9, 1362–1363.

Freeman, B., Smith, N., Curtis, C., Huckett, L., Mill, J., Craig, I.W., 2003. DNA from buccal swabs recruited by mail: evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. Behav. Genet. 33, 67–72.

Fusar-Poli, P., Placentino, A., Carletti, F., Landi, P., Allen, P., Surguladze, S., et al., 2009. Functional atlas of emotional faces processing: a voxel-based meta-analysis of 105 functional magnetic resonance imaging studies. J. Psychiatry Neurosci. 34, 418–432.

Gabriel, S.B., Schaffner, S.F., Nguyen, H., Moore, J.M., Roy, J., Blumenstiel, B., et al., 2002. The structure of haplotype blocks in the human genome. Science 296, 2225–2229.

Gross, C., Zhuang, X., Stark, K., Ramboz, S., Oosting, R., Kirby, L., et al., 2002. Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. Nature 416, 396–400.

Gur, R.C., Erwin, R.J., Gur, R.E., Zwil, A.S., Heimberg, C., Kraemer, H.C., 1992. Facial emotion discrimination: II. Behavioral findings in depression. Psychiatry Res. 42, 241–251.

Hall, H., Lundkvist, C., Halldin, C., Farde, L., Pike, V.W., McCarron, J.A., et al., 1997. Autoradiographic localization of 5-HT1A receptors in the post-mortem human brain using [3H]WAY-100635 and [11C]way-100635. Brain Res. 745, 96–108.

Harmer, C.J., 2008. Serotonin and emotional processing: does it help explain antidepressant drug action? Neuropharmacology 55, 1023–1028.

Harmer, C.J., Bhagwagar, Z., Perrett, D.I., Vollm, B.A., Cowen, P. J., Goodwin, G.M., 2003. Acute SSRI administration affects the processing of social cues in healthy volunteers. Neuropsychopharmacology 28, 148–152.

Harmer, C.J., Shelley, N.C., Cowen, P.J., Goodwin, G.M., 2004. Increased positive versus negative affective perception and memory in healthy volunteers following selective serotonin and norepinephrine reuptake inhibition. Am. J. Psychiatry 161, 1256 1263.

Heisler, L.K., Chu, H.M., Brennan, T.J., Danao, J.A., Bajwa, P., Parsons, L.H., et al., 1998. Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. Proc. Natl Acad. Sci. USA 95, 15049–15054.

Hettema, J.M., 2008. The nosologic relationship between generalized anxiety disorder and major depression. Depress. Anxiety 25, 300–316.

Please cite this article as: Mekli, K., et al., The HTR1A and HTR1B receptor genes influence stress-related information processing. Eur. Neuropsychopharmacol. (2010), doi:10.1016/j.euroneuro.2010.06.013

Hettema, J.M., An, S.S., van den Oord, E.J., Neale, M.C., Kendler, K.S., Chen, X., 2008. Association study between the serotonin 1A receptor (HTR1A) gene and neuroticism, major depression, and anxiety disorders. Am. J. Med. Genet. B Neuropsychiatr. Genet. 147B, 661–666.

Hong, C.J., Chen, T.J., Yu, Y.W., Tsai, S.J., 2006. Response to fluoxetine and serotonin 1A receptor (C-1019G) polymorphism in Taiwan Chinese major depressive disorder. Pharmacogenomics J. 6, 27–33.

Huang, Y.Y., Oquendo, M.A., Friedman, J.M., Greenhill, L.L., Brodsky, B., Malone, K.M., et al., 2003. Substance abuse disorder and major depression are associated with the human 5-HT1B receptor gene (HTR1B) G861C polymorphism. Neuropsychopharmacology 28, 163–169.

Illi, A., Setala-Soikkeli, E., Viikki, M., Poutanen, O., Huhtala, H., Mononen, N., et al., 2009. 5-HTR1A, 5-HTR2A, 5-HTR6, TPH1 and TPH2 polymorphisms and major depression. NeuroReport 20, 1125–1128.

Juhasz, G., Chase, D., Pegg, E., Downey, D., Toth, Z.G., Stones, K., et al., 2009. CNR1 gene is associated with high neuroticism and low agreeableness and interacts with recent negative life events to predict current depressive symptoms. Neuropsychopharmacology 34, 2019–2027.

Kato, M., Fukuda, T., Wakeno, M., Okugawa, G., Takekita, Y., Watanabe, S., et al., 2009. Effect of 5-HT1A gene polymorphisms on antidepressant response in major depressive disorder. Am. J. Med. Genet. B Neuropsychiatr. Genet. 150B, 115–123.

Kendler, K.S., 1996. Major depression and generalised anxiety disorder. Same genes, (partly)different environments — revisited. Br. J. Psychiatry Suppl. 68–75.

Kendler, K.S., Gardner, C.O., Gatz, M., Pedersen, N.L., 2007. The sources of co-morbidity between major depression and generalized anxiety disorder in a Swedish national twin sample. Psychol. Med. 37, 453–462.

Kennett, G.A., Marcou, M., Dourish, C.T., Curzon, G., 1987. Single administration of 5-HT1A agonists decreases 5-HT1A presynaptic, but not postsynaptic receptor-mediated responses: relationship to antidepressant-like action. Eur. J. Pharmacol. 138, 53–60.

Kraus, M.R., Al Taie, O., Schafer, A., Pfersdorff, M., Lesch, K.P., Scheurlen, M., 2007. Serotonin-1A receptor gene HTR1A variation predicts interferon-induced depression in chronic hepatitis C. Gastroenterology 132, 1279–1286.

Lanfumey, L., Hamon, M., 2004. 5-HT1 receptors. Curr. Drug Targets CNS Neurol. Disord. 3, 1–10.

Lazary, J., Lazary, A., Gonda, X., Benko, A., Molnar, E., Juhasz, G., et al., 2008. New evidence for the association of the serotonin transporter gene (SLC6A4) haplotypes, threatening life events, and depressive phenotype. Biol. Psychiatry 64, 498–504.

Le Francois, B., Czesak, M., Steubl, D., Albert, P.R., 2008. Transcriptional regulation at a HTR1A polymorphism associated with mental illness. Neuropharmacology 55, 977–985.

Lemonde, S., Turecki, G., Bakish, D., Du, L., Hrdina, P.D., Bown, C.D., et al., 2003. Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. J. Neurosci. 23, 8788–8799.

Lemonde, S., Du, L., Bakish, D., Hrdina, P., Albert, P.R., 2004. Association of the C(-1019)G 5-HT1A functional promoter polymorphism with antidepressant response. Int. J. Neuropsychopharmacol. 7, 501–506.

Levinson, D.F., 2006. The genetics of depression: a review. Biol. Psychiatry 60, 84–92.

Merens, W., Willem Van der Does, A.J., Spinhoven, P., 2007. The effects of serotonin manipulations on emotional information processing and mood. J. Affect. Disord. 103, 43–62.

Mineka, S., Watson, D., Clark, L.A., 1998. Comorbidity of anxiety and unipolar mood disorders. Annu. Rev. Psychol. 49, 377–412.

Moffitt, T.E., Caspi, A., Rutter, M., 2005. Strategy for investigating interactions between measured genes and measured environments. Arch. Gen. Psychiatry 62, 473–481.

Mogg, K., Bradley, B.P., 2002. Selective orienting of attention to masked threat faces in social anxiety. Behav. Res. Ther. 40, 1403–1414.

Mogg, K., Garner, M., Bradley, B.P., 2007. Anxiety and orienting of gaze to angry and fearful faces. Biol. Psychol. 76, 163–169.

Mosing, M.A., Gordon, S.D., Medland, S.E., Statham, D.J., Nelson, E.C., Heath, A.C., et al., 2009. Genetic and environmental influences on the co-morbidity between depression, panic disorder, agoraphobia, and social phobia: a twin study. Depress. Anxiety 26, 1004–1011.

Neff, C.D., Abkevich, V., Packer, J.C., Chen, Y., Potter, J., Riley, R., et al., 2009. Evidence for HTR1A and LHPP as interacting genetic risk factors in major depression. Mol. Psychiatry 14, 621–630.

Parks, C.L., Robinson, P.S., Sibille, E., Shenk, T., Toth, M., 1998. Increased anxiety of mice lacking the serotonin1A receptor. Proc. Natl Acad. Sci. USA 95, 10734–10739.

Parsey, R.V., Olvet, D.M., Oquendo, M.A., Huang, Y.Y., Ogden, R.T., Mann, J.J., 2006a. Higher 5-HT1A receptor binding potential during a major depressive episode predicts poor treatment response: preliminary data from a naturalistic study. Neuropsychopharmacology 31, 1745–1749.

Parsey, R.V., Oquendo, M.A., Ogden, R.T., Olvet, D.M., Simpson, N., Huang, Y.Y., et al., 2006b. Altered serotonin 1A binding in major depression: a [carbonyl-C-11]WAY100635 positron emission tomography study. Biol. Psychiatry 59, 106–113.

Pazos, A., Palacios, J.M., 1985. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. Brain Res. 346, 205–230.

Plomin, R., Crabbe, J., 2000. DNA. Psychol. Bull. 126, 806–828.

Ramboz, S., Oosting, R., Amara, D.A., Kung, H.F., Blier, P., Mendelsohn, M., et al., 1998. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc. Natl Acad. Sci. USA 95, 14476–14481.

Rijsdijk, F.V., Sham, P.C., Sterne, A., Purcell, S., McGuffin, P., Farmer, A., et al., 2001. Life events and depression in a community sample of siblings. Psychol. Med. 31, 401–410.

Risch, N., Herrell, R., Lehner, T., Liang, K.Y., Eaves, L., Hoh, J., et al., 2009. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. JAMA 301, 2462–2471.

Rocha, B.A., Scearce-Levie, K., Lucas, J.J., Hiroi, N., Castanon, N., Crabbe, J.C., et al., 1998. Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. Nature 393, 175–178.

Rothe, C., Gutknecht, L., Freitag, C., Tauber, R., Mossner, R., Franke, P., et al., 2004. Association of a functional 1019C>G 5-HT1A receptor gene polymorphism with panic disorder with agoraphobia. Int. J. Neuropsychopharmacol. 7, 189–192.

Roy, M.A., Neale, M.C., Pedersen, N.L., Mathe, A.A., Kendler, K.S., 1995. A twin study of generalized anxiety disorder and major depression. Psychol. Med. 25, 1037–1049.

Rutter, M., Thapar, A., Pickles, A., 2009. Gene–environment interactions: biologically valid pathway or artifact? Arch. Gen. Psychiatry 66, 1287–1289.

Sari, Y., 2004. Serotonin1B receptors: from protein to physiological function and behavior. Neurosci. Biobehav. Rev. 28, 565–582.

Savitz, J., Lucki, I., Drevets, W.C., 2009. 5-HT(1A) receptor function in major depressive disorder. Prog. Neurobiol. 88, 17–31.

Serretti, A., Mandelli, L., Giegling, I., Schneider, B., Hartmann, A.M., Schnabel, A., et al., 2007. HTR2C and HTR1A gene variants in German and Italian suicide attempters and completers. Am. J. Med. Genet. B Neuropsychiatr. Genet. 144B, 291–299.

Serretti, A., Calati, R., Giegling, I., Hartmann, A.M., Moller, H.J., Rujescu, D., 2009. Serotonin receptor HTR1A and HTR2C variants

Please cite this article as: Mekli, K., et al., The HTR1A and HTR1B receptor genes influence stress-related information processing. Eur. Neuropsychopharmacol. (2010), doi:10.1016/j.euroneuro.2010.06.013

and personality traits in suicide attempters and controls. J. Psychiatr. Res. 43, 519–525.
Storey, J.D., Tibshirani, R., 2003. Statistical significance for genomewide studies. Proc. Natl Acad. Sci. USA 100, 9440–9445.
Strobel, A., Gutknecht, L., Rothe, C., Reif, A., Mossner, R., Zeng, Y., et al., 2003. Allelic variation in 5-HT1A receptor expression is associated with anxiety- and depression-related personality traits. J. Neural Transm. 110, 1445–1453.
Surguladze, S.A., Young, A.W., Senior, C., Brebion, G., Travis, M.J., Phillips, M.L., 2004. Recognition accuracy and response bias to happy and sad facial expressions in patients with major depression. Neuropsychology 18, 212–218.
Uher, R., McGuffin, P., 2010. The moderation by the serotonin transporter gene of environmental adversity in the etiology of depression: 2009 update. Mol. Psychiatry 15, 18–22.
Wasserman, D., Geijer, T., Sokolowski, M., Rozanov, V., Wasserman, J., 2006. The serotonin 1A receptor C(-1019)G polymorphism in relation to suicide attempt. Behav. Brain Funct. 2, 14.
Wray, N.R., James, M.R., Gordon, S.D., Dumenil, T., Ryan, L., Coventry, W.L., et al., 2009. Accurate, large-scale genotyping of 5HTTLPR and flanking single nucleotide polymorphisms in an association study of depression, anxiety, and personality measures. Biol. Psychiatry 66, 468–476.
Wu, Y., Xu, Y., Sun, Y., Wang, Y.F., Li, X., Lang, X.E., et al., 2008. Association between the serotonin 1A receptor C(-1019)G polymorphism and major depressive disorder in the northern Han ethnic group in China. Chin. Med. J. (Engl.) 121, 874–876.
Yevtushenko, O.O., Oros, M.M., Reynolds, G.P., 2010. Early response to selective serotonin reuptake inhibitors in panic disorder is associated with a functional 5-HT1A receptor gene polymorphism. J. Affect. Disord. 123, 308–311.
Yu, Y.W., Tsai, S.J., Liou, Y.J., Hong, C.J., Chen, T.J., 2006. Association study of two serotonin 1A receptor gene polymorphisms and fluoxetine treatment response in Chinese major depressive disorders. Eur. Neuropsychopharmacol. 16, 498–503.
Zalsman, G., Huang, Y.Y., Oquendo, M.A., Burke, A.K., Hu, X.Z., Brent, D.A., et al., 2006. Association of a triallelic serotonin transporter gene promoter region (5-HTTLPR) polymorphism with stressful life events and severity of depression. Am. J. Psychiatry 163, 1588–1593.
Zhang, K., Xu, Q., Xu, Y., Yang, H., Luo, J., Sun, Y., et al., 2009. The combined effects of the 5-HTTLPR and 5-HTR1A genes modulates the relationship between negative life events and major depressive disorder in a Chinese population. J. Affect. Disord. 114, 224–231.
Zhuang, X., Gross, C., Santarelli, L., Compan, V., Trillat, A.C., Hen, R., 1999. Altered emotional states in knockout mice lacking 5-HT1A or 5-HT1B receptors. Neuropsychopharmacology 21, 52S–60S.

Please cite this article as: Mekli, K., et al., The HTR1A and HTR1B receptor genes influence stress-related information processing. Eur. Neuropsychopharmacol. (2010), doi:10.1016/j.euroneuro.2010.06.013

10.9 Supplementary materials for Study 2

Supplementary Table 1: Genetic association studies investigating HTR2A and major depressive disorder (MDD)

Study	Sample size	SNPs investigated	Population	Assessment	Finding
Association with depression					
Jokela (2007)	1212 twins	rs6313 (Ser34Ser; T102C)	Young Finnish, 3-18 yrs	Modified BDI	Interaction observed between maternal nurturance and depression. T allele protective
Christiansen (2007)	684 randomly selected	rs6311 rs6314 (Tyr452His)	Elderly Danish twins, mean age 77 yrs	Modified CAMDEX	Association between rs6311 and depression score in males (230 men)
Choi (2004)	189 cases 148 controls	rs6314 (Tyr452His)	Korean, mean age 46 yrs	DSM-IV (Korean version)	G allele associated with MDD
Eley (2004)	210 cases 167 controls	rs6313 (Ser34Ser; T102C)	UK community based sample, 12-19 yrs	SMFQ	Association with depression independent of sex or environment effects. T allele protective
Jansson (2003)	377 cases 1215 controls	rs6314 (Tyr452His)	Elderly Swedish twins, mean age 73 yrs	CES-D	Association between depressed mood and rs6314 in males (123 men)
Zhang (1997)	51 cases 150 controls	rs6313 (Ser34Ser; T102C)	Japanese adults	DSM-IV	Association with MDD. T allele increases susceptibility and reduces age of onset
No association with depression					
Tencomnao (2010)	180 cases 183 controls	rs6313 (Ser34Ser; T102C)	Northeastern Thai, mean age 43 yrs	DSM-IV	No Association
Illi (2009)	106 cases 395 controls	rs6313 (Ser34Ser; T102C) rs6311, rs7997012	Finnish, mean age of cases 41 yrs, mean age of controls 44 yrs	MADRS	No association with MDD or treatment response
Zhang (2009)	401 cases 391 controls	rs6313 (Ser34Ser; T102C)	Chinese, mean age of cases 33 yrs, mean age of controls 33 yrs	DSM-IV	No association with MDD
Kishi (2009)	325 cases 802 controls	rs6313 (Ser34Ser; T102C) rs6311 rs7997012, rs1928040	Japanese, case mean age 47 yrs, mean age of controls 38 yrs	DSM-IV	No association with MDD
Yoon (2009)	181 suicidal cases 143 non-suicidal cases 176 controls	rs6313 (Ser34Ser; T102C)	Korean, mean age of suicidal cases 40 yrs, mean age on non-suicidal cases 41 yrs, mean age of controls 40 yrs	DSM-IV	No association with MDD
Shaikh (2008)	135 cases	rs6313 (Ser34Ser; T102C)	US adults	DSM-IV	No association with MDD
Oswald (2003)	142 cases 142 controls	rs6313 (Ser34Ser; T102C)	European sample taken from the European Collaboration Project on Affective Disorders (ECPAD)	DSM-III-R and DSM-IV	No association with UPAD
Minov (2001)	173 cases 121 controls	rs6313 (Ser34Ser; T102C) rs6314 (Tyr452His)	German, mean age of cases 50 yrs, mean age of controls 47 yrs	DSM-IV	No association with MDD
Frisch (1999)	102 cases 172 controls	rs6313 (Ser34Ser; T102C)	Ashkenazi and non- Ashkenazi jews	DSM-IV or SADS-L	No association with MDD
Tsai (1999)	79 cases 96 controls	rs6313 (Ser34Ser; T102C)	Taiwanese adults, 60 yrs and over	DSM-IV	No association with depressive disorders

Beck Depression Inventory (BDI); Cambridge Mental Disorders of the Elderly Examination (CAMDEX); Center for Epidemiologic Studies Depression Scale (CES-D); Montgomery–Asberg Depression Rating Scale (MADRS); short form of the Mood and Feelings Questionnaire (SMFQ); Schedule for Affective Disorders and Schizophrenia—Lifetime Version (SADS-L), Unipolar affective disorder (UPAD).

Supplementary Table 2: All possible interactions between SNPs genotyped on the Illumina 610 Quad microarray in the Dyne Steel cohort

				Beck Depression Score		Yesavage Depression Score		CMI Depression Score		CMI Anxiety Score		EPQ Neuroticsm Score	
CHR1	**SNP1**	**CHR2**	**SNP2**	**β**	**p value**	**β**	**p value**	**β**	**p value**	**β**	**p value**	**β**	**p value**
13	rs7333412	13	rs3803189	-0.1551	0.796	0.4894	0.370	0.08554	0.256	-0.00899	0.946	0.4018	0.518
13	rs7333412	13	rs6314	-0.6064	0.394	0.3409	0.595	-0.0293	0.745	0.0136	0.932	0.5261	0.475
13	rs7333412	13	rs977003	-0.3233	0.441	0.3645	0.342	0.04466	0.401	0.09466	0.312	0.101	0.815
13	rs7333412	13	rs1923884	-0.2973	0.667	-0.2684	0.669	-0.0225	0.797	0.08989	0.561	0.06795	0.921
13	rs7333412	13	rs1923885	-0.3418	0.327	0.1109	0.727	0.00652	0.883	-0.01301	0.868	-0.2641	0.456
13	rs7333412	13	rs1923886	0.6359	0.068	0.1483	0.643	0.02046	0.645	-0.0009	0.991	0.4925	0.167
13	rs7333412	13	rs1745837	0.6033	0.099	0.474	0.158	0.07987	0.087	0.07213	0.382	0.6477	0.088
13	rs7333412	13	rs622337	0.608	0.103	0.514	0.134	0.08615	0.071	0.1014	0.229	0.7031	0.067
13	rs7333412	13	rs655854	0.5817	0.116	0.4312	0.204	0.08278	0.081	0.08742	0.296	0.6618	0.086
13	rs7333412	13	rs2296972	0.6072	0.099	0.5024	0.138	0.08477	0.072	0.096	0.249	0.6748	0.078
13	rs7333412	13	rs4942578	0.8191	0.070	0.6538	0.113	0.01669	0.771	0.2412	0.017	0.8964	0.050
13	rs7333412	13	rs1928042	-0.3068	0.458	-0.362	0.330	-0.0087	0.866	-0.02653	0.770	-0.6672	0.103
13	rs7333412	13	rs2760345	-0.3815	0.557	-0.1722	0.772	-0.064	0.439	0.03728	0.799	-0.415	0.533
13	rs7333412	13	rs2770296	0.9575	0.017	0.5927	0.107	0.0112	0.827	0.01187	0.896	0.3246	0.437
13	rs7333412	13	rs1328674	0.4169	0.631	0.0478	0.952	-0.0921	0.399	0.1095	0.570	-0.3422	0.692
13	rs7333412	13	rs9316235	0.9193	0.026	0.6551	0.082	0.01077	0.837	-0.04227	0.648	0.3045	0.478
13	rs7333412	13	rs582385	-0.53	0.248	-0.5194	0.207	0.01443	0.802	-0.03699	0.716	-0.5071	0.268
13	rs7333412	13	rs2770298	0.9769	0.014	0.6728	0.065	0.00081	0.987	-0.01347	0.880	0.2204	0.594
13	rs7333412	13	rs972979	0.8077	0.026	0.6157	0.066	0.00559	0.904	0.1256	0.125	0.5345	0.157
13	rs7333412	13	rs731779	0.9123	0.055	0.3275	0.452	0.01294	0.831	-0.04299	0.688	0.4957	0.302
13	rs7333412	13	rs2770304	0.676	0.074	0.3853	0.270	0.00329	0.946	0.1273	0.137	0.4946	0.202
13	rs7333412	13	rs985933	0.7852	0.030	0.6168	0.065	0.00653	0.888	0.1202	0.142	0.5147	0.172
13	rs7333412	13	rs927544	0.9946	0.012	0.7405	0.041	0.04978	0.323	0.1505	0.090	0.5916	0.149
13	rs7333412	13	rs17288723	0.9227	0.071	0.1752	0.708	0.06009	0.356	0.00073	0.995	0.4774	0.355
13	rs7333412	13	rs9534505	0.6025	0.299	0.6974	0.185	-0.0345	0.637	0.06114	0.637	-0.0774	0.901
13	rs7333412	13	rs9534507	0.6158	0.407	1.121	0.095	0.01334	0.887	0.02168	0.896	0.09783	0.908
13	rs7333412	13	rs4942587	0.9669	0.026	0.4802	0.226	0.03094	0.575	0.166	0.088	0.6501	0.137
13	rs7333412	13	rs4941573	-0.4497	0.197	-0.3518	0.271	-0.011	0.803	-0.06165	0.429	0.0388	0.915
13	rs7333412	13	rs12584920	0.8826	0.061	0.4391	0.307	0.08459	0.157	0.1708	0.106	0.6741	0.155
13	rs7333412	13	rs1328684	-0.2403	0.522	0.0252	0.940	-0.009	0.847	-0.03874	0.639	-0.5329	0.159
13	rs7333412	13	rs2296973	0.647	0.099	0.397	0.271	0.02025	0.686	0.116	0.190	0.5849	0.144
13	rs7333412	13	rs2070037	0.989	0.022	0.4878	0.219	0.03567	0.518	0.1696	0.082	0.6511	0.137
13	rs7333412	13	rs9534511	0.2928	0.388	0.1221	0.696	0.02044	0.637	0.04691	0.540	0.08641	0.806

13	rs7333412	13	rs6313	-0.4991	0.152	-0.3808	0.234	-0.0169	0.702	-0.06402	0.412	-0.0658	0.856
13	rs7333412	13	rs6312	-0.4008	0.621	0.0273	0.971	-0.0311	0.766	-0.0993	0.590	0.617	0.465
13	rs7333412	13	rs6306	-0.0903	0.909	-0.8483	0.235	0.04463	0.654	0.04411	0.802	-0.4449	0.576
13	rs7333412	17	rs1906451	-0.2735	0.440	0.0768	0.814	-0.0081	0.857	-0.08627	0.281	-0.1303	0.726
13	rs7333412	17	rs1042173	-0.3304	0.354	0.0428	0.896	-0.0164	0.720	-0.0976	0.226	-0.1434	0.700
13	rs7333412	17	rs12449783	-0.3368	0.346	0.0314	0.924	-0.0166	0.716	-0.09885	0.221	-0.1437	0.699
13	rs7333412	17	rs3794808	-0.2205	0.538	0.0678	0.838	-0.0116	0.801	-0.06598	0.417	-0.0181	0.962
13	rs7333412	17	rs4583306	-0.4079	0.258	-0.0787	0.812	-0.0414	0.370	-0.06736	0.409	0.1167	0.756
13	rs7333412	17	rs140700	0.4411	0.456	0.4028	0.461	0.08268	0.270	0.09142	0.490	0.8819	0.133
13	rs7333412	17	rs6354	0.1271	0.775	-0.4657	0.255	0.01426	0.802	-0.0478	0.634	0.2979	0.509
13	rs7333412	17	rs2020936	0.0576	0.897	-0.4333	0.289	0.00972	0.864	-0.0687	0.493	0.3363	0.455
13	rs7333412	17	rs2066713	0.3502	0.331	0.3194	0.329	0.03297	0.470	0.1314	0.103	-0.3152	0.396
13	rs7333412	17	rs4251417	0.1541	0.800	1.13	0.043	-0.016	0.836	0.02143	0.875	1.398	0.023
13	rs7333412	17	rs8071667	0.1678	0.707	-0.4716	0.252	0.01194	0.835	-0.1015	0.315	0.18	0.692
13	rs7333412	17	rs1487971	0.5903	0.105	0.1031	0.757	0.04254	0.357	0.1075	0.188	0.1813	0.622
13	rs3803189	13	rs6314	-0.4128	0.684	1.292	0.152	-0.0364	0.773	-0.01219	0.956	0.7943	0.434
13	rs3803189	13	rs977003	-0.1103	0.844	0.2536	0.622	0.08108	0.253	0.06047	0.628	-0.2184	0.708
13	rs3803189	13	rs1923884	-0.5821	0.484	0.567	0.455	-0.024	0.820	0.1841	0.323	0.2351	0.775
13	rs3803189	13	rs1923885	0.2956	0.565	-0.0783	0.866	0.01184	0.854	0.01128	0.921	-0.3794	0.469
13	rs3803189	13	rs1923886	0.0779	0.880	0.0304	0.948	0.01571	0.808	-0.04091	0.721	0.5697	0.280
13	rs3803189	13	rs1745837	0.053	0.921	0.4019	0.413	0.08425	0.213	0.07404	0.536	0.3807	0.494
13	rs3803189	13	rs622337	-0.039	0.942	0.3498	0.482	0.1041	0.129	0.101	0.405	0.4635	0.413
13	rs3803189	13	rs655854	0.0062	0.991	0.276	0.579	0.1029	0.135	0.09579	0.430	0.3828	0.501
13	rs3803189	13	rs2296972	0.0264	0.961	0.3551	0.475	0.1068	0.119	0.109	0.368	0.3789	0.502
13	rs3803189	13	rs4942578	0.7663	0.170	0.5452	0.285	0.07277	0.303	0.2559	0.040	0.3487	0.537
13	rs3803189	13	rs1928042	-0.4422	0.372	0.0536	0.905	0.01898	0.759	-0.0277	0.800	-0.999	0.043
13	rs3803189	13	rs2760345	-0.877	0.256	-0.5188	0.468	-0.042	0.671	0.1603	0.358	-0.7606	0.333
13	rs3803189	13	rs2770296	0.5715	0.255	0.0613	0.894	-0.0164	0.797	0.00919	0.935	0.552	0.298
13	rs3803189	13	rs1328674	0.2914	0.782	-0.1774	0.855	-0.088	0.511	0.2195	0.354	-0.7133	0.500
13	rs3803189	13	rs9316235	0.5585	0.283	0.2776	0.558	-0.0172	0.793	-0.09005	0.438	0.5882	0.282
13	rs3803189	13	rs582385	-0.6273	0.263	-0.0013	0.998	0.03858	0.577	-0.05408	0.658	-0.7182	0.197
13	rs3803189	13	rs2770298	0.6077	0.224	0.2581	0.573	-0.0239	0.705	-0.03283	0.769	0.3764	0.473
13	rs3803189	13	rs972979	0.3489	0.447	0.1645	0.697	0.00247	0.966	0.1449	0.158	0.5504	0.246
13	rs3803189	13	rs731779	0.1732	0.775	-0.7766	0.162	-0.0802	0.298	-0.1521	0.265	0.6427	0.300
13	rs3803189	13	rs2770304	0.0232	0.961	-0.3828	0.385	-0.0322	0.597	0.1178	0.273	0.3416	0.485
13	rs3803189	13	rs985933	0.3287	0.473	0.1612	0.702	0.00321	0.956	0.1502	0.144	0.5462	0.249
13	rs3803189	13	rs927544	0.6823	0.168	0.2989	0.510	0.02421	0.701	0.1244	0.264	0.7258	0.159

13	rs3803189	13	rs17288723	0.1543	0.813	-0.8976	0.133	-0.0789	0.342	-0.2156	0.142	0.4363	0.517
13	rs3803189	13	rs9534505	0.8543	0.235	1.189	0.072	0.04194	0.647	0.206	0.203	0.1636	0.835
13	rs3803189	13	rs9534507	1.099	0.239	2.059	0.015	0.1327	0.262	0.1696	0.417	1.14	0.301
13	rs3803189	13	rs4942587	0.4416	0.418	-0.3532	0.479	-0.0399	0.565	0.08427	0.490	0.5768	0.298
13	rs3803189	13	rs4941573	0.0541	0.904	-0.3209	0.435	-0.0202	0.721	-0.08561	0.389	0.2029	0.658
13	rs3803189	13	rs12584920	0.5894	0.309	-0.0287	0.957	-0.0169	0.819	0.1383	0.289	0.8581	0.150
13	rs3803189	13	rs1328684	-0.3767	0.416	0.5959	0.154	0.04803	0.406	0.02076	0.839	-0.6443	0.166
13	rs3803189	13	rs2296973	0.0045	0.993	-0.3187	0.485	-0.0233	0.713	0.08397	0.453	0.5731	0.257
13	rs3803189	13	rs2070037	0.4541	0.405	-0.3164	0.526	-0.0371	0.592	0.08779	0.473	0.6228	0.261
13	rs3803189	13	rs9534511	-0.2889	0.507	0.0128	0.974	0.01117	0.839	0.01784	0.854	-0.0103	0.982
13	rs3803189	13	rs6313	-0.0118	0.979	-0.4477	0.276	-0.0349	0.536	-0.08927	0.370	-0.0043	0.993
13	rs3803189	13	rs6312	-1.485	0.126	-0.0502	0.956	0.04249	0.734	0.0588	0.790	0.4138	0.681
13	rs3803189	13	rs6306	-0.4971	0.585	-1.365	0.103	-0.0993	0.394	-0.1594	0.439	0.4837	0.606
13	rs3803189	17	rs1906451	0.0147	0.973	0.0994	0.807	-0.0178	0.751	-0.01257	0.899	-0.2134	0.647
13	rs3803189	17	rs1042173	0.0505	0.909	0.1336	0.743	-0.0133	0.815	-0.00013	0.999	-0.1925	0.679
13	rs3803189	17	rs12449783	0.0459	0.918	0.1255	0.758	-0.0134	0.813	-0.00087	0.993	-0.1893	0.684
13	rs3803189	17	rs3794808	0.1601	0.718	0.1878	0.647	-0.012	0.833	0.02356	0.815	-0.1086	0.815
13	rs3803189	17	rs4583306	-0.0496	0.911	-0.0003	1.000	-0.0518	0.363	0.01005	0.920	0.09957	0.831
13	rs3803189	17	rs140700	0.8086	0.303	0.3126	0.661	0.08752	0.373	-0.1233	0.477	1.112	0.145
13	rs3803189	17	rs6354	0.2327	0.669	-0.6521	0.195	-0.0195	0.780	-0.2114	0.086	0.2889	0.605
13	rs3803189	17	rs2020936	0.1744	0.749	-0.6085	0.227	-0.0191	0.784	-0.2256	0.067	0.326	0.559
13	rs3803189	17	rs2066713	-0.1025	0.818	0.3116	0.444	0.06716	0.235	0.1457	0.145	-0.3236	0.488
13	rs3803189	17	rs4251417	0.0521	0.946	0.8118	0.245	-0.0516	0.589	-0.02539	0.880	2.507	0.001
13	rs3803189	17	rs8071667	0.262	0.633	-0.6649	0.189	-0.0086	0.903	-0.2335	0.060	0.174	0.758
13	rs3803189	17	rs1487971	0.326	0.463	0.0118	0.977	0.09518	0.091	0.08065	0.417	0.2378	0.597
13	rs6314	13	rs977003	-0.6469	0.327	0.5874	0.332	0.0105	0.901	0.1493	0.317	0.4264	0.530
13	rs6314	13	rs1923884	0.2736	0.793	-1.426	0.128	-0.0197	0.881	-0.08272	0.721	-0.1966	0.845
13	rs6314	13	rs1923885	-0.9732	0.044	0.2391	0.589	-0.0328	0.597	0.00957	0.930	-0.0647	0.895
13	rs6314	13	rs1923886	1.11	0.020	0.2572	0.557	0.05732	0.349	-0.0076	0.944	0.3093	0.523
13	rs6314	13	rs1745837	1.123	0.023	0.5438	0.232	0.1072	0.093	0.02586	0.819	0.7884	0.124
13	rs6314	13	rs622337	1.185	0.019	0.6286	0.176	0.1005	0.124	0.0475	0.681	0.8036	0.119
13	rs6314	13	rs655854	1.091	0.028	0.5729	0.210	0.09751	0.129	0.0319	0.778	0.7832	0.129
13	rs6314	13	rs2296972	1.124	0.023	0.6089	0.183	0.09621	0.134	0.032	0.778	0.8168	0.112
13	rs6314	13	rs4942578	0.7062	0.287	0.6735	0.268	-0.0785	0.361	0.1672	0.270	1.549	0.024
13	rs6314	13	rs1928042	-0.0395	0.950	-0.9606	0.098	-0.0715	0.379	0.01675	0.907	-0.0131	0.984
13	rs6314	13	rs2760345	0.5783	0.561	0.4978	0.588	-0.0958	0.456	-0.1715	0.450	0.4264	0.692
13	rs6314	13	rs2770296	1.167	0.036	1.07	0.037	0.04594	0.522	0.00083	0.995	0.00093	0.999

13	rs6314	13	rs1328674	0.5076	0.687	0.4393	0.702	-0.074	0.645	-0.08136	0.774	0.3245	0.806
13	rs6314	13	rs9316235	1.113	0.052	0.912	0.083	0.04541	0.537	0.01961	0.880	-0.0677	0.907
13	rs6314	13	rs582385	-0.3241	0.649	-1.32	0.044	-0.0516	0.575	0.04383	0.788	-0.1279	0.860
13	rs6314	13	rs2770298	1.16	0.035	0.9791	0.053	0.03415	0.630	0.00483	0.969	0.01377	0.980
13	rs6314	13	rs972979	1.246	0.017	1.059	0.028	0.00303	0.964	0.06984	0.558	0.4382	0.419
13	rs6314	13	rs731779	1.451	0.023	1.419	0.015	0.118	0.149	0.08517	0.556	0.2543	0.690
13	rs6314	13	rs2770304	1.343	0.012	1.25	0.012	0.04207	0.544	0.1134	0.355	0.6319	0.255
13	rs6314	13	rs985933	1.222	0.019	1.062	0.027	0.00388	0.954	0.0525	0.659	0.4005	0.459
13	rs6314	13	rs927544	1.178	0.039	1.127	0.032	0.06912	0.347	0.1508	0.245	0.2991	0.606
13	rs6314	13	rs17288723	1.457	0.032	1.247	0.046	0.1987	0.023	0.2382	0.124	0.4505	0.507
13	rs6314	13	rs9534505	0.1359	0.868	-0.0549	0.942	-0.1245	0.235	-0.1583	0.393	-0.3403	0.689
13	rs6314	13	rs9534507	-0.1414	0.894	-0.3118	0.748	-0.1448	0.286	-0.1944	0.417	-0.9907	0.368
13	rs6314	13	rs4942587	1.376	0.025	1.381	0.014	0.111	0.159	0.2329	0.094	0.6214	0.313
13	rs6314	13	rs4941573	-0.9537	0.056	-0.3099	0.497	0.01734	0.786	-0.03527	0.754	-0.1747	0.736
13	rs6314	13	rs12584920	1.149	0.123	1.043	0.122	0.2104	0.026	0.2441	0.143	0.3856	0.598
13	rs6314	13	rs1328684	-0.0102	0.986	-0.8138	0.111	-0.11	0.125	-0.1017	0.423	-0.2828	0.622
13	rs6314	13	rs2296973	1.319	0.019	1.194	0.021	0.06623	0.360	0.1382	0.280	0.5307	0.358
13	rs6314	13	rs2070037	1.406	0.022	1.351	0.016	0.1169	0.138	0.2366	0.089	0.5674	0.357
13	rs6314	13	rs9534511	0.9052	0.059	0.2049	0.644	0.01304	0.834	0.09591	0.381	0.199	0.691
13	rs6314	13	rs6313	-0.9743	0.050	-0.2118	0.641	0.0234	0.713	-0.03786	0.736	-0.1261	0.807
13	rs6314	13	rs6312	1.37	0.251	0.1392	0.901	-0.1481	0.345	-0.3068	0.267	0.7929	0.532
13	rs6314	13	rs6306	0.7886	0.553	0.4138	0.727	0.2965	0.074	0.4854	0.098	-2.188	0.097
13	rs6314	17	rs1906451	-0.6284	0.220	0.0274	0.954	0.01403	0.832	-0.1666	0.155	-0.0232	0.966
13	rs6314	17	rs1042173	-0.7984	0.121	-0.0978	0.837	-0.011	0.869	-0.2089	0.077	-0.083	0.879
13	rs6314	17	rs12449783	-0.8048	0.118	-0.1095	0.818	-0.0113	0.865	-0.2103	0.074	-0.0869	0.874
13	rs6314	17	rs3794808	-0.6905	0.186	-0.1048	0.829	-0.0028	0.966	-0.1781	0.137	0.1192	0.829
13	rs6314	17	rs4583306	-0.7967	0.129	-0.1562	0.747	-0.01	0.883	-0.1633	0.173	0.1198	0.828
13	rs6314	17	rs140700	-0.0132	0.988	0.4879	0.537	0.06541	0.550	0.3485	0.071	0.4739	0.575
13	rs6314	17	rs6354	-0.0808	0.901	-0.1148	0.850	0.04961	0.558	0.2192	0.142	0.3391	0.608
13	rs6314	17	rs2020936	-0.1451	0.823	-0.109	0.857	0.03956	0.639	0.1913	0.199	0.3693	0.576
13	rs6314	17	rs2066713	0.9504	0.078	0.278	0.572	-0.0225	0.746	0.0763	0.533	-0.3332	0.547
13	rs6314	17	rs4251417	0.2853	0.736	1.227	0.117	0.03778	0.729	0.06781	0.725	-0.4158	0.628
13	rs6314	17	rs8071667	-0.0356	0.957	-0.107	0.860	0.02964	0.727	0.1269	0.397	0.2561	0.700
13	rs6314	17	rs1487971	0.7728	0.147	0.1698	0.732	-0.0465	0.501	0.1157	0.343	0.04242	0.938
13	rs977003	13	rs1923884	-0.2706	0.636	-0.2231	0.670	-0.029	0.690	0.00251	0.984	-0.3002	0.602
13	rs977003	13	rs1923885	-0.1289	0.677	0.3447	0.228	0.02413	0.542	0.06151	0.377	0.04514	0.887
13	rs977003	13	rs1923886	0.4077	0.164	-0.0427	0.875	-0.0082	0.826	-0.02107	0.750	0.2921	0.329

13	rs977003	13	rs1745837	0.4093	0.199	0.2757	0.350	0.02875	0.481	0.01492	0.835	0.3294	0.322
13	rs977003	13	rs622337	0.3089	0.345	0.1918	0.526	0.03109	0.458	0.03394	0.645	0.3717	0.272
13	rs977003	13	rs655854	0.3043	0.350	0.1613	0.591	0.03009	0.472	0.03173	0.666	0.3861	0.256
13	rs977003	13	rs2296972	0.3153	0.332	0.2063	0.491	0.03175	0.445	0.03739	0.609	0.3785	0.262
13	rs977003	13	rs4942578	0.1399	0.713	0.4146	0.238	-0.0311	0.522	0.05655	0.508	0.1984	0.613
13	rs977003	13	rs1928042	0.1316	0.690	-0.1543	0.611	0.0629	0.133	-0.07369	0.317	-0.2178	0.512
13	rs977003	13	rs2760345	-0.4128	0.436	0.3052	0.533	0.04069	0.548	-0.1069	0.370	-0.7707	0.157
13	rs977003	13	rs2770296	0.8682	0.009	0.559	0.070	0.04371	0.307	0.01745	0.817	0.5262	0.122
13	rs977003	13	rs1328674	0.024	0.974	0.4904	0.459	0.1821	0.046	0.0716	0.655	-0.4243	0.556
13	rs977003	13	rs9316235	0.9637	0.005	0.3929	0.215	-0.018	0.681	-0.0392	0.611	0.5417	0.124
13	rs977003	13	rs582385	0.072	0.841	-0.2376	0.472	0.02863	0.533	-0.05913	0.464	0.09414	0.795
13	rs977003	13	rs2770298	0.943	0.004	0.53	0.083	0.04075	0.336	-0.01808	0.808	0.4356	0.198
13	rs977003	13	rs972979	0.7236	0.016	0.7269	0.009	0.03357	0.386	0.04455	0.512	0.4136	0.187
13	rs977003	13	rs731779	1.011	0.009	0.4322	0.226	0.03579	0.469	0.02916	0.738	0.8137	0.037
13	rs977003	13	rs2770304	0.6874	0.029	0.6838	0.019	0.06897	0.087	0.07178	0.312	0.4234	0.196
13	rs977003	13	rs985933	0.7102	0.018	0.7331	0.009	0.03323	0.390	0.04316	0.526	0.3936	0.210
13	rs977003	13	rs927544	0.8912	0.007	0.6506	0.031	0.03388	0.418	0.1114	0.130	0.6407	0.058
13	rs977003	13	rs17288723	1.303	0.002	0.24	0.531	0.04941	0.350	0.07694	0.409	1.07	0.010
13	rs977003	13	rs9534505	0.2134	0.670	0.5474	0.231	0.00769	0.903	-0.02117	0.850	-0.215	0.677
13	rs977003	13	rs9534507	0.1963	0.764	0.4975	0.401	-0.1395	0.091	-0.09893	0.496	-0.0365	0.958
13	rs977003	13	rs4942587	0.8964	0.011	0.5563	0.090	0.06951	0.127	0.1568	0.050	0.7202	0.048
13	rs977003	13	rs4941573	-0.7236	0.014	-0.4439	0.104	-0.0469	0.212	0.0314	0.634	-0.2242	0.460
13	rs977003	13	rs12584920	1.005	0.007	0.5488	0.115	0.09456	0.050	0.1712	0.043	0.8439	0.028
13	rs977003	13	rs1328684	0.0633	0.838	0.0752	0.790	0.00252	0.949	-0.1535	0.026	-0.3229	0.300
13	rs977003	13	rs2296973	0.6965	0.033	0.5681	0.062	0.02906	0.491	0.06342	0.393	0.5165	0.132
13	rs977003	13	rs2070037	0.9074	0.010	0.5467	0.096	0.06817	0.134	0.1619	0.043	0.7173	0.048
13	rs977003	13	rs9534511	0.6233	0.029	0.2611	0.326	0.03811	0.299	-0.03883	0.547	0.2907	0.327
13	rs977003	13	rs6313	-0.8074	0.006	-0.4985	0.069	-0.0528	0.162	0.0349	0.599	-0.3021	0.321
13	rs977003	13	rs6312	-0.2275	0.740	0.6912	0.272	-0.112	0.203	-0.3184	0.039	-0.0477	0.947
13	rs977003	13	rs6306	0.8622	0.144	0.0206	0.970	0.03485	0.644	0.03542	0.790	0.4919	0.416
13	rs977003	17	rs1906451	0.3084	0.304	0.0192	0.945	0.04275	0.266	-0.07122	0.294	0.3301	0.291
13	rs977003	17	rs1042173	0.2977	0.320	0.0031	0.991	0.04154	0.281	-0.07338	0.279	0.3307	0.288
13	rs977003	17	rs12449783	0.3057	0.309	0.0173	0.950	0.042	0.276	-0.07183	0.290	0.3277	0.294
13	rs977003	17	rs3794808	0.2942	0.332	0.0929	0.743	0.05639	0.150	-0.06565	0.342	0.4221	0.186
13	rs977003	17	rs4583306	0.1177	0.700	0.0389	0.891	0.03496	0.373	-0.0518	0.453	0.4785	0.132
13	rs977003	17	rs140700	-0.0521	0.916	-0.3257	0.475	-0.0179	0.776	0.1011	0.359	0.205	0.676
13	rs977003	17	rs6354	-0.1644	0.653	-0.4652	0.169	-0.0193	0.681	-0.00145	0.986	-0.2203	0.552

13	rs977003	17	rs2020936	-0.1558	0.671	-0.3927	0.246	-0.0205	0.663	-0.00126	0.988	-0.129	0.727
13	rs977003	17	rs2066713	-0.0016	0.996	0.3145	0.260	-0.0208	0.591	0.06325	0.353	-0.3786	0.223
13	rs977003	17	rs4251417	0.5144	0.322	0.6388	0.185	0.00591	0.929	0.2307	0.049	1.008	0.063
13	rs977003	17	rs8071667	-0.1168	0.752	-0.4188	0.221	-0.0242	0.611	-0.01831	0.827	-0.1528	0.684
13	rs977003	17	rs1487971	0.2187	0.479	0.181	0.527	-0.0268	0.497	-0.04565	0.510	-0.6097	0.054
13	rs1923884	13	rs1923885	-0.7478	0.210	-0.4759	0.387	-0.0521	0.497	-0.08314	0.539	-0.8539	0.156
13	rs1923884	13	rs1923886	0.5673	0.322	0.3017	0.570	0.1471	0.045	0.1688	0.194	0.9427	0.106
13	rs1923884	13	rs1745837	0.5796	0.266	0.324	0.501	-0.0099	0.883	0.1615	0.170	0.6817	0.204
13	rs1923884	13	rs622337	0.8919	0.143	0.2364	0.675	-0.0003	0.997	0.1403	0.310	0.8862	0.160
13	rs1923884	13	rs655854	0.6478	0.272	0.1187	0.828	-0.0297	0.696	0.04292	0.748	0.7745	0.207
13	rs1923884	13	rs2296972	0.5836	0.319	0.0871	0.873	-0.0195	0.796	0.06354	0.633	0.7525	0.217
13	rs1923884	13	rs4942578	-0.4295	0.429	-0.6425	0.203	-0.019	0.787	-0.00071	0.995	-0.442	0.438
13	rs1923884	13	rs1928042	0.6457	0.242	0.5731	0.266	0.01395	0.846	0.1213	0.338	0.1944	0.730
13	rs1923884	13	rs2760345	0.3258	0.683	-0.9463	0.206	-0.1131	0.273	-0.1163	0.524	-0.3024	0.713
13	rs1923884	13	rs2770296	-0.7235	0.151	-1.305	0.005	-0.0802	0.217	-0.2421	0.035	-0.8882	0.081
13	rs1923884	13	rs1328674	1.271	0.304	-0.5367	0.648	-0.03	0.852	-0.2071	0.464	0.439	0.726
13	rs1923884	13	rs9316235	-1.003	0.052	-1.031	0.031	-0.0221	0.740	-0.1665	0.156	-1.053	0.044
13	rs1923884	13	rs582385	0.6011	0.324	0.5876	0.299	-0.0447	0.574	0.1138	0.418	0.2391	0.702
13	rs1923884	13	rs2770298	-0.7014	0.156	-1.106	0.016	-0.0544	0.392	-0.1858	0.097	-0.9133	0.067
13	rs1923884	13	rs972979	-0.9009	0.051	-1.654	0.000	-0.1016	0.086	-0.1795	0.086	-0.9788	0.039
13	rs1923884	13	rs731779	-1.319	0.022	-1.487	0.006	-0.1354	0.071	-0.3058	0.021	-1.316	0.024
13	rs1923884	13	rs2770304	-0.9676	0.042	-1.657	0.000	-0.1434	0.019	-0.2087	0.053	-1.049	0.033
13	rs1923884	13	rs985933	-0.8266	0.075	-1.68	0.000	-0.1009	0.091	-0.1669	0.113	-0.8962	0.061
13	rs1923884	13	rs927544	-1.076	0.032	-1.507	0.001	-0.074	0.253	-0.1792	0.117	-0.7604	0.134
13	rs1923884	13	rs17288723	-1.619	0.007	-1.37	0.014	-0.1449	0.064	-0.3221	0.020	-1.367	0.025
13	rs1923884	13	rs9534505	1.154	0.183	-0.6616	0.413	0.1236	0.267	-0.00711	0.971	0.5906	0.499
13	rs1923884	13	rs9534507	0.9278	0.438	-0.7931	0.468	0.2409	0.115	0.1735	0.521	0.7432	0.536
13	rs1923884	13	rs4942587	-1.236	0.018	-1.506	0.002	-0.1127	0.096	-0.2266	0.058	-1.014	0.056
13	rs1923884	13	rs4941573	0.4169	0.372	1.157	0.007	0.1057	0.077	0.07197	0.495	0.7672	0.103
13	rs1923884	13	rs12584920	-1.159	0.034	-1.444	0.004	-0.1185	0.094	-0.2013	0.108	-0.8786	0.113
13	rs1923884	13	rs1328684	0.6957	0.165	0.0069	0.988	-0.0051	0.937	0.1256	0.271	0.1336	0.793
13	rs1923884	13	rs2296973	-1.254	0.011	-1.7	0.000	-0.1393	0.028	-0.1883	0.093	-1.226	0.016
13	rs1923884	13	rs2070037	-1.222	0.020	-1.466	0.003	-0.1096	0.105	-0.2232	0.062	-0.9632	0.069
13	rs1923884	13	rs9534511	-0.737	0.102	-0.9302	0.025	-0.1221	0.035	-0.03823	0.709	-0.7501	0.100
13	rs1923884	13	rs6313	0.6575	0.160	1.152	0.007	0.117	0.050	0.07502	0.477	0.7876	0.094
13	rs1923884	13	rs6312	-0.6588	0.481	-1.306	0.132	-0.1407	0.243	0.023	0.914	-1.03	0.292
13	rs1923884	13	rs6306	-0.9494	0.225	-0.4658	0.524	-0.0513	0.618	-0.1807	0.320	-1.1	0.169

13	rs1923884	17	rs1906451	-0.0487	0.912	-0.1632	0.690	-0.057	0.310	0.00115	0.991	0.4112	0.365
13	rs1923884	17	rs1042173	-0.0092	0.984	-0.1239	0.763	-0.0522	0.359	0.01468	0.884	0.4375	0.335
13	rs1923884	17	rs12449783	-0.016	0.971	-0.1358	0.741	-0.0525	0.356	0.01338	0.894	0.4364	0.337
13	rs1923884	17	rs3794808	-0.0623	0.889	-0.2273	0.586	-0.0625	0.279	0.03319	0.745	0.1055	0.820
13	rs1923884	17	rs4583306	-0.0138	0.975	-0.2231	0.589	-0.0622	0.278	0.04211	0.678	0.2034	0.657
13	rs1923884	17	rs140700	-0.1783	0.804	0.0545	0.935	0.01813	0.843	-0.03306	0.838	-0.7629	0.278
13	rs1923884	17	rs6354	-0.1006	0.857	-0.1098	0.832	0.00692	0.923	0.05599	0.658	-0.8274	0.139
13	rs1923884	17	rs2020936	0.0047	0.993	-0.0223	0.965	0.01567	0.826	0.09843	0.433	-0.7547	0.174
13	rs1923884	17	rs2066713	0.0579	0.899	0.3021	0.484	0.0647	0.278	-0.102	0.333	0.3244	0.485
13	rs1923884	17	rs4251417	-0.0982	0.897	-0.2763	0.690	-0.0498	0.605	0.02967	0.861	0.2704	0.729
13	rs1923884	17	rs8071667	-0.0281	0.960	0.0301	0.953	0.00892	0.901	0.09017	0.475	-0.7155	0.200
13	rs1923884	17	rs1487971	-0.2191	0.642	0.3346	0.444	0.09004	0.137	-0.08538	0.425	0.5108	0.286
13	rs1923885	13	rs1923886	0.2623	0.441	-0.198	0.528	-0.0269	0.538	0.00147	0.985	0.4771	0.171
13	rs1923885	13	rs1745837	0.2242	0.569	0.045	0.901	-0.0221	0.661	0.03919	0.660	0.3754	0.360
13	rs1923885	13	rs622337	0.0703	0.862	-0.1668	0.655	-0.021	0.686	0.04297	0.639	0.4021	0.340
13	rs1923885	13	rs655854	0.1046	0.796	-0.1631	0.661	-0.0213	0.681	0.04663	0.610	0.4109	0.329
13	rs1923885	13	rs2296972	0.0964	0.811	-0.1463	0.694	-0.0198	0.702	0.05079	0.577	0.3777	0.367
13	rs1923885	13	rs4942578	-0.059	0.884	0.0247	0.947	-0.0437	0.401	-0.06179	0.501	-0.4833	0.237
13	rs1923885	13	rs1928042	0.2222	0.507	0.1127	0.715	0.08606	0.043	-0.04746	0.528	0.00051	0.999
13	rs1923885	13	rs2760345	-0.2938	0.581	0.4916	0.318	0.05437	0.424	-0.07076	0.557	-0.5262	0.332
13	rs1923885	13	rs2770296	0.5119	0.139	0.0091	0.977	-0.0129	0.772	-0.0785	0.317	0.1056	0.763
13	rs1923885	13	rs1328674	-0.1944	0.788	0.2894	0.664	0.1724	0.061	0.04044	0.804	-0.5349	0.456
13	rs1923885	13	rs9316235	0.5935	0.098	-0.1021	0.757	-0.0743	0.104	-0.1136	0.159	0.2367	0.514
13	rs1923885	13	rs582385	0.3161	0.394	0.0815	0.810	0.05226	0.269	-0.04186	0.616	0.2638	0.475
13	rs1923885	13	rs2770298	0.5334	0.118	0.0164	0.958	-0.0086	0.844	-0.09213	0.231	0.1196	0.729
13	rs1923885	13	rs972979	0.4472	0.151	0.2815	0.330	-0.0143	0.721	-0.06198	0.379	-0.0814	0.799
13	rs1923885	13	rs731779	0.7582	0.061	-0.1555	0.676	-0.0225	0.663	-0.07203	0.430	0.3537	0.381
13	rs1923885	13	rs2770304	0.4833	0.134	0.2499	0.404	0.02426	0.558	-0.03266	0.655	-0.0646	0.845
13	rs1923885	13	rs985933	0.4411	0.156	0.2828	0.327	-0.015	0.706	-0.05667	0.421	-0.0915	0.774
13	rs1923885	13	rs927544	0.53	0.123	0.0441	0.890	-0.0416	0.344	-0.01663	0.831	-0.0108	0.976
13	rs1923885	13	rs17288723	0.9796	0.026	-0.4573	0.258	-0.0213	0.704	-0.05252	0.597	0.6027	0.170
13	rs1923885	13	rs9534505	-0.1378	0.780	0.259	0.568	-0.0035	0.956	-0.05045	0.650	-0.3663	0.466
13	rs1923885	13	rs9534507	-0.1476	0.820	0.2158	0.716	-0.1598	0.053	-0.1186	0.417	-0.2486	0.715
13	rs1923885	13	rs4942587	0.6218	0.094	-0.0621	0.856	-0.0003	0.994	0.01243	0.882	-0.0088	0.981
13	rs1923885	13	rs4941573	-0.5907	0.049	-0.2268	0.413	-0.0185	0.629	0.08251	0.222	-0.0296	0.923
13	rs1923885	13	rs12584920	0.8692	0.027	-0.015	0.967	0.01253	0.803	0.07568	0.394	0.2925	0.463
13	rs1923885	13	rs1328684	0.1616	0.610	0.3157	0.274	0.02091	0.602	-0.1184	0.094	-0.0167	0.958

13	rs1923885	13	rs2296973	0.5482	0.102	0.1684	0.588	-0.0215	0.618	-0.04535	0.552	0.03544	0.918
13	rs1923885	13	rs2070037	0.6236	0.093	-0.0585	0.864	-0.0038	0.937	0.01709	0.839	0.01163	0.975
13	rs1923885	13	rs9534511	0.5821	0.043	0.185	0.486	0.01863	0.614	-0.08671	0.183	0.1519	0.604
13	rs1923885	13	rs6313	-0.6831	0.023	-0.3158	0.257	-0.0249	0.519	0.09684	0.154	-0.0921	0.764
13	rs1923885	13	rs6312	0.1948	0.778	1.035	0.102	-0.0794	0.366	-0.2266	0.144	0.3475	0.627
13	rs1923885	13	rs6306	1.038	0.096	-0.4914	0.387	-0.0154	0.845	-0.1152	0.410	0.6009	0.336
13	rs1923885	17	rs1906451	0.7325	0.019	0.1772	0.540	0.0477	0.231	-0.02101	0.766	0.4348	0.180
13	rs1923885	17	rs1042173	0.7038	0.025	0.1451	0.617	0.04503	0.263	-0.02875	0.686	0.4237	0.189
13	rs1923885	17	rs12449783	0.7173	0.023	0.1652	0.570	0.04576	0.256	-0.0265	0.710	0.4234	0.192
13	rs1923885	17	rs3794808	0.5978	0.057	0.2288	0.434	0.04607	0.255	-0.03081	0.667	0.5481	0.093
13	rs1923885	17	rs4583306	0.5081	0.107	0.25	0.391	0.029	0.473	-0.02513	0.725	0.6163	0.057
13	rs1923885	17	rs140700	-0.3957	0.449	-0.7161	0.132	-0.0427	0.517	-0.09841	0.398	0.00984	0.985
13	rs1923885	17	rs6354	-0.3593	0.350	-0.7013	0.049	-0.0295	0.551	-0.1613	0.065	-0.4547	0.236
13	rs1923885	17	rs2020936	-0.3284	0.393	-0.6223	0.080	-0.0274	0.580	-0.1533	0.079	-0.3647	0.342
13	rs1923885	17	rs2066713	-0.275	0.378	0.2447	0.396	-0.0135	0.734	0.1322	0.060	-0.3697	0.243
13	rs1923885	17	rs4251417	0.6229	0.249	0.2573	0.605	-0.014	0.838	0.1933	0.111	1.134	0.039
13	rs1923885	17	rs8071667	-0.2909	0.455	-0.6131	0.089	-0.0255	0.612	-0.1442	0.104	-0.3044	0.435
13	rs1923885	17	rs1487971	-0.0253	0.936	0.2165	0.458	-0.0145	0.718	-0.04165	0.557	-0.5868	0.065
13	rs1923886	13	rs1745837	-0.2657	0.433	-0.1511	0.631	0.02129	0.626	-0.1024	0.184	-0.5156	0.141
13	rs1923886	13	rs622337	-0.186	0.603	0.0744	0.822	0.02231	0.628	-0.09129	0.261	-0.5982	0.106
13	rs1923886	13	rs655854	-0.1075	0.761	0.118	0.718	0.03382	0.458	-0.05454	0.497	-0.5591	0.127
13	rs1923886	13	rs2296972	-0.0818	0.817	0.1104	0.736	0.02924	0.519	-0.06554	0.413	-0.5298	0.146
13	rs1923886	13	rs4942578	0.2536	0.500	0.3828	0.269	0.04615	0.342	0.06778	0.429	0.5774	0.126
13	rs1923886	13	rs1928042	-0.6051	0.063	-0.5307	0.077	-0.1104	0.008	-0.00575	0.938	-0.206	0.524
13	rs1923886	13	rs2760345	0.189	0.713	0.1061	0.824	-0.0062	0.925	0.1057	0.364	0.7357	0.157
13	rs1923886	13	rs2770296	-0.1117	0.740	0.6307	0.042	0.05538	0.199	0.1944	0.011	0.3665	0.277
13	rs1923886	13	rs1328674	-0.2557	0.721	-0.0075	0.991	-0.1665	0.067	0.02849	0.859	0.5973	0.394
13	rs1923886	13	rs9316235	-0.1261	0.721	0.6066	0.060	0.09215	0.040	0.2068	0.009	0.2168	0.539
13	rs1923886	13	rs582385	-0.6085	0.087	-0.5802	0.076	-0.0594	0.193	-0.01639	0.839	-0.475	0.177
13	rs1923886	13	rs2770298	-0.2156	0.521	0.5442	0.077	0.03927	0.360	0.1931	0.011	0.3147	0.349
13	rs1923886	13	rs972979	0.0343	0.911	0.5815	0.040	0.06722	0.088	0.156	0.025	0.6342	0.042
13	rs1923886	13	rs731779	-0.2062	0.601	0.854	0.019	0.08589	0.089	0.2504	0.005	0.2717	0.488
13	rs1923886	13	rs2770304	-0.0957	0.763	0.6064	0.038	0.0411	0.313	0.1576	0.028	0.6224	0.052
13	rs1923886	13	rs985933	0.0051	0.987	0.5819	0.040	0.06757	0.087	0.1447	0.038	0.6052	0.052
13	rs1923886	13	rs927544	0.0448	0.894	0.6787	0.027	0.08302	0.052	0.117	0.122	0.4438	0.189
13	rs1923886	13	rs17288723	-0.1915	0.647	1.09	0.005	0.09418	0.079	0.2475	0.009	0.1265	0.762
13	rs1923886	13	rs9534505	0.0497	0.917	0.0513	0.907	-0.0197	0.746	0.01846	0.863	0.3124	0.512

13	rs1923886	13	rs9534507	0.3522	0.568	0.1009	0.859	0.1123	0.155	0.00924	0.947	0.1068	0.865
13	rs1923886	13	rs4942587	-0.0915	0.800	0.8247	0.013	0.05588	0.229	0.1434	0.080	0.5614	0.122
13	rs1923886	13	rs4941573	0.4749	0.109	-0.1584	0.562	-0.0153	0.687	-0.1202	0.071	-0.2629	0.381
13	rs1923886	13	rs12584920	-0.3295	0.385	0.7826	0.025	0.0518	0.286	0.07863	0.360	0.2896	0.448
13	rs1923886	13	rs1328684	-0.439	0.154	-0.4415	0.118	-0.0287	0.464	0.04669	0.500	-0.1181	0.702
13	rs1923886	13	rs2296973	-0.0222	0.947	0.7132	0.020	0.08845	0.039	0.1682	0.026	0.578	0.087
13	rs1923886	13	rs2070037	-0.1014	0.780	0.7984	0.017	0.05735	0.217	0.1372	0.094	0.5133	0.158
13	rs1923886	13	rs9534511	-0.419	0.137	0.0715	0.784	0.01616	0.655	0.121	0.058	0.07588	0.790
13	rs1923886	13	rs6313	0.4682	0.116	-0.0763	0.781	-0.0135	0.723	-0.1379	0.040	-0.2062	0.493
13	rs1923886	13	rs6312	0.2469	0.714	-0.0756	0.903	0.1522	0.076	0.1907	0.208	0.2156	0.756
13	rs1923886	13	rs6306	-0.4172	0.450	0.7262	0.153	0.0468	0.509	0.2613	0.037	0.1768	0.748
13	rs1923886	17	rs1906451	-0.5338	0.075	-0.0375	0.893	-0.0056	0.884	0.0227	0.737	-0.4038	0.188
13	rs1923886	17	rs1042173	-0.5321	0.077	-0.0297	0.915	-0.006	0.877	0.02266	0.740	-0.4079	0.183
13	rs1923886	17	rs12449783	-0.5391	0.074	-0.0417	0.881	-0.0063	0.870	0.02131	0.755	-0.4062	0.186
13	rs1923886	17	rs3794808	-0.3901	0.196	-0.0694	0.805	-0.0005	0.989	0.01519	0.825	-0.3622	0.240
13	rs1923886	17	rs4583306	-0.3465	0.249	-0.0593	0.831	0.01184	0.759	0.00466	0.946	-0.4675	0.125
13	rs1923886	17	rs140700	0.5217	0.304	0.8924	0.057	0.03153	0.625	0.1338	0.241	0.3128	0.529
13	rs1923886	17	rs6354	0.3524	0.344	0.7293	0.035	0.02922	0.543	0.1499	0.077	0.6869	0.066
13	rs1923886	17	rs2020936	0.2904	0.434	0.6111	0.077	0.02512	0.600	0.1278	0.131	0.5661	0.129
13	rs1923886	17	rs2066713	0.1178	0.698	-0.4692	0.096	-0.0305	0.433	-0.1044	0.128	0.06827	0.822
13	rs1923886	17	rs4251417	-0.5472	0.310	-0.1359	0.784	0.04829	0.482	-0.2091	0.085	-0.9248	0.085
13	rs1923886	17	rs8071667	0.2593	0.490	0.5617	0.107	0.02576	0.595	0.1212	0.157	0.4762	0.207
13	rs1923886	17	rs1487971	0.0516	0.866	-0.319	0.257	-0.0369	0.345	0.06333	0.358	0.3234	0.289
13	rs1745837	13	rs622337	0.1764	0.611	0.0424	0.895	0.02304	0.605	-0.08343	0.289	-0.1625	0.651
13	rs1745837	13	rs655854	0.1936	0.574	0.0464	0.884	0.03025	0.496	-0.06699	0.392	-0.182	0.611
13	rs1745837	13	rs2296972	0.2167	0.528	0.0545	0.864	0.02833	0.521	-0.0714	0.360	-0.1389	0.696
13	rs1745837	13	rs4942578	0.0297	0.938	0.3572	0.311	-0.0382	0.441	0.09288	0.288	0.7372	0.058
13	rs1745837	13	rs1928042	-0.8222	0.019	-0.8021	0.014	-0.1159	0.011	-0.08055	0.315	-0.9089	0.011
13	rs1745837	13	rs2760345	-0.1168	0.832	0.0834	0.871	0.00837	0.907	-0.01302	0.918	0.3711	0.512
13	rs1745837	13	rs2770296	-0.1494	0.677	0.3549	0.285	0.08958	0.052	0.09564	0.241	0.0444	0.904
13	rs1745837	13	rs1328674	-0.913	0.265	-0.2033	0.789	-0.1236	0.241	0.03795	0.839	-0.0933	0.910
13	rs1745837	13	rs9316235	0.0718	0.843	0.3205	0.336	0.09084	0.050	0.06646	0.416	0.07331	0.842
13	rs1745837	13	rs582385	-0.7391	0.053	-0.8361	0.018	-0.0649	0.189	-0.06822	0.434	-0.9495	0.014
13	rs1745837	13	rs2770298	-0.1539	0.664	0.3105	0.342	0.06952	0.126	0.07197	0.369	0.04833	0.894
13	rs1745837	13	rs972979	-0.0603	0.853	0.3055	0.310	0.05644	0.176	0.05958	0.418	0.4395	0.193
13	rs1745837	13	rs731779	-0.2698	0.515	0.4101	0.283	0.09092	0.087	0.07256	0.441	-0.0433	0.918
13	rs1745837	13	rs2770304	-0.3127	0.346	0.2704	0.382	0.02495	0.561	0.02748	0.717	0.3525	0.306

13	rs1745837	13	rs985933	-0.0967	0.765	0.3107	0.301	0.05698	0.171	0.04581	0.533	0.4093	0.224
13	rs1745837	13	rs927544	-0.0201	0.954	0.3065	0.341	0.05442	0.224	0.04208	0.595	0.2385	0.506
13	rs1745837	13	rs17288723	-0.1717	0.701	0.7535	0.068	0.1205	0.037	0.1177	0.249	-0.0095	0.983
13	rs1745837	13	rs9534505	0.0386	0.940	0.1253	0.790	0.00864	0.895	0.08583	0.457	0.1453	0.780
13	rs1745837	13	rs9534507	0.7316	0.256	0.3749	0.528	0.1207	0.145	0.1168	0.425	0.3805	0.567
13	rs1745837	13	rs4942587	-0.3165	0.407	0.2984	0.398	0.00998	0.839	0.02374	0.784	0.2022	0.605
13	rs1745837	13	rs4941573	0.6251	0.049	0.2464	0.405	-0.0041	0.921	0.03306	0.648	0.3144	0.340
13	rs1745837	13	rs12584920	-0.5478	0.191	0.3528	0.360	0.03155	0.557	0.00103	0.991	0.09043	0.834
13	rs1745837	13	rs1328684	-0.4345	0.183	-0.4955	0.101	-0.0097	0.818	-0.02414	0.745	-0.4614	0.161
13	rs1745837	13	rs2296973	-0.1373	0.692	0.3559	0.269	0.05631	0.209	0.01602	0.840	0.4079	0.260
13	rs1745837	13	rs2070037	-0.3169	0.407	0.2945	0.404	0.01714	0.727	0.01558	0.858	0.1592	0.684
13	rs1745837	13	rs9534511	-0.5174	0.085	-0.2884	0.301	-0.0019	0.962	-0.02441	0.722	-0.2794	0.367
13	rs1745837	13	rs6313	0.6066	0.059	0.304	0.308	-0.011	0.790	0.00336	0.963	0.2753	0.405
13	rs1745837	13	rs6312	0.4898	0.481	0.4668	0.468	0.1667	0.064	0.00397	0.980	1.258	0.085
13	rs1745837	13	rs6306	-1.038	0.101	-0.1107	0.849	0.03269	0.687	0.06086	0.671	-0.7172	0.266
13	rs1745837	17	rs1906451	-0.4307	0.172	0.2294	0.434	0.01937	0.633	0.052	0.469	-0.3606	0.277
13	rs1745837	17	rs1042173	-0.4643	0.144	0.2107	0.474	0.01439	0.725	0.04715	0.515	-0.3626	0.273
13	rs1745837	17	rs12449783	-0.4675	0.142	0.2024	0.492	0.01413	0.730	0.04614	0.524	-0.3618	0.275
13	rs1745837	17	rs3794808	-0.3984	0.216	0.1586	0.597	0.01298	0.755	0.03623	0.622	-0.2836	0.399
13	rs1745837	17	rs4583306	-0.4145	0.202	0.1394	0.643	0.01532	0.714	0.0263	0.722	-0.3958	0.239
13	rs1745837	17	rs140700	0.7758	0.151	1.074	0.030	0.08147	0.235	0.1367	0.259	0.4525	0.404
13	rs1745837	17	rs6354	0.1909	0.626	0.1167	0.750	-0.0055	0.913	0.02736	0.761	0.3066	0.449
13	rs1745837	17	rs2020936	0.2316	0.554	0.1349	0.712	-0.0015	0.977	0.03596	0.689	0.3205	0.428
13	rs1745837	17	rs2066713	0.2246	0.488	-0.3329	0.268	-0.0185	0.657	-0.03255	0.657	0.04505	0.892
13	rs1745837	17	rs4251417	-0.3486	0.528	0.2743	0.580	0.05992	0.385	-0.1155	0.344	-0.4616	0.411
13	rs1745837	17	rs8071667	0.2115	0.594	0.1028	0.780	-0.0071	0.890	0.01431	0.874	0.2596	0.524
13	rs1745837	17	rs1487971	-0.2121	0.516	-0.4373	0.146	-0.0416	0.319	0.05694	0.440	0.2909	0.385
13	rs622337	13	rs655854	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
13	rs622337	13	rs2296972	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
13	rs622337	13	rs4942578	0.1157	0.767	0.4288	0.231	-0.0475	0.346	0.05646	0.526	0.7489	0.055
13	rs622337	13	rs1928042	-0.8846	0.014	-0.8027	0.016	-0.0992	0.033	-0.03666	0.655	-0.9092	0.012
13	rs622337	13	rs2760345	-0.1234	0.825	0.1144	0.826	0.00366	0.960	-0.03072	0.810	0.4053	0.473
13	rs622337	13	rs2770296	-0.166	0.652	0.4771	0.162	0.106	0.025	0.1666	0.047	0.2514	0.505
13	rs622337	13	rs1328674	-1.044	0.207	-0.0672	0.930	-0.1169	0.272	0.07392	0.695	-0.1455	0.860
13	rs622337	13	rs9316235	0.0783	0.834	0.4253	0.217	0.113	0.018	0.1366	0.106	0.3147	0.409
13	rs622337	13	rs582385	-0.7361	0.059	-0.8289	0.022	-0.0513	0.308	-0.0394	0.657	-0.9689	0.014
13	rs622337	13	rs2770298	-0.1841	0.613	0.43	0.202	0.0896	0.056	0.1457	0.078	0.2648	0.478

13	rs622337	13	rs972979	-0.0462	0.890	0.3946	0.199	0.06874	0.107	0.07726	0.305	0.6019	0.080
13	rs622337	13	rs731779	-0.2785	0.518	0.5576	0.161	0.1075	0.052	0.1539	0.116	0.2116	0.628
13	rs622337	13	rs2770304	-0.3224	0.344	0.3617	0.254	0.03579	0.417	0.04519	0.562	0.5123	0.146
13	rs622337	13	rs985933	-0.0847	0.798	0.3999	0.192	0.0693	0.104	0.06272	0.405	0.57	0.097
13	rs622337	13	rs927544	0.0204	0.955	0.4043	0.220	0.07226	0.115	0.07216	0.373	0.3975	0.281
13	rs622337	13	rs17288723	-0.2436	0.596	0.8985	0.034	0.1335	0.024	0.1842	0.079	0.1532	0.742
13	rs622337	13	rs9534505	0.0207	0.968	0.1858	0.694	0.01285	0.844	0.09935	0.390	0.1365	0.793
13	rs622337	13	rs9534507	0.7733	0.227	0.3712	0.530	0.1242	0.131	0.1157	0.426	0.3799	0.565
13	rs622337	13	rs4942587	-0.3271	0.408	0.4151	0.256	0.02617	0.608	0.05643	0.530	0.3915	0.335
13	rs622337	13	rs4941573	0.6095	0.059	0.1575	0.601	-0.0269	0.520	-0.00633	0.932	0.1579	0.635
13	rs622337	13	rs12584920	-0.6641	0.124	0.453	0.256	0.04645	0.402	0.00683	0.945	0.1486	0.739
13	rs622337	13	rs1328684	-0.4287	0.195	-0.4735	0.123	0.00097	0.982	-0.00255	0.973	-0.4355	0.192
13	rs622337	13	rs2296973	-0.1144	0.750	0.4398	0.187	0.06962	0.133	0.02798	0.733	0.6045	0.105
13	rs622337	13	rs2070037	-0.3287	0.406	0.4067	0.266	0.03361	0.510	0.0476	0.597	0.3405	0.402
13	rs622337	13	rs9534511	-0.504	0.101	-0.2371	0.407	0.01723	0.666	0.00773	0.913	-0.1416	0.653
13	rs622337	13	rs6313	0.5884	0.071	0.2122	0.484	-0.0342	0.417	-0.04026	0.588	0.1167	0.727
13	rs622337	13	rs6312	0.6789	0.344	0.423	0.524	0.1617	0.081	-0.04424	0.787	1.359	0.066
13	rs622337	13	rs6306	-1.077	0.087	-0.1224	0.832	0.05493	0.495	0.1355	0.341	-0.6669	0.297
13	rs622337	17	rs1906451	-0.2836	0.383	0.3233	0.286	0.03523	0.399	0.07919	0.285	-0.3163	0.353
13	rs622337	17	rs1042173	-0.3221	0.325	0.3009	0.321	0.02967	0.483	0.07324	0.327	-0.3199	0.347
13	rs622337	17	rs12449783	-0.3246	0.322	0.293	0.335	0.0294	0.487	0.07222	0.334	-0.3193	0.348
13	rs622337	17	rs3794808	-0.2589	0.434	0.2235	0.470	0.02798	0.514	0.05357	0.480	-0.2147	0.535
13	rs622337	17	rs4583306	-0.2775	0.403	0.1866	0.545	0.03063	0.474	0.04652	0.539	-0.3409	0.323
13	rs622337	17	rs140700	0.6815	0.206	1.068	0.031	0.08083	0.237	0.1046	0.386	0.4143	0.444
13	rs622337	17	rs6354	0.0991	0.804	0.2104	0.572	-0.0233	0.652	-0.02444	0.789	0.2528	0.540
13	rs622337	17	rs2020936	0.0492	0.901	0.1652	0.657	-0.0256	0.620	-0.04082	0.654	0.1867	0.650
13	rs622337	17	rs2066713	0.2159	0.514	-0.4032	0.189	-0.0176	0.679	-0.00482	0.949	0.07379	0.828
13	rs622337	17	rs4251417	-0.4945	0.398	0.1022	0.849	0.06866	0.358	-0.0855	0.517	-0.584	0.332
13	rs622337	17	rs8071667	0.0451	0.911	0.1195	0.749	-0.0319	0.539	-0.06758	0.461	0.1188	0.774
13	rs622337	17	rs1487971	-0.0573	0.863	-0.456	0.136	-0.0411	0.333	0.07949	0.290	0.2875	0.399
13	rs655854	13	rs2296972	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
13	rs655854	13	rs4942578	0.0864	0.823	0.4337	0.221	-0.0483	0.335	0.05269	0.551	0.7121	0.067
13	rs655854	13	rs1928042	-0.875	0.016	-0.8168	0.015	-0.0945	0.043	-0.03498	0.672	-1.033	0.005
13	rs655854	13	rs2760345	-0.0517	0.926	0.2298	0.659	0.00516	0.943	-0.02473	0.847	0.3177	0.576
13	rs655854	13	rs2770296	-0.1871	0.611	0.5494	0.105	0.1113	0.019	0.1797	0.031	0.2951	0.435
13	rs655854	13	rs1328674	-1.042	0.218	0.1318	0.867	-0.1003	0.357	0.1018	0.596	-0.2176	0.796
13	rs655854	13	rs9316235	0.0471	0.899	0.4589	0.182	0.1149	0.016	0.147	0.082	0.3693	0.334

13	rs655854	13	rs582385	-0.7229	0.063	-0.876	0.015	-0.0493	0.327	-0.04413	0.619	-1.079	0.006
13	rs655854	13	rs2770298	-0.2023	0.577	0.5001	0.136	0.09487	0.043	0.1596	0.053	0.3055	0.413
13	rs655854	13	rs972979	-0.0776	0.815	0.4329	0.157	0.07012	0.100	0.08402	0.263	0.6163	0.073
13	rs655854	13	rs731779	-0.3111	0.470	0.598	0.132	0.1078	0.052	0.1597	0.103	0.2957	0.499
13	rs655854	13	rs2770304	-0.3473	0.307	0.3988	0.207	0.03626	0.411	0.04933	0.525	0.5349	0.130
13	rs655854	13	rs985933	-0.0995	0.764	0.4368	0.152	0.07113	0.094	0.07122	0.342	0.5846	0.089
13	rs655854	13	rs927544	-0.0255	0.943	0.4065	0.216	0.07344	0.109	0.07953	0.325	0.4518	0.221
13	rs655854	13	rs17288723	-0.3389	0.462	0.9052	0.033	0.1322	0.027	0.1792	0.089	0.2051	0.661
13	rs655854	13	rs9534505	0.0563	0.912	0.2744	0.562	0.02534	0.700	0.1197	0.302	0.1007	0.847
13	rs655854	13	rs9534507	0.7915	0.216	0.3725	0.528	0.13	0.115	0.1316	0.365	0.3558	0.590
13	rs655854	13	rs4942587	-0.3932	0.319	0.4169	0.253	0.02558	0.616	0.05807	0.518	0.4676	0.251
13	rs655854	13	rs4941573	0.6062	0.060	0.15	0.616	-0.0302	0.469	-0.01258	0.864	0.2207	0.510
13	rs655854	13	rs12584920	-0.7408	0.085	0.4383	0.269	0.04481	0.419	0.00505	0.959	0.2263	0.612
13	rs655854	13	rs1328684	-0.3884	0.243	-0.473	0.125	0.00496	0.908	0.00255	0.973	-0.5595	0.097
13	rs655854	13	rs2296973	-0.1516	0.672	0.4431	0.182	0.06633	0.153	0.02781	0.734	0.6441	0.084
13	rs655854	13	rs2070037	-0.3951	0.317	0.4086	0.262	0.03293	0.518	0.04909	0.585	0.417	0.306
13	rs655854	13	rs9534511	-0.5108	0.095	-0.2601	0.360	0.01677	0.673	0.00584	0.934	-0.1868	0.555
13	rs655854	13	rs6313	0.6011	0.064	0.2196	0.465	-0.0359	0.391	-0.04153	0.574	0.1709	0.610
13	rs655854	13	rs6312	0.7428	0.296	0.4423	0.501	0.149	0.106	-0.04767	0.769	1.228	0.094
13	rs655854	13	rs6306	-1.132	0.075	-0.0005	0.999	0.05741	0.481	0.1454	0.312	-0.5737	0.375
13	rs655854	17	rs1906451	-0.3576	0.270	0.3179	0.291	0.03741	0.370	0.07436	0.313	-0.3894	0.253
13	rs655854	17	rs1042173	-0.3946	0.226	0.2964	0.326	0.03198	0.449	0.06874	0.356	-0.3922	0.249
13	rs655854	17	rs12449783	-0.3974	0.223	0.2883	0.340	0.03172	0.452	0.06769	0.363	-0.3917	0.251
13	rs655854	17	rs3794808	-0.2904	0.381	0.2508	0.417	0.03119	0.468	0.05546	0.465	-0.2771	0.425
13	rs655854	17	rs4583306	-0.3139	0.344	0.2188	0.476	0.0338	0.430	0.0475	0.529	-0.4085	0.237
13	rs655854	17	rs140700	0.7543	0.162	1.047	0.034	0.08838	0.197	0.08452	0.484	0.5288	0.329
13	rs655854	17	rs6354	0.2435	0.542	0.1809	0.627	-0.0241	0.642	-0.03335	0.715	0.2899	0.485
13	rs655854	17	rs2020936	0.1914	0.631	0.1356	0.715	-0.0264	0.609	-0.04984	0.584	0.2233	0.589
13	rs655854	17	rs2066713	0.1476	0.654	-0.4113	0.177	-0.0206	0.626	0.00016	0.998	0.1173	0.730
13	rs655854	17	rs4251417	-0.4921	0.398	0.1064	0.842	0.06957	0.351	-0.08316	0.527	-0.6222	0.299
13	rs655854	17	rs8071667	0.1885	0.641	0.0885	0.812	-0.033	0.527	-0.07694	0.401	0.1568	0.706
13	rs655854	17	rs1487971	-0.0942	0.778	-0.4747	0.123	-0.0451	0.293	0.07857	0.299	0.2956	0.390
13	rs2296972	13	rs4942578	0.1002	0.795	0.3683	0.298	-0.051	0.306	0.05277	0.549	0.714	0.064
13	rs2296972	13	rs1928042	-0.8365	0.020	-0.806	0.016	-0.0974	0.035	-0.03979	0.627	-0.9634	0.008
13	rs2296972	13	rs2760345	-0.0711	0.898	0.0645	0.901	-0.0028	0.969	-0.02878	0.821	0.3622	0.519
13	rs2296972	13	rs2770296	-0.1908	0.603	0.4863	0.152	0.1069	0.024	0.1676	0.044	0.2894	0.442
13	rs2296972	13	rs1328674	-0.9896	0.233	-0.0766	0.921	-0.1177	0.271	0.07802	0.679	-0.1338	0.871

13	rs2296972	13	rs9316235	0.0395	0.916	0.4386	0.202	0.1143	0.017	0.1376	0.104	0.3231	0.397
13	rs2296972	13	rs582385	-0.7002	0.071	-0.8322	0.021	-0.0495	0.323	-0.04534	0.608	-1.034	0.008
13	rs2296972	13	rs2770298	-0.2082	0.566	0.44	0.190	0.09021	0.053	0.1465	0.075	0.2755	0.460
13	rs2296972	13	rs972979	-0.0747	0.822	0.3814	0.213	0.06735	0.113	0.07628	0.309	0.5941	0.084
13	rs2296972	13	rs731779	-0.3219	0.454	0.5532	0.163	0.1085	0.049	0.1461	0.135	0.236	0.588
13	rs2296972	13	rs2770304	-0.3455	0.310	0.3345	0.290	0.03401	0.439	0.03851	0.620	0.4879	0.166
13	rs2296972	13	rs985933	-0.0966	0.770	0.3852	0.208	0.06837	0.107	0.0635	0.397	0.5623	0.101
13	rs2296972	13	rs927544	-0.0139	0.969	0.4058	0.217	0.0734	0.108	0.07226	0.371	0.4048	0.271
13	rs2296972	13	rs17288723	-0.345	0.454	0.8833	0.038	0.1345	0.024	0.1652	0.117	0.1299	0.781
13	rs2296972	13	rs9534505	0.0636	0.901	0.2014	0.670	0.01395	0.831	0.1175	0.310	0.1888	0.716
13	rs2296972	13	rs9534507	0.7937	0.217	0.4034	0.497	0.1265	0.126	0.1455	0.319	0.4762	0.472
13	rs2296972	13	rs4942587	-0.3849	0.330	0.3995	0.274	0.02651	0.602	0.04622	0.606	0.3812	0.348
13	rs2296972	13	rs4941573	0.5887	0.067	0.1722	0.565	-0.0271	0.515	-0.00428	0.953	0.2133	0.522
13	rs2296972	13	rs12584920	-0.7442	0.084	0.4207	0.290	0.04518	0.414	-0.00878	0.928	0.1349	0.762
13	rs2296972	13	rs1328684	-0.3724	0.260	-0.4816	0.116	0.00114	0.979	0.00249	0.974	-0.4803	0.151
13	rs2296972	13	rs2296973	-0.1528	0.670	0.4101	0.217	0.06765	0.144	0.02006	0.806	0.5765	0.121
13	rs2296972	13	rs2070037	-0.3871	0.327	0.3911	0.284	0.03389	0.505	0.03728	0.678	0.3307	0.415
13	rs2296972	13	rs9534511	-0.5002	0.102	-0.2598	0.362	0.01736	0.662	-0.00113	0.987	-0.2118	0.502
13	rs2296972	13	rs6313	0.5838	0.071	0.2257	0.453	-0.0338	0.419	-0.03581	0.628	0.1741	0.602
13	rs2296972	13	rs6312	0.7059	0.320	0.3565	0.587	0.1512	0.099	-0.03926	0.809	1.256	0.085
13	rs2296972	13	rs6306	-1.206	0.055	-0.1882	0.745	0.05367	0.505	0.09738	0.494	-0.6731	0.292
13	rs2296972	17	rs1906451	-0.2917	0.367	0.3434	0.254	0.03625	0.383	0.0812	0.270	-0.3226	0.342
13	rs2296972	17	rs1042173	-0.3227	0.322	0.3253	0.281	0.0315	0.454	0.0737	0.321	-0.3371	0.321
13	rs2296972	17	rs12449783	-0.3254	0.318	0.3173	0.294	0.03123	0.458	0.07266	0.328	-0.3365	0.322
13	rs2296972	17	rs3794808	-0.22	0.507	0.2759	0.372	0.03076	0.473	0.06076	0.423	-0.2239	0.518
13	rs2296972	17	rs4583306	-0.2469	0.456	0.2407	0.433	0.03342	0.434	0.05284	0.484	-0.3586	0.297
13	rs2296972	17	rs140700	0.6886	0.200	1.019	0.039	0.08572	0.208	0.08775	0.466	0.4948	0.359
13	rs2296972	17	rs6354	0.1403	0.724	0.1718	0.644	-0.0242	0.639	-0.03431	0.706	0.2458	0.550
13	rs2296972	17	rs2020936	0.0903	0.820	0.127	0.732	-0.0266	0.606	-0.05069	0.577	0.1802	0.661
13	rs2296972	17	rs2066713	0.1511	0.646	-0.4284	0.160	-0.02	0.635	-0.00421	0.955	0.09917	0.769
13	rs2296972	17	rs4251417	-0.4117	0.480	0.1547	0.773	0.06769	0.363	-0.08913	0.498	-0.6044	0.312
13	rs2296972	17	rs8071667	0.0847	0.833	0.0791	0.832	-0.033	0.524	-0.07761	0.396	0.1126	0.785
13	rs2296972	17	rs1487971	-0.1035	0.755	-0.4938	0.106	-0.0423	0.320	0.0746	0.320	0.3319	0.329
13	rs4942578	13	rs1928042	-0.2156	0.623	-0.5991	0.141	-0.0787	0.163	-0.1189	0.232	-0.474	0.282
13	rs4942578	13	rs2760345	0.2308	0.704	0.0021	0.997	-0.0181	0.816	-0.0393	0.775	0.7499	0.216
13	rs4942578	13	rs2770296	0.3699	0.394	0.5855	0.144	0.03581	0.521	0.1303	0.186	-0.0594	0.892
13	rs4942578	13	rs1328674	-0.8671	0.276	-0.3931	0.597	-0.1379	0.176	-0.06248	0.729	-0.5723	0.473

13	rs4942578	13	rs9316235	0.72	0.133	0.9605	0.029	0.01122	0.856	0.2025	0.062	0.2613	0.590
13	rs4942578	13	rs582385	0.2945	0.617	-0.7328	0.177	-0.0833	0.272	-0.2056	0.125	-0.475	0.424
13	rs4942578	13	rs2770298	0.2961	0.492	0.6127	0.125	0.01932	0.728	0.1407	0.151	-0.0116	0.979
13	rs4942578	13	rs972979	0.7698	0.049	0.8213	0.024	0.1008	0.045	0.2026	0.022	0.69	0.088
13	rs4942578	13	rs731779	0.8025	0.122	0.9935	0.037	0.00983	0.883	0.1946	0.099	0.6036	0.247
13	rs4942578	13	rs2770304	0.7028	0.073	0.7352	0.043	0.07855	0.119	0.1879	0.035	0.9236	0.022
13	rs4942578	13	rs985933	0.7492	0.055	0.8174	0.024	0.1013	0.044	0.2073	0.019	0.6881	0.088
13	rs4942578	13	rs927544	0.8178	0.051	0.7873	0.039	0.04929	0.354	0.2842	0.002	0.293	0.481
13	rs4942578	13	rs17288723	0.5897	0.284	0.4981	0.326	-0.0484	0.496	0.2371	0.059	0.3303	0.551
13	rs4942578	13	rs9534505	-0.3489	0.537	-0.1239	0.812	0.02831	0.696	-0.00142	0.991	-0.833	0.143
13	rs4942578	13	rs9534507	0.7359	0.511	0.4478	0.658	0.2074	0.143	0.1205	0.630	-1.797	0.116
13	rs4942578	13	rs4942587	0.741	0.088	0.6415	0.106	0.03067	0.579	0.2802	0.004	0.4896	0.255
13	rs4942578	13	rs4941573	-0.8373	0.039	-0.5289	0.160	-0.0534	0.305	-0.1339	0.145	-0.4583	0.273
13	rs4942578	13	rs12584920	0.3233	0.484	0.2109	0.621	0.04258	0.473	0.2406	0.022	0.251	0.588
13	rs4942578	13	rs1328684	0.2649	0.500	-0.0705	0.845	0.02732	0.585	-0.08671	0.327	0.0173	0.965
13	rs4942578	13	rs2296973	1.012	0.010	0.8078	0.027	0.09485	0.061	0.2182	0.015	1.074	0.008
13	rs4942578	13	rs2070037	0.7519	0.083	0.6184	0.119	0.03874	0.483	0.2727	0.005	0.4354	0.311
13	rs4942578	13	rs9534511	0.926	0.013	0.5257	0.130	0.02989	0.535	0.1585	0.062	0.7222	0.060
13	rs4942578	13	rs6313	-0.9142	0.025	-0.5765	0.127	-0.0579	0.269	-0.1769	0.056	-0.4727	0.261
13	rs4942578	13	rs6312	1.596	0.038	1.087	0.123	0.2227	0.022	0.03003	0.861	2.778	0.000
13	rs4942578	13	rs6306	-0.9404	0.236	-0.6073	0.409	-0.0687	0.503	0.05036	0.782	-0.5143	0.531
13	rs4942578	17	rs1906451	0.269	0.484	0.4101	0.252	0.03641	0.459	0.159	0.068	0.2261	0.571
13	rs4942578	17	rs1042173	0.3133	0.418	0.4499	0.211	0.04238	0.394	0.1722	0.050	0.2484	0.533
13	rs4942578	17	rs12449783	0.3082	0.427	0.4413	0.220	0.04235	0.394	0.1715	0.051	0.2525	0.527
13	rs4942578	17	rs3794808	0.2944	0.454	0.5714	0.120	0.0352	0.487	0.1205	0.178	0.4778	0.240
13	rs4942578	17	rs4583306	0.4795	0.225	0.7312	0.047	0.04165	0.413	0.1115	0.214	0.5706	0.157
13	rs4942578	17	rs140700	-0.233	0.718	0.5083	0.388	-0.0276	0.735	-0.184	0.201	0.5894	0.349
13	rs4942578	17	rs6354	-0.1218	0.807	-0.1127	0.806	-0.0221	0.729	-0.07833	0.487	-0.3073	0.542
13	rs4942578	17	rs2020936	-0.2089	0.675	-0.1013	0.825	-0.0246	0.699	-0.09775	0.386	-0.2841	0.572
13	rs4942578	17	rs2066713	-0.3324	0.402	-0.5673	0.123	0.00602	0.906	0.00094	0.992	-0.2908	0.476
13	rs4942578	17	rs4251417	0.1606	0.816	-0.0034	0.996	0.06638	0.452	0.1531	0.326	0.3267	0.648
13	rs4942578	17	rs8071667	-0.0745	0.882	0.0303	0.948	-0.0218	0.735	-0.1251	0.270	-0.2629	0.603
13	rs4942578	17	rs1487971	-0.0982	0.804	-0.1945	0.596	0.00389	0.939	0.00942	0.917	-0.3955	0.326
13	rs1928042	13	rs2760345	-0.7235	0.193	-0.8028	0.119	-0.059	0.406	-0.02303	0.854	-1.099	0.048
13	rs1928042	13	rs2770296	-0.4031	0.306	-0.5521	0.131	-0.0275	0.587	0.077	0.389	-0.5673	0.153
13	rs1928042	13	rs1328674	-2.156	0.058	-1.835	0.079	-0.1317	0.357	-0.2152	0.394	-2.296	0.037
13	rs1928042	13	rs9316235	-0.2175	0.636	0.0034	0.994	-0.0109	0.853	0.1328	0.200	0.06896	0.883

13	rs1928042	13	rs582385	0.3273	0.454	0.1345	0.741	0.03175	0.573	-0.06706	0.500	-0.2393	0.590
13	rs1928042	13	rs2770298	-0.3686	0.328	-0.3695	0.296	0.00164	0.973	0.06952	0.416	-0.3343	0.385
13	rs1928042	13	rs972979	-0.255	0.472	-0.4584	0.166	-0.0483	0.288	0.04005	0.617	-0.4321	0.231
13	rs1928042	13	rs731779	0.3161	0.586	0.0648	0.904	-0.0122	0.871	0.1686	0.202	0.5646	0.342
13	rs1928042	13	rs2770304	-0.0068	0.985	-0.3013	0.375	-0.0064	0.891	-0.00738	0.929	-0.1148	0.756
13	rs1928042	13	rs985933	-0.2786	0.431	-0.4564	0.167	-0.0481	0.289	0.02437	0.761	-0.4598	0.202
13	rs1928042	13	rs927544	-0.3632	0.431	-0.3074	0.475	-0.102	0.085	0.02786	0.790	-0.3006	0.525
13	rs1928042	13	rs17288723	-0.0918	0.884	-0.2268	0.700	-0.0302	0.711	0.08179	0.571	-0.2949	0.647
13	rs1928042	13	rs9534505	-1.148	0.029	-0.9042	0.062	-0.0813	0.224	0.00754	0.949	-1.465	0.005
13	rs1928042	13	rs9534507	-2.238	0.030	-1.659	0.081	-0.3251	0.014	0.1952	0.402	-3.071	0.004
13	rs1928042	13	rs4942587	0.1682	0.735	-0.0095	0.984	-0.0333	0.602	-0.01697	0.880	0.3807	0.452
13	rs1928042	13	rs4941573	0.2364	0.573	0.5465	0.162	0.09151	0.090	0.00137	0.989	0.9379	0.029
13	rs1928042	13	rs12584920	0.0178	0.973	-0.1394	0.775	-0.0482	0.473	-0.074	0.533	-0.0878	0.869
13	rs1928042	13	rs1328684	-0.2385	0.516	-0.4054	0.233	-0.054	0.250	0.01391	0.867	-0.8515	0.022
13	rs1928042	13	rs2296973	0.2015	0.662	0.0759	0.859	-0.0386	0.514	0.03549	0.734	0.4688	0.322
13	rs1928042	13	rs2070037	0.181	0.715	0.0295	0.949	-0.0303	0.634	-0.01335	0.906	0.4293	0.396
13	rs1928042	13	rs9534511	0.4155	0.221	0.0843	0.791	-0.0316	0.472	0.02197	0.777	0.2951	0.397
13	rs1928042	13	rs6313	0.3232	0.438	0.5449	0.161	0.08485	0.114	-0.00498	0.958	0.7689	0.073
13	rs1928042	13	rs6312	0.5006	0.611	-0.0285	0.975	-0.0671	0.593	0.1825	0.410	0.28	0.783
13	rs1928042	13	rs6306	-0.0071	0.993	-0.2904	0.692	-0.1447	0.156	0.1461	0.417	0.9403	0.239
13	rs1928042	17	rs1906451	0.854	0.013	0.3123	0.332	0.04683	0.290	0.06214	0.428	-0.0555	0.876
13	rs1928042	17	rs1042173	0.8717	0.012	0.3142	0.328	0.05077	0.255	0.07306	0.354	-0.0204	0.954
13	rs1928042	17	rs12449783	0.892	0.010	0.344	0.287	0.05216	0.244	0.07636	0.334	-0.026	0.942
13	rs1928042	17	rs3794808	1.044	0.003	0.6091	0.067	0.06013	0.191	0.1558	0.055	0.146	0.689
13	rs1928042	17	rs4583306	0.8004	0.024	0.4714	0.154	0.02874	0.531	0.1375	0.089	0.2079	0.566
13	rs1928042	17	rs140700	-1.014	0.067	-0.8503	0.094	-0.1061	0.129	-0.0633	0.608	0.1104	0.838
13	rs1928042	17	rs6354	-0.5884	0.151	-0.7855	0.037	-0.0769	0.141	-0.1653	0.072	-0.5069	0.213
13	rs1928042	17	rs2020936	-0.6184	0.130	-0.8344	0.026	-0.0821	0.115	-0.1814	0.048	-0.5165	0.203
13	rs1928042	17	rs2066713	-0.3285	0.345	0.1622	0.616	0.02601	0.562	-0.00537	0.946	0.2347	0.510
13	rs1928042	17	rs4251417	0.7711	0.161	0.3049	0.549	0.01457	0.837	0.07493	0.548	0.5204	0.353
13	rs1928042	17	rs8071667	-0.6519	0.112	-0.8446	0.027	-0.0745	0.159	-0.1696	0.069	-0.6177	0.135
13	rs1928042	17	rs1487971	-0.1804	0.604	-0.0393	0.903	0.01873	0.674	-0.03552	0.651	0.2262	0.525
13	rs2760345	13	rs2770296	-0.5127	0.342	-0.4968	0.321	0.02024	0.772	-0.02915	0.813	-1.06	0.053
13	rs2760345	13	rs1328674	-2.275	0.077	-1.054	0.377	-0.1444	0.386	0.03728	0.899	-1.908	0.126
13	rs2760345	13	rs9316235	-0.3281	0.689	0.0867	0.910	-0.0239	0.822	-0.1147	0.541	-1.234	0.157
13	rs2760345	13	rs582385	0.4034	0.693	-0.9516	0.301	-0.0675	0.596	-0.08537	0.704	0.2039	0.839
13	rs2760345	13	rs2770298	-0.6437	0.232	-0.4995	0.319	-0.0127	0.855	-0.02335	0.850	-1.187	0.030

13	rs2760345	13	rs972979	-0.0485	0.944	0.6081	0.343	0.03355	0.706	0.041	0.794	-0.5183	0.462
13	rs2760345	13	rs731779	0.1883	0.841	0.2869	0.744	-0.0302	0.804	-0.0892	0.678	-0.327	0.743
13	rs2760345	13	rs2770304	0.0808	0.907	0.7422	0.251	-0.0119	0.894	0.07509	0.636	-0.0066	0.993
13	rs2760345	13	rs985933	-0.0421	0.951	0.6004	0.349	0.03294	0.711	0.04192	0.790	-0.5246	0.456
13	rs2760345	13	rs927544	0.5868	0.452	0.6699	0.359	0.06201	0.539	0.03537	0.843	-0.5389	0.512
13	rs2760345	13	rs17288723	0.5629	0.591	-0.8978	0.355	-0.1267	0.350	-0.01276	0.958	-0.9067	0.412
13	rs2760345	13	rs9534505	-0.9457	0.134	-0.69	0.237	-0.0017	0.983	-0.00873	0.952	-1.369	0.032
13	rs2760345	13	rs9534507	-0.7631	0.646	-0.8094	0.599	0.2673	0.214	-0.1977	0.603	-3.11	0.071
13	rs2760345	13	rs4942587	0.8592	0.303	0.9006	0.248	0.017	0.875	0.1024	0.592	0.2343	0.792
13	rs2760345	13	rs4941573	0.0664	0.926	-0.1802	0.787	0.03979	0.667	-0.02264	0.890	0.5825	0.430
13	rs2760345	13	rs12584920	0.8369	0.357	0.6422	0.449	-0.001	0.993	0.1753	0.400	-0.1022	0.914
13	rs2760345	13	rs1328684	-0.6897	0.324	-0.4882	0.451	-0.0471	0.600	-0.06793	0.669	-0.7161	0.312
13	rs2760345	13	rs2296973	1.034	0.060	1.055	0.040	0.03916	0.583	0.08081	0.522	1.129	0.048
13	rs2760345	13	rs2070037	0.8698	0.297	0.9332	0.231	0.0195	0.857	0.1053	0.582	0.2742	0.757
13	rs2760345	13	rs9534511	0.8529	0.093	0.688	0.148	-0.0301	0.648	0.09618	0.409	1.001	0.061
13	rs2760345	13	rs6313	-0.0407	0.955	-0.2905	0.663	0.03936	0.669	-0.1194	0.463	0.248	0.736
13	rs2760345	13	rs6312	1.452	0.132	1.972	0.024	0.1936	0.112	0.1565	0.469	2.846	0.002
13	rs2760345	13	rs6306	-1.757	0.240	-1.756	0.206	-0.2655	0.172	-0.1009	0.769	0.1853	0.910
13	rs2760345	17	rs1906451	0.6949	0.171	0.4209	0.378	0.1679	0.010	0.1653	0.153	-0.1249	0.812
13	rs2760345	17	rs1042173	0.7418	0.147	0.4613	0.335	0.1751	0.008	0.1778	0.128	-0.1028	0.845
13	rs2760345	17	rs12449783	0.7378	0.150	0.4542	0.343	0.1751	0.008	0.1772	0.129	-0.1	0.849
13	rs2760345	17	rs3794808	0.8915	0.101	0.8457	0.099	0.1807	0.010	0.1746	0.162	0.3379	0.547
13	rs2760345	17	rs4583306	1.061	0.055	0.9258	0.075	0.1504	0.035	0.1081	0.393	0.2038	0.718
13	rs2760345	17	rs140700	-0.9913	0.164	-0.8324	0.211	-0.1124	0.218	-0.07427	0.645	-0.0781	0.911
13	rs2760345	17	rs6354	-0.6256	0.306	-0.9015	0.112	-0.0576	0.462	0.05571	0.687	-0.6877	0.261
13	rs2760345	17	rs2020936	-0.6938	0.256	-0.9387	0.097	-0.0649	0.406	0.03848	0.781	-0.6865	0.261
13	rs2760345	17	rs2066713	-0.4989	0.342	-0.0103	0.983	-0.0805	0.236	-0.09468	0.431	0.5861	0.277
13	rs2760345	17	rs4251417	-0.3162	0.719	-0.6932	0.397	0.1153	0.307	0.2419	0.226	-0.7909	0.380
13	rs2760345	17	rs8071667	-0.6571	0.283	-0.8156	0.154	-0.0518	0.512	0.08665	0.535	-0.6731	0.276
13	rs2760345	17	rs1487971	0.0665	0.900	0.0686	0.890	-0.0204	0.765	-0.03306	0.784	0.7925	0.139
13	rs2770296	13	rs1328674	-1.03	0.334	-1.009	0.308	-0.1256	0.361	-0.2189	0.368	-2.197	0.040
13	rs2770296	13	rs9316235	0.3662	0.343	0.3493	0.324	-0.0243	0.623	-0.1153	0.186	0.3675	0.350
13	rs2770296	13	rs582385	-0.5251	0.344	-0.4139	0.417	-0.1228	0.084	0.1687	0.179	-0.5094	0.365
13	rs2770296	13	rs2770298	0.1876	0.616	0.3397	0.324	0.03076	0.521	-0.1628	0.054	0.2306	0.545
13	rs2770296	13	rs972979	0.2561	0.496	0.4995	0.149	0.04278	0.376	-0.1954	0.022	0.017	0.965
13	rs2770296	13	rs731779	0.5526	0.277	0.8027	0.087	0.03474	0.595	-0.2165	0.061	0.4868	0.342
13	rs2770296	13	rs2770304	0.2585	0.464	0.2369	0.469	-0.0132	0.773	-0.1492	0.064	-0.3024	0.403

13	rs2770296	13	rs985933	0.3011	0.423	0.4788	0.166	0.04278	0.376	-0.1904	0.025	0.03725	0.922
13	rs2770296	13	rs927544	0.7721	0.039	0.6296	0.066	0.02481	0.604	-0.1647	0.051	0.5137	0.175
13	rs2770296	13	rs17288723	0.7284	0.216	1.223	0.024	-0.0132	0.862	-0.1764	0.188	0.8064	0.169
13	rs2770296	13	rs9534505	-0.2198	0.745	-0.022	0.972	0.03728	0.667	-0.1973	0.198	-0.136	0.844
13	rs2770296	13	rs9534507	0.2771	0.768	0.6494	0.449	0.159	0.185	-0.2152	0.309	1.575	0.105
13	rs2770296	13	rs4942587	0.8061	0.043	0.6499	0.078	-0.0099	0.847	-0.1623	0.073	0.3598	0.376
13	rs2770296	13	rs4941573	-0.1713	0.675	-0.4276	0.257	0.01405	0.789	0.099	0.284	0.1273	0.760
13	rs2770296	13	rs12584920	0.8101	0.062	0.6466	0.107	-0.0494	0.377	-0.1715	0.082	0.331	0.453
13	rs2770296	13	rs1328684	-0.2188	0.523	-0.3512	0.267	-0.0086	0.846	0.04912	0.527	-0.4867	0.163
13	rs2770296	13	rs2296973	0.4611	0.204	0.4687	0.164	-0.0392	0.404	-0.1145	0.167	-0.0673	0.856
13	rs2770296	13	rs2070037	0.8107	0.042	0.6311	0.087	-0.0116	0.822	-0.1596	0.078	0.3458	0.394
13	rs2770296	13	rs9534511	0.3113	0.350	0.3048	0.325	-0.0872	0.043	0.01731	0.820	-0.2707	0.427
13	rs2770296	13	rs6313	-0.1946	0.636	-0.4605	0.225	0.0148	0.779	0.08126	0.383	0.2101	0.617
13	rs2770296	13	rs6312	-0.8207	0.283	-0.6176	0.382	-0.1018	0.302	-0.0456	0.793	-1.92	0.014
13	rs2770296	13	rs6306	1.184	0.189	1.613	0.050	-0.0676	0.558	-0.1123	0.582	1.827	0.041
13	rs2770296	17	rs1906451	-0.6353	0.060	-0.3367	0.279	0.03875	0.367	-0.1458	0.055	-0.4372	0.210
13	rs2770296	17	rs1042173	-0.5912	0.082	-0.3098	0.321	0.04474	0.304	-0.1359	0.077	-0.4263	0.221
13	rs2770296	17	rs12449783	-0.5854	0.085	-0.2991	0.338	0.04498	0.301	-0.1349	0.079	-0.4269	0.221
13	rs2770296	17	rs3794808	-0.4471	0.204	-0.1308	0.689	0.06855	0.130	-0.1768	0.027	-0.1762	0.628
13	rs2770296	17	rs4583306	-0.4827	0.172	0.0000	1.000	0.08673	0.056	-0.1338	0.095	-0.1595	0.661
13	rs2770296	17	rs140700	0.2152	0.700	0.7928	0.125	0.1293	0.071	-0.03483	0.783	-0.3448	0.535
13	rs2770296	17	rs6354	0.6885	0.108	0.6034	0.128	0.04588	0.405	0.01277	0.896	0.3275	0.452
13	rs2770296	17	rs2020936	0.6624	0.121	0.5731	0.148	0.04101	0.456	-0.00775	0.936	0.3018	0.487
13	rs2770296	17	rs2066713	0.0311	0.929	-0.411	0.203	-0.1033	0.021	0.1226	0.121	0.01146	0.974
13	rs2770296	17	rs4251417	-0.9477	0.120	-0.1747	0.755	0.1322	0.088	-0.02934	0.831	-0.5122	0.408
13	rs2770296	17	rs8071667	0.5232	0.224	0.5005	0.209	0.03495	0.528	0.0094	0.924	0.2746	0.530
13	rs2770296	17	rs1487971	0.069	0.842	-0.2406	0.453	-0.0294	0.510	-0.05697	0.469	-0.1411	0.691
13	rs1328674	13	rs9316235	0.8977	0.453	0.5053	0.653	-0.0223	0.886	-0.09543	0.727	-0.3843	0.757
13	rs1328674	13	rs582385	0.8014	0.619	-0.639	0.643	-0.0617	0.748	-0.296	0.384	-0.4336	0.763
13	rs1328674	13	rs2770298	-1.226	0.245	-1.124	0.254	-0.1651	0.225	-0.1908	0.427	-2.249	0.035
13	rs1328674	13	rs972979	-0.2755	0.785	0.4244	0.651	-0.0701	0.589	-0.05256	0.818	-1.341	0.189
13	rs1328674	13	rs731779	1.957	0.160	1.457	0.260	0.2514	0.163	-0.1263	0.692	0.9048	0.529
13	rs1328674	13	rs2770304	-0.0737	0.943	0.7068	0.457	0.03086	0.814	-0.02805	0.904	-0.6969	0.499
13	rs1328674	13	rs985933	-0.2689	0.790	0.4162	0.657	-0.0707	0.585	-0.0516	0.822	-1.348	0.186
13	rs1328674	13	rs927544	1.787	0.107	1.325	0.198	0.0238	0.867	-0.0865	0.731	-0.0863	0.939
13	rs1328674	13	rs17288723	2.474	0.097	0.8878	0.522	0.02041	0.916	-0.1415	0.679	-0.1364	0.927
13	rs1328674	13	rs9534505	-2.978	0.017	-2.507	0.030	-0.3727	0.020	-0.198	0.487	-3.523	0.004

13	rs1328674	13	rs9534507	-0.9191	0.662	-1.188	0.542	-0.4475	0.100	-0.1059	0.826	-2.905	0.177
13	rs1328674	13	rs4942587	2.292	0.055	1.87	0.092	0.1717	0.262	-0.07087	0.793	0.7945	0.517
13	rs1328674	13	rs4941573	0.1871	0.856	0.0263	0.978	0.1502	0.259	0.1637	0.485	1.832	0.086
13	rs1328674	13	rs12584920	2.815	0.025	1.756	0.134	0.02475	0.878	-0.04353	0.879	0.0602	0.962
13	rs1328674	13	rs1328684	-1.922	0.066	-1.425	0.138	-0.2732	0.040	-0.125	0.595	-2.284	0.027
13	rs1328674	13	rs2296973	2.027	0.067	2.494	0.015	0.2075	0.144	0.09509	0.705	1.353	0.228
13	rs1328674	13	rs2070037	2.302	0.054	1.901	0.087	0.174	0.256	-0.06807	0.801	0.8331	0.496
13	rs1328674	13	rs9534511	1.721	0.082	1.793	0.050	0.08845	0.484	-0.02902	0.897	1.127	0.256
13	rs1328674	13	rs6313	-0.0441	0.966	-0.2994	0.755	0.1197	0.365	0.1366	0.558	1.526	0.148
13	rs1328674	13	rs6312	0.1665	0.937	3.205	0.100	0.223	0.413	0.5813	0.227	2.23	0.271
13	rs1328674	13	rs6306	0.1695	0.939	0.3875	0.851	-0.3154	0.273	0.00132	0.998	2.093	0.328
13	rs1328674	17	rs1906451	0.2461	0.736	-0.1107	0.871	0.08209	0.379	0.09084	0.583	0.02357	0.975
13	rs1328674	17	rs1042173	0.2773	0.706	-0.0811	0.906	0.08597	0.364	0.1015	0.544	0.04182	0.955
13	rs1328674	17	rs12449783	0.2735	0.711	-0.088	0.898	0.0859	0.364	0.1009	0.546	0.04434	0.953
13	rs1328674	17	rs3794808	0.6793	0.409	0.7672	0.318	0.143	0.177	0.1224	0.514	0.9794	0.249
13	rs1328674	17	rs4583306	0.3697	0.653	0.7584	0.323	0.1154	0.274	0.1467	0.432	0.7009	0.408
13	rs1328674	17	rs140700	-0.4107	0.670	-1.079	0.241	-0.0282	0.819	-0.1731	0.427	-0.0136	0.988
13	rs1328674	17	rs6354	0.0892	0.916	-0.6427	0.415	-0.0439	0.682	-0.1669	0.378	0.07421	0.928
13	rs1328674	17	rs2020936	0.067	0.937	-0.6448	0.413	-0.0485	0.650	-0.1773	0.349	0.09611	0.907
13	rs1328674	17	rs2066713	-0.4636	0.550	0.0092	0.990	-0.0411	0.681	0.05827	0.742	-0.3626	0.642
13	rs1328674	17	rs4251417	-0.1505	0.900	-0.8162	0.455	0.1186	0.437	0.3464	0.199	-0.6483	0.581
13	rs1328674	17	rs8071667	-0.0129	0.988	-0.7037	0.381	-0.0445	0.683	-0.1587	0.411	0.01507	0.986
13	rs1328674	17	rs1487971	0.3934	0.589	-0.2243	0.741	0.05085	0.583	-0.1577	0.336	-0.02	0.978
13	rs9316235	13	rs582385	-0.5323	0.347	-0.3175	0.543	-0.1018	0.162	0.2312	0.072	-0.3475	0.550
13	rs9316235	13	rs2770298	0.2936	0.465	0.3395	0.356	0.01607	0.754	-0.1478	0.102	0.4565	0.263
13	rs9316235	13	rs972979	0.2615	0.487	0.2136	0.539	-0.0217	0.654	-0.1633	0.055	-0.1344	0.728
13	rs9316235	13	rs731779	0.3332	0.518	0.5026	0.290	0.00545	0.934	-0.1826	0.119	0.3753	0.469
13	rs9316235	13	rs2770304	0.2426	0.504	0.126	0.708	-0.0753	0.107	-0.1226	0.138	-0.3981	0.282
13	rs9316235	13	rs985933	0.2923	0.436	0.1975	0.570	-0.0218	0.651	-0.1601	0.060	-0.1109	0.774
13	rs9316235	13	rs927544	0.5458	0.158	0.3066	0.390	0.0323	0.513	-0.1502	0.084	0.3722	0.348
13	rs9316235	13	rs17288723	0.355	0.550	0.8994	0.100	-0.0012	0.987	-0.122	0.366	0.6574	0.267
13	rs9316235	13	rs9534505	0.6596	0.216	0.4325	0.378	0.0265	0.697	-0.02696	0.823	0.7708	0.158
13	rs9316235	13	rs9534507	0.4937	0.613	0.8189	0.357	0.2316	0.062	-0.2164	0.324	2.298	0.024
13	rs9316235	13	rs4942587	0.5795	0.155	0.3341	0.377	-0.0137	0.795	-0.1503	0.105	0.1406	0.736
13	rs9316235	13	rs4941573	-0.1641	0.700	-0.3822	0.330	0.01354	0.803	0.06579	0.492	-0.0047	0.991
13	rs9316235	13	rs12584920	0.5038	0.254	0.3171	0.438	-0.0264	0.642	-0.1534	0.126	0.1474	0.743
13	rs9316235	13	rs1328684	-0.0757	0.838	-0.0832	0.807	-0.0029	0.951	0.07106	0.395	-0.1429	0.706

13	rs9316235	13	rs2296973	0.2165	0.565	0.1004	0.774	-0.0576	0.236	-0.1288	0.133	-0.4049	0.293
13	rs9316235	13	rs2070037	0.5834	0.152	0.3093	0.413	-0.0158	0.764	-0.1477	0.111	0.119	0.775
13	rs9316235	13	rs9534511	0.0675	0.849	0.1127	0.731	-0.0567	0.213	0.01819	0.821	-0.3964	0.272
13	rs9316235	13	rs6313	-0.1254	0.770	-0.3538	0.370	0.02102	0.701	0.042	0.664	0.05825	0.894
13	rs9316235	13	rs6312	-0.9428	0.214	-1.051	0.133	-0.1561	0.109	-0.1335	0.439	-2.458	0.001
13	rs9316235	13	rs6306	0.804	0.363	1.183	0.143	-0.0578	0.607	-0.03315	0.868	1.492	0.088
13	rs9316235	17	rs1906451	-0.6291	0.075	-0.2404	0.455	0.02837	0.524	-0.1989	0.011	-0.5245	0.143
13	rs9316235	17	rs1042173	-0.583	0.101	-0.2133	0.509	0.0346	0.442	-0.1889	0.017	-0.514	0.151
13	rs9316235	17	rs12449783	-0.5764	0.106	-0.2016	0.533	0.03485	0.439	-0.1879	0.018	-0.5153	0.150
13	rs9316235	17	rs3794808	-0.5101	0.162	-0.1791	0.594	0.05559	0.233	-0.2327	0.005	-0.4744	0.204
13	rs9316235	17	rs4583306	-0.4973	0.176	-0.05	0.882	0.08315	0.075	-0.1885	0.022	-0.3775	0.312
13	rs9316235	17	rs140700	0.3653	0.520	1.079	0.040	0.1241	0.087	0.1043	0.416	-0.1721	0.760
13	rs9316235	17	rs6354	0.6201	0.160	0.6079	0.136	0.02514	0.657	0.1061	0.288	0.3999	0.371
13	rs9316235	17	rs2020936	0.5965	0.176	0.5767	0.157	0.02089	0.712	0.0861	0.388	0.3687	0.409
13	rs9316235	17	rs2066713	0.1129	0.757	-0.4029	0.231	-0.0911	0.051	0.1096	0.184	0.1219	0.745
13	rs9316235	17	rs4251417	-0.8998	0.156	0.0579	0.917	0.05832	0.450	-0.1708	0.211	-0.2679	0.665
13	rs9316235	17	rs8071667	0.4628	0.296	0.5016	0.220	0.01267	0.824	0.09851	0.327	0.3675	0.413
13	rs9316235	17	rs1487971	0.0873	0.813	-0.0979	0.773	-0.0314	0.505	0.03274	0.694	0.0042	0.991
13	rs582385	13	rs2770298	-0.4623	0.397	-0.3384	0.500	-0.1073	0.125	0.1791	0.147	-0.3833	0.489
13	rs582385	13	rs972979	-0.4969	0.322	-0.8718	0.060	-0.152	0.018	0.07989	0.482	-0.3016	0.555
13	rs582385	13	rs731779	0.0794	0.902	0.0362	0.952	-0.0478	0.565	0.2018	0.170	0.316	0.633
13	rs582385	13	rs2770304	-0.1012	0.848	-0.6946	0.155	-0.1177	0.083	0.0058	0.962	0.1431	0.790
13	rs582385	13	rs985933	-0.5155	0.304	-0.8651	0.061	-0.1507	0.019	0.06328	0.577	-0.3322	0.515
13	rs582385	13	rs927544	-0.8379	0.121	-0.7537	0.133	-0.1476	0.035	0.06059	0.624	-0.478	0.390
13	rs582385	13	rs17288723	-0.3772	0.586	-0.1043	0.872	-0.0322	0.721	0.06842	0.667	-0.3946	0.580
13	rs582385	13	rs9534505	-1.536	0.105	-1.109	0.191	-0.1982	0.092	0.08103	0.697	-1.798	0.052
13	rs582385	13	rs9534507	-2.343	0.040	-1.277	0.221	-0.2288	0.112	0.2836	0.266	-2.435	0.038
13	rs582385	13	rs4942587	-0.3154	0.591	-0.562	0.303	-0.1045	0.170	-0.00441	0.974	0.1213	0.841
13	rs582385	13	rs4941573	0.1617	0.734	0.5357	0.225	0.07739	0.206	-0.00784	0.942	0.5666	0.247
13	rs582385	13	rs12584920	-0.5867	0.344	-0.7087	0.218	-0.1034	0.198	-0.1209	0.394	-0.268	0.672
13	rs582385	13	rs1328684	0.0292	0.947	-0.1334	0.743	-0.0051	0.928	0.00956	0.924	-0.4945	0.268
13	rs582385	13	rs2296973	-0.1545	0.778	-0.6792	0.180	-0.1034	0.143	0.04126	0.741	0.2391	0.671
13	rs582385	13	rs2070037	-0.304	0.605	-0.5262	0.335	-0.1019	0.181	-0.00119	0.993	0.1656	0.784
13	rs582385	13	rs9534511	0.118	0.794	-0.2378	0.569	-0.0116	0.842	-0.02102	0.837	0.0619	0.894
13	rs582385	13	rs6313	0.3031	0.525	0.6117	0.166	0.06953	0.257	-0.01485	0.891	0.6115	0.213
13	rs582385	13	rs6312	0.7268	0.523	-1.149	0.265	-0.081	0.570	0.154	0.541	0.2492	0.829
13	rs582385	13	rs6306	0.2393	0.782	-0.1088	0.893	-0.059	0.601	0.09109	0.647	0.2564	0.772

13	rs582385	17	rs1906451	0.6712	0.075	0.2163	0.537	0.03207	0.509	0.06432	0.454	-0.085	0.828
13	rs582385	17	rs1042173	0.6937	0.068	0.2291	0.515	0.03636	0.459	0.07522	0.386	-0.054	0.890
13	rs582385	17	rs12449783	0.7038	0.064	0.2438	0.489	0.03656	0.457	0.07666	0.377	-0.0596	0.879
13	rs582385	17	rs3794808	0.7769	0.044	0.3316	0.357	0.03521	0.481	0.1625	0.066	-0.0705	0.859
13	rs582385	17	rs4583306	0.5349	0.158	0.1519	0.666	-0.0024	0.961	0.1071	0.215	0.02004	0.959
13	rs582385	17	rs140700	-0.4272	0.473	-0.2447	0.654	-0.0962	0.203	-0.05643	0.673	0.4016	0.492
13	rs582385	17	rs6354	-0.2355	0.591	-0.309	0.446	-0.0249	0.659	-0.1404	0.158	-0.397	0.368
13	rs582385	17	rs2020936	-0.263	0.548	-0.3116	0.442	-0.0306	0.586	-0.1531	0.123	-0.3695	0.401
13	rs582385	17	rs2066713	-0.3638	0.326	0.0181	0.958	0.0095	0.842	-0.02734	0.745	0.2217	0.559
13	rs582385	17	rs4251417	0.6025	0.332	0.4081	0.476	0.04134	0.604	-0.03203	0.820	0.3013	0.634
13	rs582385	17	rs8071667	-0.2768	0.530	-0.3112	0.444	-0.023	0.684	-0.1438	0.150	-0.4525	0.307
13	rs582385	17	rs1487971	-0.3421	0.361	-0.0399	0.909	-0.0243	0.613	-0.08479	0.318	0.1452	0.706
13	rs2770298	13	rs972979	0.2048	0.559	0.2122	0.512	-0.011	0.808	-0.1564	0.048	-0.295	0.412
13	rs2770298	13	rs731779	0.5144	0.309	0.6901	0.139	0.03875	0.551	-0.1909	0.096	0.4755	0.351
13	rs2770298	13	rs2770304	0.2057	0.553	0.0953	0.767	-0.0415	0.356	-0.1354	0.087	-0.5034	0.157
13	rs2770298	13	rs985933	0.2319	0.507	0.1952	0.546	-0.0111	0.805	-0.1538	0.052	-0.2777	0.440
13	rs2770298	13	rs927544	0.7323	0.043	0.4294	0.200	0.00393	0.933	-0.1539	0.060	0.3202	0.389
13	rs2770298	13	rs17288723	0.618	0.284	0.9961	0.061	0.00127	0.986	-0.1349	0.303	0.598	0.300
13	rs2770298	13	rs9534505	-0.2949	0.661	-0.1187	0.848	0.01096	0.899	-0.1887	0.215	-0.1947	0.778
13	rs2770298	13	rs9534507	0.2954	0.752	0.5487	0.520	0.1384	0.246	-0.2175	0.302	1.516	0.119
13	rs2770298	13	rs4942587	0.7828	0.046	0.4862	0.183	-0.0226	0.656	-0.1531	0.087	0.1895	0.637
13	rs2770298	13	rs4941573	-0.1024	0.797	-0.3662	0.320	0.01829	0.720	0.08084	0.368	0.2511	0.537
13	rs2770298	13	rs12584920	0.7721	0.070	0.4536	0.251	-0.0556	0.311	-0.1517	0.118	0.1043	0.810
13	rs2770298	13	rs1328684	-0.2674	0.432	-0.2399	0.448	0.00434	0.921	0.04854	0.529	-0.4038	0.245
13	rs2770298	13	rs2296973	0.4156	0.245	0.3502	0.293	-0.0574	0.215	-0.1076	0.188	-0.2517	0.492
13	rs2770298	13	rs2070037	0.7883	0.045	0.471	0.197	-0.024	0.636	-0.1502	0.093	0.1795	0.655
13	rs2770298	13	rs9534511	0.2761	0.402	0.3357	0.271	-0.0701	0.098	0.02207	0.768	-0.2417	0.470
13	rs2770298	13	rs6313	-0.1055	0.792	-0.3959	0.285	0.02199	0.668	0.05541	0.540	0.266	0.516
13	rs2770298	13	rs6312	-0.8791	0.241	-0.564	0.416	-0.1267	0.190	-0.05659	0.740	-2.073	0.007
13	rs2770298	13	rs6306	0.871	0.321	1.304	0.105	-0.1	0.371	-0.03459	0.861	1.84	0.035
13	rs2770298	17	rs1906451	-0.4716	0.156	-0.1765	0.562	0.05799	0.168	-0.1576	0.034	-0.4492	0.186
13	rs2770298	17	rs1042173	-0.4265	0.203	-0.1481	0.628	0.06409	0.132	-0.1469	0.050	-0.4372	0.198
13	rs2770298	17	rs12449783	-0.4209	0.209	-0.1386	0.650	0.06433	0.131	-0.146	0.052	-0.4372	0.198
13	rs2770298	17	rs3794808	-0.306	0.379	0.0329	0.919	0.091	0.041	-0.1889	0.016	-0.2394	0.503
13	rs2770298	17	rs4583306	-0.3478	0.319	0.149	0.642	0.1104	0.013	-0.143	0.069	-0.1985	0.578
13	rs2770298	17	rs140700	0.0302	0.955	0.6512	0.194	0.1082	0.119	0.03705	0.762	-0.2058	0.702
13	rs2770298	17	rs6354	0.4965	0.236	0.404	0.298	0.01945	0.718	0.05419	0.569	0.3748	0.378

13	rs2770298	17	rs2020936	0.4703	0.260	0.3754	0.332	0.01456	0.787	0.03364	0.723	0.3517	0.407
13	rs2770298	17	rs2066713	0.0322	0.926	-0.4349	0.176	-0.1116	0.012	0.1089	0.167	0.01834	0.959
13	rs2770298	17	rs4251417	-0.8335	0.169	-0.1113	0.836	0.0927	0.214	-0.07559	0.567	-0.3957	0.504
13	rs2770298	17	rs8071667	0.3284	0.435	0.2982	0.443	0.00853	0.875	0.05042	0.598	0.3283	0.441
13	rs2770298	17	rs1487971	0.0908	0.793	-0.2409	0.452	-0.0398	0.371	-0.01338	0.865	-0.0505	0.887
13	rs972979	13	rs731779	0.295	0.549	0.6441	0.157	0.01294	0.838	-0.2089	0.061	0.07405	0.882
13	rs972979	13	rs2770304	0.2776	0.412	0.6317	0.044	0.04891	0.261	-0.03754	0.625	-0.0568	0.870
13	rs972979	13	rs985933	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
13	rs972979	13	rs927544	0.431	0.242	0.5937	0.080	0.07213	0.127	-0.1021	0.220	0.2372	0.526
13	rs972979	13	rs17288723	0.4499	0.417	0.3758	0.464	-0.0685	0.338	-0.1814	0.150	0.06356	0.910
13	rs972979	13	rs9534505	0.1713	0.794	0.404	0.503	0.1118	0.181	-0.2069	0.161	-0.1957	0.771
13	rs972979	13	rs9534507	0.4132	0.643	0.3442	0.673	0.2392	0.035	-0.343	0.087	0.7513	0.417
13	rs972979	13	rs4942587	0.3629	0.390	0.6954	0.074	0.05561	0.305	-0.05191	0.586	0.07887	0.854
13	rs972979	13	rs4941573	-0.207	0.559	-0.3918	0.234	-0.0065	0.887	0.03956	0.621	0.0774	0.831
13	rs972979	13	rs12584920	0.254	0.590	0.5037	0.247	-0.0041	0.946	-0.04682	0.661	-0.1145	0.810
13	rs972979	13	rs1328684	0.1688	0.588	-0.2108	0.465	-0.0046	0.909	0.00263	0.970	-0.1384	0.661
13	rs972979	13	rs2296973	0.3456	0.350	0.7437	0.029	0.06699	0.159	-0.02603	0.757	0.131	0.730
13	rs972979	13	rs2070037	0.3744	0.375	0.7235	0.063	0.05783	0.286	-0.04908	0.608	0.1152	0.788
13	rs972979	13	rs9534511	0.2276	0.451	0.293	0.298	-0.0572	0.143	0.07882	0.252	-0.0894	0.774
13	rs972979	13	rs6313	-0.2372	0.506	-0.3886	0.240	-0.0009	0.984	0.01146	0.887	0.1102	0.763
13	rs972979	13	rs6312	0.2814	0.749	1.052	0.198	0.1249	0.272	0.06061	0.762	0.3407	0.708
13	rs972979	13	rs6306	0.1624	0.842	0.3654	0.627	-0.0982	0.348	-0.335	0.069	0.2527	0.760
13	rs972979	17	rs1906451	-0.2596	0.401	0.1172	0.683	0.07651	0.052	0.00166	0.981	-0.1595	0.620
13	rs972979	17	rs1042173	-0.1968	0.527	0.1698	0.555	0.08454	0.034	0.01689	0.810	-0.1444	0.653
13	rs972979	17	rs12449783	-0.2003	0.521	0.1622	0.574	0.08454	0.034	0.01593	0.821	-0.1404	0.663
13	rs972979	17	rs3794808	-0.0412	0.897	0.3565	0.230	0.1	0.014	0.00307	0.966	0.1375	0.676
13	rs972979	17	rs4583306	0.071	0.824	0.5658	0.056	0.1139	0.005	-0.00258	0.972	0.2295	0.484
13	rs972979	17	rs140700	-0.1229	0.814	0.7486	0.122	0.09552	0.154	-0.06961	0.556	-0.2346	0.655
13	rs972979	17	rs6354	0.1281	0.741	0.1795	0.618	0.04869	0.330	0.05181	0.557	-0.018	0.964
13	rs972979	17	rs2020936	0.0609	0.875	0.1002	0.780	0.04328	0.385	0.0288	0.743	-0.07	0.859
13	rs972979	17	rs2066713	-0.1101	0.726	-0.5739	0.049	-0.1181	0.003	-0.00882	0.901	-0.0234	0.942
13	rs972979	17	rs4251417	-0.4107	0.455	0.2928	0.569	0.1281	0.070	0.1136	0.362	-0.0848	0.879
13	rs972979	17	rs8071667	0.0049	0.990	0.0999	0.782	0.04385	0.382	0.02741	0.757	-0.1501	0.705
13	rs972979	17	rs1487971	0.1275	0.688	-0.1816	0.538	-0.004	0.922	-0.02935	0.682	0.06416	0.845
13	rs731779	13	rs2770304	0.6665	0.179	0.6444	0.160	0.02712	0.671	-0.1434	0.204	-0.0957	0.849
13	rs731779	13	rs985933	0.3136	0.524	0.6345	0.163	0.01227	0.846	-0.2084	0.062	0.06864	0.891
13	rs731779	13	rs927544	0.5027	0.312	0.7049	0.121	0.05866	0.355	-0.1558	0.164	0.5483	0.270

13	rs731779	13	rs17288723	1.035	0.108	0.931	0.118	0.03821	0.646	-0.07522	0.609	0.7199	0.259
13	rs731779	13	rs9534505	-0.2857	0.761	0.6027	0.481	0.04475	0.708	-0.3148	0.135	1.179	0.224
13	rs731779	13	rs9534507	-1.554	0.198	-0.0022	0.998	-0.0795	0.601	-0.4194	0.118	1.436	0.251
13	rs731779	13	rs4942587	0.9767	0.054	0.7962	0.088	0.08004	0.218	-0.0905	0.430	0.4	0.431
13	rs731779	13	rs4941573	-0.5976	0.233	-0.7377	0.110	0.01183	0.854	0.06928	0.540	-0.3913	0.440
13	rs731779	13	rs12584920	0.7183	0.153	0.4199	0.365	0.03852	0.551	-0.1344	0.239	0.3775	0.455
13	rs731779	13	rs1328684	-0.2183	0.664	-0.1075	0.815	-0.0961	0.131	0.02667	0.813	0.00921	0.986
13	rs731779	13	rs2296973	0.5811	0.243	0.4749	0.302	-0.004	0.950	-0.1128	0.320	-0.2578	0.608
13	rs731779	13	rs2070037	0.962	0.056	0.6908	0.136	0.07062	0.274	-0.08947	0.433	0.3011	0.551
13	rs731779	13	rs9534511	0.8455	0.076	0.4658	0.290	-0.0085	0.889	0.08439	0.435	-0.0488	0.920
13	rs731779	13	rs6313	-0.6847	0.172	-0.6856	0.138	0.01193	0.852	0.02162	0.849	-0.3722	0.464
13	rs731779	13	rs6312	-0.958	0.244	-1.204	0.112	-0.2455	0.020	-0.13	0.486	-2.384	0.004
13	rs731779	13	rs6306	1.854	0.061	1.424	0.121	0.06957	0.589	-0.01248	0.956	1.452	0.143
13	rs731779	17	rs1906451	-0.5884	0.140	-0.1683	0.649	0.05039	0.324	-0.2608	0.004	-0.6324	0.125
13	rs731779	17	rs1042173	-0.5559	0.167	-0.1371	0.712	0.05512	0.286	-0.2471	0.007	-0.6086	0.139
13	rs731779	17	rs12449783	-0.5539	0.169	-0.1396	0.707	0.05495	0.287	-0.2478	0.007	-0.6055	0.142
13	rs731779	17	rs3794808	-0.4132	0.311	-0.1003	0.792	0.06734	0.201	-0.2615	0.005	-0.4571	0.278
13	rs731779	17	rs4583306	-0.4427	0.283	0.0338	0.929	0.09259	0.081	-0.23	0.014	-0.3891	0.359
13	rs731779	17	rs140700	0.1681	0.796	1.187	0.047	-0.0201	0.808	0.107	0.464	-0.321	0.619
13	rs731779	17	rs6354	0.6862	0.169	0.8889	0.054	0.0299	0.641	0.1637	0.149	0.4329	0.391
13	rs731779	17	rs2020936	0.6689	0.180	0.8509	0.065	0.02684	0.675	0.144	0.204	0.3817	0.449
13	rs731779	17	rs2066713	0.1296	0.752	-0.6414	0.090	-0.0985	0.061	0.1003	0.281	0.1725	0.683
13	rs731779	17	rs4251417	-1.018	0.146	0.1568	0.806	0.08644	0.329	-0.1524	0.331	-0.4074	0.577
13	rs731779	17	rs8071667	0.5243	0.297	0.7644	0.099	0.0162	0.802	0.1544	0.175	0.3751	0.459
13	rs731779	17	rs1487971	0.0754	0.853	0.2089	0.579	-0.0636	0.223	0.06579	0.476	0.02528	0.952
13	rs2770304	13	rs985933	0.2854	0.400	0.64	0.041	0.04805	0.270	-0.03549	0.645	-0.0632	0.856
13	rs2770304	13	rs927544	0.2547	0.463	0.5042	0.115	0.00636	0.887	-0.00518	0.948	-0.0349	0.921
13	rs2770304	13	rs17288723	0.8872	0.114	0.0615	0.906	-0.0393	0.588	-0.1071	0.403	-0.1551	0.784
13	rs2770304	13	rs9534505	-0.1699	0.729	-0.0575	0.899	0.05878	0.350	-0.1229	0.268	-0.2214	0.657
13	rs2770304	13	rs9534507	-1.008	0.302	-0.5018	0.571	-0.0241	0.846	-0.4575	0.036	-0.4726	0.638
13	rs2770304	13	rs4942587	0.5613	0.183	0.6411	0.099	0.07331	0.175	0.02297	0.810	-0.0299	0.944
13	rs2770304	13	rs4941573	-0.3929	0.300	-0.4321	0.220	0.0081	0.868	0.03435	0.689	0.03164	0.935
13	rs2770304	13	rs12584920	0.4223	0.372	0.333	0.447	0.03326	0.585	0.02365	0.826	-0.2391	0.617
13	rs2770304	13	rs1328684	0.1568	0.634	-0.1438	0.637	-0.0233	0.583	-0.04066	0.587	0.0451	0.892
13	rs2770304	13	rs2296973	0.4996	0.171	0.6779	0.044	0.05398	0.250	0.03313	0.690	0.0768	0.837
13	rs2770304	13	rs2070037	0.5712	0.175	0.6639	0.087	0.07519	0.165	0.0254	0.791	0.001	0.998
13	rs2770304	13	rs9534511	0.5646	0.081	0.3921	0.193	-0.0469	0.262	0.0928	0.208	0.08578	0.796

13	rs2770304	13	rs6313	-0.4729	0.214	-0.4643	0.190	0.01147	0.814	-0.0132	0.878	-0.0286	0.942
13	rs2770304	13	rs6312	0.3304	0.712	1.114	0.181	-0.0176	0.880	0.08422	0.681	0.5133	0.578
13	rs2770304	13	rs6306	0.5824	0.498	0.0602	0.940	-0.0531	0.633	-0.2864	0.145	0.00238	0.998
13	rs2770304	17	rs1906451	-0.0218	0.946	0.3047	0.309	0.09365	0.022	-0.01163	0.873	-0.1674	0.617
13	rs2770304	17	rs1042173	0.0244	0.940	0.3467	0.248	0.1001	0.016	0.00465	0.950	-0.139	0.678
13	rs2770304	17	rs12449783	0.0224	0.945	0.341	0.257	0.1001	0.016	0.00383	0.958	-0.1348	0.687
13	rs2770304	17	rs3794808	0.1884	0.566	0.5634	0.067	0.1107	0.009	0.00605	0.936	0.1676	0.623
13	rs2770304	17	rs4583306	0.2848	0.389	0.7603	0.014	0.1211	0.004	-0.00471	0.950	0.2649	0.437
13	rs2770304	17	rs140700	-0.4805	0.365	0.5564	0.257	-0.0322	0.635	-0.03346	0.780	-0.2019	0.706
13	rs2770304	17	rs6354	-0.0885	0.822	0.0749	0.838	0.0223	0.660	0.09792	0.274	-0.0296	0.941
13	rs2770304	17	rs2020936	-0.1304	0.739	-0.0108	0.976	0.0172	0.733	0.07374	0.408	-0.0942	0.813
13	rs2770304	17	rs2066713	-0.1087	0.735	-0.6443	0.032	-0.1027	0.013	-0.03803	0.604	0.0064	0.985
13	rs2770304	17	rs4251417	-0.3064	0.579	0.3583	0.489	0.1	0.160	0.1253	0.319	-0.0364	0.949
13	rs2770304	17	rs8071667	-0.169	0.668	0.0138	0.970	0.01872	0.713	0.07195	0.423	-0.1796	0.654
13	rs2770304	17	rs1487971	0.1178	0.715	0.1021	0.734	-0.0075	0.856	0.02337	0.749	0.1779	0.595
13	rs985933	13	rs927544	0.4264	0.248	0.605	0.074	0.0711	0.132	-0.1027	0.219	0.2335	0.533
13	rs985933	13	rs17288723	0.5419	0.328	0.3551	0.488	-0.0688	0.334	-0.169	0.180	0.129	0.818
13	rs985933	13	rs9534505	0.1775	0.786	0.3961	0.511	0.1112	0.184	-0.206	0.163	-0.2025	0.763
13	rs985933	13	rs9534507	0.4196	0.638	0.336	0.680	0.2386	0.036	-0.342	0.088	0.744	0.421
13	rs985933	13	rs4942587	0.373	0.378	0.713	0.067	0.05489	0.312	-0.0494	0.606	0.0739	0.863
13	rs985933	13	rs4941573	-0.1945	0.584	-0.3718	0.259	-0.0054	0.906	0.04975	0.535	0.1249	0.731
13	rs985933	13	rs12584920	0.2715	0.564	0.4946	0.255	-0.0048	0.937	-0.0463	0.665	-0.1197	0.802
13	rs985933	13	rs1328684	0.1485	0.633	-0.2129	0.460	-0.0045	0.911	-0.01222	0.863	-0.169	0.592
13	rs985933	13	rs2296973	0.3582	0.332	0.7349	0.031	0.06639	0.163	-0.02543	0.762	0.1255	0.741
13	rs985933	13	rs2070037	0.3845	0.364	0.7416	0.057	0.05715	0.293	-0.04659	0.628	0.1108	0.796
13	rs985933	13	rs9534511	0.2173	0.471	0.2989	0.287	-0.0573	0.141	0.06791	0.323	-0.1236	0.690
13	rs985933	13	rs6313	-0.2373	0.506	-0.399	0.228	-0.0002	0.996	0.01766	0.827	0.1348	0.713
13	rs985933	13	rs6312	0.2878	0.743	1.044	0.201	0.1243	0.274	0.06151	0.759	0.3332	0.714
13	rs985933	13	rs6306	0.204	0.802	0.3505	0.642	-0.099	0.343	-0.3349	0.070	0.2483	0.765
13	rs985933	17	rs1906451	-0.2327	0.452	0.1072	0.709	0.07674	0.052	0.01166	0.868	-0.1279	0.691
13	rs985933	17	rs1042173	-0.1701	0.585	0.1596	0.579	0.08476	0.034	0.02676	0.705	-0.1131	0.725
13	rs985933	17	rs12449783	-0.1735	0.578	0.152	0.598	0.08476	0.034	0.02583	0.715	-0.109	0.735
13	rs985933	17	rs3794808	-0.0223	0.944	0.3534	0.235	0.1013	0.013	0.00728	0.920	0.1647	0.617
13	rs985933	17	rs4583306	0.0977	0.760	0.558	0.060	0.1145	0.005	0.00737	0.919	0.2643	0.421
13	rs985933	17	rs140700	-0.1421	0.785	0.7574	0.117	0.09668	0.148	-0.08905	0.451	-0.2705	0.606
13	rs985933	17	rs6354	0.1027	0.791	0.1833	0.611	0.04999	0.317	0.04533	0.607	-0.0367	0.926
13	rs985933	17	rs2020936	0.0545	0.888	0.0985	0.783	0.04353	0.381	0.01935	0.826	-0.094	0.811

13	rs985933	17	rs2066713	-0.1351	0.667	-0.5657	0.052	-0.1189	0.003	-0.01259	0.860	-0.0426	0.896
13	rs985933	17	rs4251417	-0.4416	0.421	0.2951	0.565	0.129	0.067	0.1208	0.332	-0.085	0.879
13	rs985933	17	rs8071667	-0.0016	0.997	0.0987	0.784	0.04413	0.378	0.01775	0.841	-0.1744	0.659
13	rs985933	17	rs1487971	0.096	0.762	-0.1687	0.567	-0.0034	0.933	-0.03878	0.588	0.0595	0.856
13	rs927544	13	rs17288723	0.6383	0.274	0.7425	0.168	-0.0281	0.708	-0.1666	0.209	0.7067	0.225
13	rs927544	13	rs9534505	0.9936	0.059	0.5826	0.228	0.03058	0.649	-0.1027	0.387	0.6449	0.228
13	rs927544	13	rs9534507	0.7031	0.456	0.6981	0.416	0.1923	0.108	-0.3335	0.115	1.697	0.081
13	rs927544	13	rs4942587	0.2157	0.614	0.483	0.220	0.05605	0.307	-0.06141	0.526	0.1842	0.669
13	rs927544	13	rs4941573	-0.1783	0.660	-0.3678	0.324	-0.0099	0.848	0.07598	0.404	-0.0138	0.973
13	rs927544	13	rs12584920	0.1194	0.805	0.3267	0.464	0.00264	0.966	-0.08419	0.444	0.07636	0.875
13	rs927544	13	rs1328684	0.1282	0.722	-0.214	0.520	-0.026	0.574	-0.00497	0.952	-0.2543	0.489
13	rs927544	13	rs2296973	0.0908	0.803	0.3693	0.270	0.02559	0.584	-0.00477	0.954	-0.0989	0.787
13	rs927544	13	rs2070037	0.2241	0.600	0.5023	0.202	0.05758	0.294	-0.0593	0.541	0.2099	0.627
13	rs927544	13	rs9534511	-0.0893	0.792	0.0734	0.815	-0.0403	0.355	0.0428	0.578	-0.3663	0.290
13	rs927544	13	rs6313	-0.187	0.647	-0.3704	0.324	-0.0053	0.920	0.06344	0.490	0.07295	0.860
13	rs927544	13	rs6312	-0.5457	0.549	-0.4289	0.614	0.00128	0.991	-0.00274	0.990	-2.175	0.024
13	rs927544	13	rs6306	0.8891	0.302	1.047	0.187	-0.0208	0.851	-0.3205	0.100	0.657	0.444
13	rs927544	17	rs1906451	-0.623	0.066	-0.1225	0.694	0.01516	0.724	-0.05216	0.493	-0.0463	0.894
13	rs927544	17	rs1042173	-0.5684	0.095	-0.0775	0.804	0.0218	0.616	-0.03983	0.604	-0.04	0.909
13	rs927544	17	rs12449783	-0.5707	0.095	-0.0837	0.789	0.02164	0.619	-0.04068	0.597	-0.0359	0.918
13	rs927544	17	rs3794808	-0.4189	0.225	0.0267	0.934	0.04289	0.333	-0.05292	0.500	0.09196	0.796
13	rs927544	17	rs4583306	-0.3276	0.342	0.2371	0.454	0.07214	0.102	-0.03	0.700	0.2562	0.468
13	rs927544	17	rs140700	0.5532	0.335	1.348	0.011	0.1934	0.008	-0.03164	0.806	-0.241	0.671
13	rs927544	17	rs6354	0.4876	0.250	0.6251	0.111	0.09031	0.097	0.03388	0.725	0.3642	0.396
13	rs927544	17	rs2020936	0.4619	0.275	0.5437	0.165	0.08631	0.112	0.01166	0.903	0.2936	0.493
13	rs927544	17	rs2066713	0.0373	0.913	-0.587	0.063	-0.106	0.016	0.01682	0.828	-0.3766	0.286
13	rs927544	17	rs4251417	-0.2869	0.630	0.4917	0.369	0.1022	0.179	0.00896	0.947	0.2549	0.676
13	rs927544	17	rs8071667	0.3767	0.377	0.4849	0.218	0.0813	0.138	-0.01343	0.890	0.1899	0.660
13	rs927544	17	rs1487971	0.202	0.565	-0.2443	0.450	0.00619	0.890	-0.04281	0.589	-0.2681	0.455
13	rs17288723	13	rs9534505	0.0045	0.997	1.293	0.161	-0.1114	0.388	-0.2824	0.216	0.8815	0.382
13	rs17288723	13	rs9534507	-1.428	0.278	1.521	0.196	-0.1656	0.314	-0.354	0.223	1.737	0.182
13	rs17288723	13	rs4942587	1.028	0.090	0.5912	0.293	0.00944	0.904	-0.09778	0.481	0.5181	0.392
13	rs17288723	13	rs4941573	-0.3612	0.513	-0.3065	0.548	0.07827	0.269	0.0926	0.459	0.03765	0.947
13	rs17288723	13	rs12584920	0.4037	0.484	0.0537	0.921	0.00553	0.942	-0.2521	0.058	0.1259	0.828
13	rs17288723	13	rs1328684	-0.5286	0.365	-0.2161	0.689	-0.0932	0.214	0.00849	0.949	-0.489	0.412
13	rs17288723	13	rs2296973	0.6217	0.278	-0.0672	0.899	-0.0479	0.518	-0.07996	0.541	-0.1782	0.757
13	rs17288723	13	rs2070037	0.9993	0.096	0.4228	0.447	-0.0048	0.950	-0.09465	0.490	0.3554	0.552

13	rs17288723	13	rs9534511	0.6265	0.235	-0.1229	0.801	-0.0258	0.704	0.08126	0.498	-0.4004	0.456
13	rs17288723	13	rs6313	-0.4033	0.470	-0.263	0.610	0.08059	0.260	0.03887	0.759	0.1437	0.801
13	rs17288723	13	rs6312	-1.244	0.269	-2.675	0.010	-0.2163	0.137	-0.09808	0.703	-3.725	0.002
13	rs17288723	13	rs6306	1.558	0.156	1.498	0.142	-0.0067	0.963	0.03035	0.905	1.435	0.184
13	rs17288723	17	rs1906451	-0.7743	0.072	-0.4825	0.225	0.04914	0.371	-0.2565	0.008	-0.9938	0.025
13	rs17288723	17	rs1042173	-0.742	0.086	-0.4513	0.258	0.05377	0.334	-0.2432	0.013	-0.9702	0.029
13	rs17288723	17	rs12449783	-0.7406	0.087	-0.4542	0.256	0.05361	0.335	-0.2439	0.013	-0.9674	0.029
13	rs17288723	17	rs3794808	-0.5444	0.211	-0.351	0.386	0.06482	0.248	-0.2536	0.011	-0.713	0.113
13	rs17288723	17	rs4583306	-0.6306	0.152	-0.3172	0.436	0.06845	0.228	-0.243	0.015	-0.6795	0.135
13	rs17288723	17	rs140700	0.3907	0.583	1.342	0.039	-0.0298	0.740	0.2454	0.123	-0.0925	0.894
13	rs17288723	17	rs6354	0.9474	0.079	1.293	0.009	0.02337	0.736	0.2458	0.045	0.7331	0.177
13	rs17288723	17	rs2020936	0.9337	0.083	1.251	0.012	0.02112	0.761	0.2259	0.065	0.668	0.218
13	rs17288723	17	rs2066713	0.1361	0.764	-0.7164	0.085	-0.0953	0.100	0.05862	0.568	0.1862	0.688
13	rs17288723	17	rs4251417	-1.05	0.155	-0.0283	0.967	0.1066	0.259	-0.1373	0.411	-0.7378	0.344
13	rs17288723	17	rs8071667	0.7983	0.143	1.186	0.018	0.01019	0.884	0.2396	0.052	0.6607	0.224
13	rs17288723	17	rs1487971	0.3373	0.443	0.0259	0.950	-0.0692	0.221	0.07099	0.478	-0.0436	0.924
13	rs9534505	13	rs9534507	1.885	0.108	0.9815	0.365	0.3242	0.032	0.04439	0.868	1.067	0.387
13	rs9534505	13	rs4942587	0.4227	0.610	1.063	0.159	0.00058	0.996	-0.3203	0.083	0.8791	0.297
13	rs9534505	13	rs4941573	0.6334	0.347	0.0407	0.948	0.01879	0.828	0.1411	0.353	1.124	0.106
13	rs9534505	13	rs12584920	0.7808	0.372	1.315	0.098	-0.1214	0.271	-0.2567	0.188	0.4451	0.609
13	rs9534505	13	rs1328684	-0.8888	0.179	-0.7194	0.236	-0.0137	0.872	0.05788	0.698	-1.614	0.016
13	rs9534505	13	rs2296973	0.3779	0.622	1.294	0.064	0.1347	0.167	-0.2194	0.202	0.9428	0.228
13	rs9534505	13	rs2070037	0.4333	0.601	1.096	0.146	0.00307	0.977	-0.3174	0.086	0.9193	0.275
13	rs9534505	13	rs9534511	-0.6563	0.325	0.4634	0.449	-0.0434	0.609	-0.1331	0.374	-0.35	0.606
13	rs9534505	13	rs6313	0.6252	0.353	-0.0951	0.879	0.01829	0.832	0.1619	0.288	1.23	0.077
13	rs9534505	13	rs6312	0.0345	0.982	1.641	0.255	0.5856	0.004	0.2405	0.500	0.8631	0.589
13	rs9534505	13	rs6306	-0.6167	0.647	0.9653	0.421	-0.243	0.147	-0.1734	0.559	1.379	0.281
13	rs9534505	17	rs1906451	-0.4305	0.390	-0.5096	0.269	0.02768	0.664	0.09565	0.396	0.04286	0.934
13	rs9534505	17	rs1042173	-0.3736	0.458	-0.4668	0.312	0.03375	0.600	0.09918	0.383	0.0197	0.970
13	rs9534505	17	rs12449783	-0.3785	0.453	-0.4748	0.304	0.03371	0.600	0.09853	0.386	0.02304	0.965
13	rs9534505	17	rs3794808	-0.2814	0.597	-0.1834	0.710	0.06773	0.322	0.04809	0.691	0.3814	0.490
13	rs9534505	17	rs4583306	-0.3308	0.534	-0.1159	0.813	0.06897	0.310	0.08402	0.485	0.2799	0.608
13	rs9534505	17	rs140700	0.0938	0.900	-0.2188	0.754	0.23	0.016	-0.2139	0.204	-0.2337	0.751
13	rs9534505	17	rs6354	0.2915	0.628	-0.1071	0.847	0.03619	0.637	-0.2019	0.136	-0.0305	0.960
13	rs9534505	17	rs2020936	0.2663	0.658	-0.1098	0.844	0.03099	0.686	-0.2135	0.115	-0.0059	0.992
13	rs9534505	17	rs2066713	-0.1076	0.831	0.1656	0.722	-0.0798	0.216	0.09037	0.427	-0.1938	0.707
13	rs9534505	17	rs4251417	-0.3878	0.660	-0.5945	0.461	0.1498	0.183	0.1835	0.356	-0.499	0.571

13	rs9534505	17	rs8071667	0.1769	0.769	-0.1827	0.744	0.03094	0.689	-0.2019	0.140	-0.0415	0.946
13	rs9534505	17	rs1487971	0.0605	0.900	-0.695	0.117	0.02089	0.734	-0.1971	0.070	-0.2471	0.615
13	rs9534507	13	rs4942587	-0.8761	0.427	0.3789	0.702	-0.13	0.347	-0.496	0.042	1.011	0.365
13	rs9534507	13	rs4941573	0.8996	0.310	0.1078	0.894	-0.0749	0.508	0.129	0.517	0.4857	0.598
13	rs9534507	13	rs12584920	-0.6365	0.583	0.8973	0.386	-0.2159	0.135	-0.402	0.115	0.8544	0.456
13	rs9534507	13	rs1328684	-0.3676	0.682	-0.3638	0.658	0.1702	0.137	0.1921	0.342	-1.216	0.192
13	rs9534507	13	rs2296973	-0.8076	0.433	0.2649	0.775	0.06721	0.605	-0.4638	0.043	0.6353	0.548
13	rs9534507	13	rs2070037	-0.8654	0.432	0.4111	0.678	-0.1277	0.356	-0.4931	0.043	1.05	0.346
13	rs9534507	13	rs9534511	-2.16	0.014	-0.565	0.483	-0.1213	0.279	-0.2117	0.284	-1.422	0.118
13	rs9534507	13	rs6313	1.077	0.226	0.1199	0.883	-0.0608	0.593	0.1866	0.352	0.8846	0.338
13	rs9534507	13	rs6312	-0.2204	0.921	-0.2879	0.888	0.9081	0.002	-0.1478	0.771	-1.299	0.602
13	rs9534507	13	rs6306	-1.392	0.437	1.243	0.420	-0.2103	0.330	-0.3075	0.421	1.026	0.539
13	rs9534507	17	rs1906451	-1.037	0.119	-0.7773	0.198	-0.0381	0.649	0.0982	0.508	0.0202	0.977
13	rs9534507	17	rs1042173	-0.9566	0.152	-0.7234	0.232	-0.0303	0.720	0.09673	0.517	-0.0345	0.960
13	rs9534507	17	rs12449783	-0.9616	0.150	-0.7314	0.227	-0.0303	0.720	0.09607	0.520	-0.0311	0.964
13	rs9534507	17	rs3794808	-0.9821	0.147	-0.7783	0.212	-0.005	0.954	0.00269	0.986	-0.0866	0.901
13	rs9534507	17	rs4583306	-0.8459	0.213	-0.6536	0.291	0.01499	0.862	0.04503	0.768	-0.0588	0.932
13	rs9534507	17	rs140700	1.287	0.227	0.8776	0.369	0.5317	0.000	-0.2289	0.343	-0.2817	0.795
13	rs9534507	17	rs6354	0.8038	0.312	0.5179	0.479	0.1205	0.238	-0.1987	0.271	-0.1177	0.886
13	rs9534507	17	rs2020936	0.7784	0.328	0.5151	0.482	0.1153	0.259	-0.2103	0.244	-0.0931	0.910
13	rs9534507	17	rs2066713	-0.0558	0.931	0.1502	0.798	-0.0951	0.244	0.09428	0.513	-0.0323	0.962
13	rs9534507	17	rs4251417	-0.6898	0.576	-0.3265	0.772	0.1534	0.329	-0.01204	0.965	-0.224	0.857
13	rs9534507	17	rs8071667	0.6898	0.386	0.4155	0.570	0.1097	0.283	-0.2064	0.253	-0.0753	0.927
13	rs9534507	17	rs1487971	-0.0397	0.950	-0.9747	0.090	0.01386	0.864	-0.2172	0.127	-0.3974	0.544
13	rs4942587	13	rs4941573	-0.3474	0.442	-0.4093	0.326	0.00081	0.989	0.07829	0.441	-0.1798	0.695
13	rs4942587	13	rs12584920	0.2721	0.583	0.1842	0.688	0.04724	0.460	-0.0264	0.815	-0.0479	0.923
13	rs4942587	13	rs1328684	-0.0522	0.912	-0.0562	0.898	-0.0963	0.113	-0.07746	0.471	0.158	0.745
13	rs4942587	13	rs2296973	0.3096	0.463	0.3526	0.364	0.04913	0.365	0.02324	0.808	-0.1765	0.678
13	rs4942587	13	rs2070037	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
13	rs4942587	13	rs9534511	0.3689	0.387	0.0746	0.849	0.0093	0.865	0.06481	0.501	-0.1439	0.740
13	rs4942587	13	rs6313	-0.4028	0.377	-0.4151	0.323	0.00304	0.958	0.04582	0.655	-0.172	0.710
13	rs4942587	13	rs6312	-0.5127	0.590	-0.4946	0.577	-0.1512	0.222	0.03905	0.858	-2.112	0.037
13	rs4942587	13	rs6306	1.271	0.162	0.5805	0.492	0.04232	0.720	-0.28	0.179	0.2074	0.819
13	rs4942587	17	rs1906451	-0.4109	0.257	0.0616	0.855	0.03894	0.401	-0.08906	0.278	-0.1318	0.725
13	rs4942587	17	rs1042173	-0.3749	0.305	0.0954	0.777	0.04378	0.351	-0.0748	0.366	-0.1088	0.772
13	rs4942587	17	rs12449783	-0.3754	0.305	0.0911	0.787	0.04365	0.352	-0.07552	0.362	-0.1045	0.781
13	rs4942587	17	rs3794808	-0.1923	0.602	0.2298	0.503	0.05961	0.210	-0.05955	0.478	0.06041	0.874

13	rs4942587	17	rs4583306	-0.1335	0.717	0.4369	0.199	0.08703	0.066	-0.04691	0.574	0.236	0.532
13	rs4942587	17	rs140700	0.2135	0.727	1.159	0.039	0.02464	0.751	0.02457	0.857	-0.188	0.756
13	rs4942587	17	rs6354	0.2859	0.525	0.5908	0.156	0.05377	0.353	0.09565	0.348	0.4528	0.318
13	rs4942587	17	rs2020936	0.2661	0.554	0.5016	0.227	0.05107	0.377	0.07436	0.465	0.3665	0.419
13	rs4942587	17	rs2066713	0.0701	0.848	-0.728	0.031	-0.0922	0.050	-0.00956	0.908	-0.3541	0.346
13	rs4942587	17	rs4251417	-0.1283	0.838	0.5463	0.345	0.0799	0.319	-0.01502	0.915	0.3217	0.621
13	rs4942587	17	rs8071667	0.1973	0.663	0.47	0.261	0.04595	0.430	0.0462	0.653	0.2481	0.586
13	rs4942587	17	rs1487971	0.2215	0.547	-0.0239	0.944	0.00175	0.971	0.0328	0.695	-0.2501	0.509
13	rs4941573	13	rs12584920	0.0409	0.934	0.0115	0.980	0.06611	0.297	0.09977	0.372	0.3372	0.505
13	rs4941573	13	rs1328684	0.2346	0.525	0.2668	0.435	0.04728	0.317	-0.01772	0.832	0.6521	0.084
13	rs4941573	13	rs2296973	-0.3968	0.335	-0.5274	0.165	-0.0154	0.770	0.01928	0.835	-0.2889	0.491
13	rs4941573	13	rs2070037	-0.3686	0.416	-0.4654	0.265	-0.0034	0.953	0.07305	0.474	-0.2519	0.583
13	rs4941573	13	rs9534511	-0.1357	0.667	-0.0081	0.978	0.03003	0.458	-0.00994	0.889	0.07027	0.828
13	rs4941573	13	rs6313	-0.049	0.871	-0.046	0.869	-0.0342	0.376	0.00067	0.992	-0.3174	0.305
13	rs4941573	13	rs6312	-0.5738	0.524	-0.3998	0.630	-0.0397	0.731	-0.17	0.404	-0.27	0.769
13	rs4941573	13	rs6306	-0.2476	0.739	0.024	0.972	0.1574	0.099	0.205	0.223	-0.4619	0.546
13	rs4941573	17	rs1906451	-0.3782	0.223	-0.3465	0.229	-0.1152	0.004	-0.0598	0.395	0.1625	0.615
13	rs4941573	17	rs1042173	-0.4522	0.146	-0.3965	0.169	-0.1261	0.002	-0.08168	0.249	0.1212	0.707
13	rs4941573	17	rs12449783	-0.4609	0.140	-0.4122	0.154	-0.1266	0.002	-0.08326	0.240	0.1236	0.702
13	rs4941573	17	rs3794808	-0.6404	0.044	-0.6919	0.020	-0.1458	0.000	-0.1177	0.106	-0.1129	0.734
13	rs4941573	17	rs4583306	-0.6224	0.049	-0.7716	0.009	-0.1343	0.001	-0.09982	0.166	-0.2997	0.361
13	rs4941573	17	rs140700	0.6513	0.214	-0.5239	0.285	-0.0166	0.807	0.08582	0.472	-0.1923	0.716
13	rs4941573	17	rs6354	0.2311	0.532	0.1861	0.591	-0.0022	0.963	0.03746	0.658	0.2121	0.577
13	rs4941573	17	rs2020936	0.2918	0.427	0.2618	0.446	0.00779	0.870	0.07035	0.403	0.2417	0.522
13	rs4941573	17	rs2066713	0.424	0.186	0.5877	0.050	0.1186	0.004	0.05729	0.432	-0.0165	0.961
13	rs4941573	17	rs4251417	-0.2525	0.622	-0.5586	0.237	-0.1142	0.079	-0.06774	0.555	-0.3736	0.472
13	rs4941573	17	rs8071667	0.3601	0.333	0.2471	0.478	0.00097	0.984	0.06511	0.445	0.3835	0.316
13	rs4941573	17	rs1487971	0.2132	0.502	0.2774	0.347	0.02521	0.536	0.07828	0.275	-0.1717	0.598
13	rs12584920	13	rs1328684	-0.2727	0.594	-0.17	0.719	-0.1191	0.069	-0.09798	0.398	-0.2768	0.595
13	rs12584920	13	rs2296973	0.0757	0.874	0.0502	0.910	0.02468	0.689	0.02288	0.834	-0.3112	0.519
13	rs12584920	13	rs2070037	0.2569	0.601	0.0873	0.848	0.0389	0.539	-0.02502	0.823	-0.1332	0.787
13	rs12584920	13	rs9534511	0.0008	0.999	-0.3778	0.379	-0.0118	0.843	0.01841	0.861	-0.5095	0.282
13	rs12584920	13	rs6313	-0.0779	0.875	0.0235	0.959	0.0671	0.288	0.09184	0.411	0.3614	0.474
13	rs12584920	13	rs6312	-0.8976	0.380	-0.9076	0.342	-0.0994	0.457	0.1163	0.622	-2.166	0.046
13	rs12584920	13	rs6306	0.9141	0.346	0.4713	0.600	-0.047	0.709	-0.503	0.024	-0.1855	0.845
13	rs12584920	17	rs1906451	-0.5524	0.151	-0.0037	0.992	0.04064	0.409	-0.05796	0.506	-0.2916	0.465
13	rs12584920	17	rs1042173	-0.5179	0.181	0.0294	0.935	0.0454	0.362	-0.04427	0.615	-0.2684	0.501

13	rs12584920	17	rs12449783	-0.5184	0.181	0.0252	0.944	0.04526	0.363	-0.04494	0.610	-0.2646	0.508
13	rs12584920	17	rs3794808	-0.3147	0.420	0.1923	0.597	0.06477	0.199	-0.03048	0.733	-0.0046	0.991
13	rs12584920	17	rs4583306	-0.2905	0.459	0.3397	0.350	0.07238	0.152	-0.03853	0.666	0.1674	0.680
13	rs12584920	17	rs140700	0.4905	0.456	1.418	0.019	0.01429	0.864	0.06076	0.681	0.1046	0.872
13	rs12584920	17	rs6354	0.3547	0.475	0.7446	0.104	0.04656	0.464	0.1041	0.354	0.5725	0.252
13	rs12584920	17	rs2020936	0.3363	0.498	0.6375	0.164	0.04467	0.482	0.08138	0.469	0.4621	0.354
13	rs12584920	17	rs2066713	0.1905	0.629	-0.8091	0.026	-0.0897	0.077	-0.0291	0.745	-0.3954	0.328
13	rs12584920	17	rs4251417	-0.2268	0.730	0.7109	0.241	0.07703	0.359	0.02587	0.862	0.1466	0.830
13	rs12584920	17	rs8071667	0.257	0.607	0.6294	0.172	0.04679	0.466	0.05717	0.614	0.3195	0.525
13	rs12584920	17	rs1487971	0.3504	0.363	-0.0594	0.868	-0.0055	0.912	0.0609	0.487	-0.1854	0.641
13	rs1328684	13	rs2296973	0.3841	0.291	0.1706	0.611	-0.0195	0.677	-0.02251	0.785	0.508	0.168
13	rs1328684	13	rs2070037	-0.0388	0.935	-0.0125	0.977	-0.0929	0.126	-0.07363	0.493	0.2123	0.662
13	rs1328684	13	rs9534511	0.2413	0.429	0.0408	0.885	-0.04	0.307	0.02262	0.744	0.2539	0.414
13	rs1328684	13	rs6313	0.3203	0.385	0.2919	0.392	0.04906	0.299	-0.03187	0.703	0.5642	0.135
13	rs1328684	13	rs6312	0.9795	0.258	0.7152	0.369	0.162	0.144	0.1096	0.576	1.804	0.040
13	rs1328684	13	rs6306	-0.6669	0.372	-0.4926	0.476	-0.1883	0.049	0.03663	0.829	0.2948	0.698
13	rs1328684	17	rs1906451	0.7047	0.027	0.3418	0.247	0.0952	0.019	0.1099	0.128	-0.0921	0.778
13	rs1328684	17	rs1042173	0.7511	0.019	0.3655	0.215	0.1022	0.013	0.1212	0.095	-0.0681	0.834
13	rs1328684	17	rs12449783	0.7649	0.017	0.3871	0.191	0.1035	0.012	0.1238	0.089	-0.0734	0.822
13	rs1328684	17	rs3794808	0.8323	0.011	0.5626	0.065	0.1084	0.010	0.1577	0.035	0.04357	0.897
13	rs1328684	17	rs4583306	0.7585	0.020	0.4882	0.105	0.07637	0.068	0.1224	0.098	0.09563	0.773
13	rs1328684	17	rs140700	-0.6954	0.171	-0.3285	0.477	-0.0068	0.916	-0.07544	0.505	0.2897	0.559
13	rs1328684	17	rs6354	-0.448	0.243	-0.6053	0.084	-0.0371	0.447	-0.1064	0.216	-0.5589	0.144
13	rs1328684	17	rs2020936	-0.4984	0.193	-0.6177	0.077	-0.045	0.354	-0.1256	0.143	-0.5262	0.168
13	rs1328684	17	rs2066713	-0.4912	0.131	-0.0465	0.877	-0.0511	0.220	-0.03076	0.676	0.329	0.322
13	rs1328684	17	rs4251417	0.2888	0.566	0.2054	0.658	0.05629	0.382	0.06188	0.587	0.1075	0.833
13	rs1328684	17	rs8071667	-0.5174	0.179	-0.587	0.097	-0.0344	0.485	-0.1009	0.247	-0.5868	0.130
13	rs1328684	17	rs1487971	-0.3215	0.316	-0.2807	0.342	-0.0255	0.532	-0.08248	0.253	0.3573	0.272
13	rs2296973	13	rs2070037	0.3334	0.432	0.4673	0.232	0.05894	0.280	0.02551	0.791	-0.0513	0.905
13	rs2296973	13	rs9534511	0.4199	0.275	0.1692	0.634	-0.0092	0.853	0.09888	0.257	0.06073	0.878
13	rs2296973	13	rs6313	-0.4814	0.245	-0.5229	0.171	-0.0189	0.721	-0.02829	0.763	-0.3656	0.387
13	rs2296973	13	rs6312	0.3045	0.741	0.5734	0.502	-0.0607	0.611	0.00558	0.979	0.1642	0.864
13	rs2296973	13	rs6306	0.4493	0.594	-0.193	0.805	-0.0022	0.984	-0.2808	0.146	-0.5031	0.559
13	rs2296973	17	rs1906451	-0.0643	0.849	0.338	0.283	0.0928	0.032	-0.03307	0.667	-0.2175	0.537
13	rs2296973	17	rs1042173	-0.0196	0.954	0.3783	0.231	0.09927	0.023	-0.01725	0.824	-0.1901	0.589
13	rs2296973	17	rs12449783	-0.0204	0.952	0.3737	0.237	0.09923	0.023	-0.01801	0.816	-0.1859	0.598
13	rs2296973	17	rs3794808	0.1059	0.758	0.4548	0.158	0.1048	0.018	-0.01571	0.842	-0.0164	0.964

13	rs2296973	17	rs4583306	0.265	0.446	0.6733	0.037	0.1216	0.007	-0.03331	0.674	0.1363	0.704
13	rs2296973	17	rs140700	-0.192	0.738	1.077	0.042	-0.0314	0.668	0.01721	0.894	-0.1089	0.851
13	rs2296973	17	rs6354	-0.035	0.933	0.3089	0.423	0.02561	0.632	0.1503	0.112	0.02761	0.948
13	rs2296973	17	rs2020936	-0.0772	0.852	0.2123	0.580	0.02111	0.692	0.1254	0.183	-0.0512	0.904
13	rs2296973	17	rs2066713	-0.1118	0.740	-0.7357	0.019	-0.1055	0.015	-0.05179	0.501	0.04812	0.892
13	rs2296973	17	rs4251417	-0.2875	0.625	0.5821	0.288	0.08743	0.248	0.04072	0.761	0.1511	0.803
13	rs2296973	17	rs8071667	-0.0998	0.811	0.2505	0.516	0.02093	0.697	0.1181	0.214	-0.1255	0.768
13	rs2296973	17	rs1487971	0.0641	0.850	0.0556	0.860	-0.0195	0.656	0.05671	0.463	0.1142	0.746
13	rs2070037	13	rs9534511	0.4004	0.352	0.2114	0.594	0.02049	0.710	0.07025	0.470	0.01594	0.971
13	rs2070037	13	rs6313	-0.448	0.328	-0.5468	0.195	-0.0126	0.830	0.04084	0.693	-0.3108	0.504
13	rs2070037	13	rs6312	-0.5019	0.598	-0.462	0.602	-0.1488	0.229	0.04196	0.848	-2.072	0.041
13	rs2070037	13	rs6306	1.281	0.159	0.6161	0.465	0.04497	0.703	-0.2773	0.184	0.2504	0.783
13	rs2070037	17	rs1906451	-0.3965	0.275	0.0718	0.831	0.0463	0.318	-0.09032	0.272	-0.1432	0.703
13	rs2070037	17	rs1042173	-0.3608	0.323	0.1054	0.755	0.05111	0.276	-0.07614	0.359	-0.1203	0.748
13	rs2070037	17	rs12449783	-0.3612	0.324	0.1012	0.764	0.05098	0.277	-0.07686	0.354	-0.1161	0.757
13	rs2070037	17	rs3794808	-0.1786	0.628	0.2366	0.490	0.06703	0.158	-0.06102	0.468	0.04551	0.905
13	rs2070037	17	rs4583306	-0.1212	0.742	0.4422	0.193	0.09424	0.046	-0.04871	0.560	0.2183	0.563
13	rs2070037	17	rs140700	0.2257	0.712	1.193	0.033	0.02723	0.725	0.02772	0.840	-0.1453	0.810
13	rs2070037	17	rs6354	0.2794	0.535	0.5651	0.174	0.04649	0.421	0.09925	0.331	0.4477	0.324
13	rs2070037	17	rs2020936	0.2597	0.563	0.4759	0.252	0.04381	0.448	0.07798	0.445	0.3612	0.425
13	rs2070037	17	rs2066713	0.0598	0.870	-0.7179	0.034	-0.0946	0.044	-0.01047	0.900	-0.3351	0.373
13	rs2070037	17	rs4251417	-0.1167	0.852	0.581	0.315	0.08244	0.304	-0.01182	0.934	0.3646	0.575
13	rs2070037	17	rs8071667	0.1906	0.674	0.4428	0.289	0.03849	0.508	0.04974	0.629	0.2414	0.596
13	rs2070037	17	rs1487971	0.2159	0.558	-0.0407	0.905	0.0005	0.992	0.03129	0.709	-0.2716	0.474
13	rs9534511	13	rs6313	-0.174	0.590	-0.0285	0.924	0.0256	0.536	-0.06183	0.397	0.03028	0.927
13	rs9534511	13	rs6312	0.5893	0.500	-0.2241	0.781	-0.1588	0.158	0.1955	0.325	0.08137	0.928
13	rs9534511	13	rs6306	0.4909	0.494	-0.3683	0.580	-0.0678	0.463	-0.07908	0.628	-0.0231	0.975
13	rs9534511	17	rs1906451	0.5064	0.095	0.5373	0.058	0.1146	0.003	-0.00936	0.893	-0.2877	0.366
13	rs9534511	17	rs1042173	0.551	0.070	0.565	0.046	0.122	0.002	0.01019	0.884	-0.2381	0.453
13	rs9534511	17	rs12449783	0.5624	0.065	0.5849	0.039	0.1227	0.002	0.01191	0.865	-0.2417	0.447
13	rs9534511	17	rs3794808	0.6451	0.036	0.7086	0.014	0.1264	0.002	0.06757	0.341	-0.1334	0.680
13	rs9534511	17	rs4583306	0.6375	0.037	0.7563	0.008	0.1174	0.003	0.03445	0.624	0.06548	0.837
13	rs9534511	17	rs140700	-0.5043	0.294	0.5439	0.230	-0.0949	0.127	0.04182	0.704	0.3107	0.521
13	rs9534511	17	rs6354	-0.3477	0.316	-0.2117	0.516	-0.0214	0.636	0.02758	0.730	-0.1958	0.585
13	rs9534511	17	rs2020936	-0.3922	0.256	-0.2789	0.390	-0.0283	0.529	0.00156	0.984	-0.2313	0.517
13	rs9534511	17	rs2066713	-0.2391	0.428	-0.514	0.069	-0.0801	0.040	-0.04771	0.489	0.2042	0.519
13	rs9534511	17	rs4251417	0.3594	0.477	0.7392	0.113	0.07653	0.234	-0.04997	0.660	0.4204	0.416

13	rs9534511	17	rs8071667	-0.4105	0.237	-0.2502	0.442	-0.0215	0.634	0.00871	0.913	-0.3472	0.332
13	rs9534511	17	rs1487971	-0.0694	0.815	0.0851	0.759	-0.0435	0.258	0.03114	0.646	0.3319	0.280
13	rs6313	13	rs6312	-0.6152	0.496	-0.2899	0.728	-0.0159	0.891	-0.285	0.164	-0.4357	0.636
13	rs6313	13	rs6306	-0.0995	0.895	0.1143	0.870	0.1619	0.095	0.18	0.294	-0.3225	0.679
13	rs6313	17	rs1906451	-0.3474	0.267	-0.3555	0.220	-0.1206	0.003	-0.02167	0.761	0.3029	0.352
13	rs6313	17	rs1042173	-0.4243	0.177	-0.4076	0.160	-0.1317	0.001	-0.0447	0.533	0.2593	0.424
13	rs6313	17	rs12449783	-0.433	0.170	-0.4233	0.146	-0.1323	0.001	-0.04624	0.519	0.262	0.420
13	rs6313	17	rs3794808	-0.6168	0.054	-0.7038	0.019	-0.1499	0.000	-0.09059	0.218	0.00712	0.983
13	rs6313	17	rs4583306	-0.567	0.075	-0.7616	0.010	-0.1374	0.001	-0.06021	0.408	-0.176	0.593
13	rs6313	17	rs140700	0.5514	0.294	-0.5435	0.269	-0.0223	0.742	0.05528	0.644	-0.3182	0.548
13	rs6313	17	rs6354	0.2802	0.452	0.2642	0.448	0.00413	0.932	0.04135	0.628	0.1654	0.666
13	rs6313	17	rs2020936	0.3419	0.356	0.3403	0.325	0.01437	0.765	0.07505	0.376	0.1954	0.607
13	rs6313	17	rs2066713	0.34	0.291	0.5387	0.074	0.1182	0.004	0.02197	0.765	-0.104	0.756
13	rs6313	17	rs4251417	-0.2551	0.620	-0.4762	0.314	-0.1214	0.063	-0.01604	0.890	-0.2063	0.691
13	rs6313	17	rs8071667	0.3974	0.286	0.3393	0.330	0.00602	0.901	0.0622	0.467	0.3457	0.367
13	rs6313	17	rs1487971	0.1565	0.623	0.2275	0.441	0.03491	0.392	0.06618	0.359	-0.216	0.508
13	rs6312	13	rs6306	-2.438	0.157	-3.204	0.045	-0.176	0.432	-0.2645	0.504	-3.755	0.060
13	rs6312	17	rs1906451	1.04	0.110	0.9107	0.138	0.1876	0.025	0.1356	0.360	-0.5996	0.385
13	rs6312	17	rs1042173	1.1	0.093	0.9613	0.119	0.1979	0.019	0.1496	0.318	-0.5735	0.406
13	rs6312	17	rs12449783	1.096	0.094	0.9537	0.122	0.1979	0.019	0.1489	0.320	-0.5703	0.409
13	rs6312	17	rs3794808	1.003	0.142	0.9469	0.144	0.1787	0.044	0.1345	0.392	-0.2924	0.684
13	rs6312	17	rs4583306	1.531	0.031	1.102	0.100	0.1501	0.103	0.03096	0.849	-0.3399	0.644
13	rs6312	17	rs140700	-1.376	0.184	-0.0921	0.923	-0.1795	0.175	0.00712	0.976	-0.1005	0.922
13	rs6312	17	rs6354	-1.168	0.159	-0.6473	0.396	-0.0432	0.685	0.2701	0.151	-1.425	0.096
13	rs6312	17	rs2020936	-1.28	0.121	-0.7187	0.345	-0.0528	0.619	0.2447	0.191	-1.441	0.091
13	rs6312	17	rs2066713	-0.5451	0.405	-0.4132	0.501	-0.0975	0.252	-0.1605	0.286	1.345	0.052
13	rs6312	17	rs4251417	-0.8352	0.420	0.1577	0.872	0.03988	0.766	0.108	0.648	-0.4803	0.676
13	rs6312	17	rs8071667	-1.148	0.167	-0.464	0.544	-0.0368	0.731	0.3098	0.101	-1.327	0.123
13	rs6312	17	rs1487971	-0.2848	0.680	0.378	0.555	-0.0771	0.390	0.1206	0.446	1.477	0.036
13	rs6306	17	rs1906451	-0.4914	0.394	-0.3914	0.460	0.01341	0.854	-0.2784	0.031	-0.2012	0.735
13	rs6306	17	rs1042173	-0.471	0.417	-0.3695	0.487	0.01799	0.807	-0.2662	0.041	-0.18	0.762
13	rs6306	17	rs12449783	-0.4594	0.429	-0.3647	0.493	0.01778	0.810	-0.2665	0.041	-0.1768	0.766
13	rs6306	17	rs3794808	-0.2538	0.661	-0.3158	0.559	0.03924	0.599	-0.3326	0.012	-0.2079	0.731
13	rs6306	17	rs4583306	-0.4224	0.474	-0.317	0.562	0.0439	0.562	-0.3404	0.011	-0.3851	0.530
13	rs6306	17	rs140700	0.0525	0.952	1.842	0.021	-0.0069	0.951	0.3208	0.101	-0.2751	0.750
13	rs6306	17	rs6354	0.4146	0.556	1.418	0.027	0.03188	0.722	0.2353	0.137	-0.1979	0.779
13	rs6306	17	rs2020936	0.4515	0.522	1.391	0.031	0.03356	0.708	0.2192	0.167	-0.3009	0.669

13	rs6306	17	rs2066713	0.2072	0.729	-1.018	0.062	-0.0735	0.335	0.1247	0.355	0.4119	0.507
13	rs6306	17	rs4251417	-0.941	0.333	0.145	0.872	0.0978	0.431	-0.2375	0.280	-1.98	0.069
13	rs6306	17	rs8071667	0.2669	0.706	1.274	0.048	0.03254	0.718	0.2485	0.118	-0.3393	0.631
13	rs6306	17	rs1487971	0.3831	0.500	0.1895	0.718	-0.082	0.262	0.1221	0.345	0.09446	0.873
17	rs1906451	17	rs1042173	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	rs1906451	17	rs12449783	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	rs1906451	17	rs3794808	-0.3953	0.188	-0.1551	0.581	0.1173	0.002	0.08133	0.234	-0.2793	0.373
17	rs1906451	17	rs4583306	-0.5147	0.091	-0.236	0.403	0.1032	0.008	0.05642	0.412	-0.3724	0.240
17	rs1906451	17	rs140700	-0.0981	0.883	0.373	0.544	-0.0729	0.386	-0.2006	0.178	-0.6127	0.357
17	rs1906451	17	rs6354	0.3101	0.488	0.3317	0.424	0.0346	0.545	-0.04457	0.660	0.5537	0.227
17	rs1906451	17	rs2020936	0.257	0.565	0.2486	0.549	0.03306	0.563	-0.04886	0.629	0.4663	0.309
17	rs1906451	17	rs2066713	0.6218	0.053	0.1682	0.574	-0.1459	0.000	-0.04682	0.522	0.3083	0.360
17	rs1906451	17	rs4251417	-0.6629	0.312	-0.0987	0.872	0.1757	0.035	0.107	0.468	-0.2292	0.745
17	rs1906451	17	rs8071667	0.305	0.498	0.2712	0.516	0.0323	0.575	-0.08563	0.402	0.4759	0.304
17	rs1906451	17	rs1487971	0.5088	0.111	0.381	0.201	-0.0833	0.042	-0.03141	0.665	0.44	0.189
17	rs1042173	17	rs12449783	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	rs1042173	17	rs3794808	-0.3661	0.227	-0.1371	0.627	0.1187	0.002	0.08887	0.198	-0.2698	0.389
17	rs1042173	17	rs4583306	-0.4841	0.114	-0.218	0.442	0.105	0.008	0.06417	0.356	-0.3621	0.253
17	rs1042173	17	rs140700	-0.1359	0.840	0.3726	0.548	-0.0657	0.442	-0.1869	0.216	-0.5428	0.416
17	rs1042173	17	rs6354	0.3536	0.431	0.3525	0.396	0.0491	0.395	-0.03337	0.743	0.5871	0.198
17	rs1042173	17	rs2020936	0.3004	0.503	0.2698	0.516	0.04762	0.409	-0.03764	0.712	0.5012	0.272
17	rs1042173	17	rs2066713	0.5702	0.078	0.1352	0.652	-0.1512	0.000	-0.06197	0.400	0.2792	0.407
17	rs1042173	17	rs4251417	-0.632	0.338	-0.0703	0.909	0.1793	0.033	0.1173	0.431	-0.2118	0.764
17	rs1042173	17	rs8071667	0.3642	0.422	0.3224	0.442	0.0481	0.410	-0.07116	0.490	0.5079	0.272
17	rs1042173	17	rs1487971	0.4994	0.121	0.3675	0.219	-0.0818	0.048	-0.03389	0.644	0.437	0.192
17	rs12449783	17	rs3794808	-0.3751	0.216	-0.1375	0.626	0.1193	0.002	0.08998	0.192	-0.2611	0.404
17	rs12449783	17	rs4583306	-0.4928	0.108	-0.2174	0.443	0.1056	0.007	0.06535	0.347	-0.3535	0.265
17	rs12449783	17	rs140700	-0.0708	0.916	0.3956	0.522	-0.0692	0.416	-0.1905	0.205	-0.5919	0.374
17	rs12449783	17	rs6354	0.3915	0.385	0.3884	0.351	0.04846	0.402	-0.0324	0.751	0.5657	0.217
17	rs12449783	17	rs2020936	0.3384	0.452	0.3054	0.463	0.04697	0.416	-0.03667	0.720	0.4789	0.296
17	rs12449783	17	rs2066713	0.5674	0.080	0.1261	0.675	-0.1517	0.000	-0.06321	0.391	0.282	0.403
17	rs12449783	17	rs4251417	-0.6364	0.335	-0.0769	0.900	0.1793	0.033	0.1167	0.433	-0.2088	0.767
17	rs12449783	17	rs8071667	0.3868	0.394	0.3284	0.434	0.04634	0.427	-0.07347	0.476	0.4881	0.291
17	rs12449783	17	rs1487971	0.5118	0.112	0.382	0.202	-0.0819	0.048	-0.03293	0.653	0.4363	0.193
17	rs3794808	17	rs4583306	-0.7388	0.015	-0.2568	0.365	0.08615	0.028	0.0527	0.448	-0.3537	0.264
17	rs3794808	17	rs140700	0.2528	0.710	0.2688	0.669	-0.0751	0.387	-0.106	0.490	-0.2125	0.755

17	rs3794808	17	rs6354	0.5616	0.220	0.295	0.488	0.03247	0.582	-0.00861	0.934	0.7394	0.115
17	rs3794808	17	rs2020936	0.5077	0.268	0.2092	0.623	0.03142	0.594	-0.01211	0.908	0.6467	0.168
17	rs3794808	17	rs2066713	0.8259	0.017	0.2641	0.412	-0.1219	0.006	-0.0561	0.477	0.3798	0.287
17	rs3794808	17	rs4251417	-0.891	0.183	0.0272	0.965	0.1791	0.036	0.07704	0.611	0.01592	0.982
17	rs3794808	17	rs8071667	0.5625	0.223	0.2287	0.594	0.03564	0.549	-0.02977	0.777	0.6608	0.163
17	rs3794808	17	rs1487971	0.7618	0.024	0.4202	0.184	-0.0637	0.145	0.02645	0.732	0.5719	0.104
17	rs4583306	17	rs140700	0.2896	0.667	0.6684	0.282	0.00501	0.953	-0.1129	0.455	-0.0383	0.954
17	rs4583306	17	rs6354	0.4078	0.396	0.3913	0.378	0.02594	0.673	-0.08217	0.449	0.5071	0.300
17	rs4583306	17	rs2020936	0.357	0.458	0.2987	0.502	0.02599	0.673	-0.08366	0.442	0.4008	0.414
17	rs4583306	17	rs2066713	0.748	0.034	0.2477	0.449	-0.1231	0.006	-0.02513	0.753	0.3542	0.330
17	rs4583306	17	rs4251417	-0.9567	0.158	-0.095	0.879	0.1751	0.043	0.07748	0.612	0.1004	0.889
17	rs4583306	17	rs8071667	0.446	0.358	0.3431	0.444	0.02902	0.641	-0.1107	0.314	0.4031	0.416
17	rs4583306	17	rs1487971	0.665	0.056	0.5082	0.116	-0.0553	0.217	0.04869	0.539	0.5044	0.162
17	rs140700	17	rs6354	-0.4764	0.483	0.0046	0.994	0.00297	0.973	0.3326	0.029	0.00824	0.990
17	rs140700	17	rs2020936	-0.2215	0.744	0.1822	0.771	0.02178	0.800	0.4055	0.008	0.1679	0.800
17	rs140700	17	rs2066713	0.1268	0.851	-0.3231	0.605	0.02762	0.748	-0.3065	0.043	0.07407	0.912
17	rs140700	17	rs4251417	-1.263	0.266	-0.2216	0.831	-0.1225	0.393	-0.6279	0.013	-1.313	0.253
17	rs140700	17	rs8071667	-0.1013	0.878	0.263	0.670	0.02332	0.782	0.4241	0.004	0.1318	0.840
17	rs140700	17	rs1487971	-0.7322	0.153	-0.8464	0.073	0.05365	0.408	-0.00108	0.993	-0.7675	0.125
17	rs6354	17	rs2020936	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	rs6354	17	rs2066713	-0.2375	0.621	-0.1496	0.738	0.01623	0.792	-0.1242	0.253	-0.2838	0.561
17	rs6354	17	rs4251417	-0.1283	0.884	1.004	0.211	-0.1097	0.325	-0.09567	0.627	0.804	0.363
17	rs6354	17	rs8071667	-0.226	0.631	-0.1849	0.671	-0.0442	0.465	0.1632	0.127	-0.0033	0.995
17	rs6354	17	rs1487971	0.0767	0.837	-0.2061	0.552	-0.0151	0.753	-0.01976	0.815	-0.7684	0.042
17	rs2020936	17	rs2066713	-0.3159	0.516	-0.2129	0.636	0.01925	0.757	-0.1528	0.165	-0.4383	0.372
17	rs2020936	17	rs4251417	-0.292	0.741	1.056	0.191	-0.109	0.331	-0.08712	0.660	0.8671	0.329
17	rs2020936	17	rs8071667	-0.0098	0.983	-0.0205	0.962	-0.0284	0.638	0.2312	0.030	0.1738	0.714
17	rs2020936	17	rs1487971	0.0288	0.938	-0.227	0.510	-0.0174	0.715	-0.04089	0.627	-0.8235	0.028
17	rs2066713	17	rs4251417	1.236	0.076	-0.6966	0.279	-0.1296	0.145	-0.01899	0.904	-0.4289	0.551
17	rs2066713	17	rs8071667	-0.3296	0.502	-0.196	0.666	0.01237	0.844	-0.152	0.171	-0.3852	0.437
17	rs2066713	17	rs1487971	-0.8723	0.006	-0.3653	0.213	0.03964	0.329	-0.01084	0.880	0.101	0.758
17	rs4251417	17	rs8071667	-0.2166	0.807	1.087	0.181	-0.1086	0.336	-0.1565	0.432	0.665	0.458
17	rs4251417	17	rs1487971	0.8994	0.192	0.0301	0.962	-0.0405	0.647	0.04538	0.771	0.3234	0.649
17	rs8071667	17	rs1487971	0.0022	0.995	-0.2591	0.456	-0.0193	0.689	-0.02753	0.746	-0.7741	0.042

CHR1: chromosome of SNP1
CHR2: chromosome of SNP2
P values are uncorrected

Supplementary Table3: All possible interactions between SNPs genotyped in the New Mood cohort

				BSI Depression Score		BSI Anxiety Score		Big5 Neuroticism Score	
CHR1	SNP1	CHR2	SNP2	β	p value	β	p value	β	p value
13	rs3125	13	rs6314	0.3983	0.053	0.2293	0.195	0.2733	0.121
13	rs3125	13	rs9316232	0.06079	0.542	0.1041	0.225	0.07467	0.382
13	rs3125	13	rs2296972	-0.02654	0.799	0.02948	0.741	0.02089	0.814
13	rs3125	13	rs2770296	0.1029	0.286	-0.01333	0.872	-0.003767	0.964
13	rs3125	13	rs1928040	-0.07334	0.386	-0.04478	0.536	-0.05477	0.444
13	rs3125	13	rs731779	0.06033	0.589	-0.04014	0.676	0.01495	0.875
13	rs3125	13	rs985934	0.03617	0.693	-0.07428	0.344	-0.06225	0.425
13	rs3125	13	rs6310	0.0825	0.690	0.01788	0.920	0.0115	0.948
13	rs3125	13	rs6311	-0.06207	0.475	-0.005663	0.940	-0.03057	0.680
13	rs3125	17	rs1042173	0.1406	0.100	0.08713	0.236	0.08641	0.236
13	rs3125	17	rs3794808	0.1216	0.140	0.05022	0.478	0.05327	0.449
13	rs3125	17	rs140700	0.2897	0.060	0.2339	0.077	0.2028	0.123
13	rs3125	17	rs2020942	-0.1495	0.081	-0.08618	0.243	-0.09771	0.182
13	rs3125	17	rs6354	0.06698	0.540	0.104	0.267	0.05072	0.587
13	rs3125	17	rs2020934	-0.009939	0.903	-0.04392	0.530	-0.08428	0.225
13	rs3125	17	rs25531	0.1593	0.358	0.2541	0.088	0.03897	0.793
13	rs3125	17	5-HTT_LPR	-0.04471	0.586	-0.04275	0.544	-0.05195	0.459
13	rs6314	13	rs9316232	-0.002244	0.984	-0.02856	0.761	-0.04346	0.645
13	rs6314	13	rs2296972	-0.01534	0.871	-0.07519	0.355	-0.05014	0.539
13	rs6314	13	rs2770296	0.2699	0.012	0.2455	0.008	0.1805	0.054
13	rs6314	13	rs1928040	-0.1673	0.069	-0.1745	0.028	-0.03763	0.633
13	rs6314	13	rs731779	0.2406	0.045	0.1936	0.062	0.1194	0.251
13	rs6314	13	rs985934	0.1623	0.081	0.2097	0.009	0.08965	0.264
13	rs6314	13	rs6310	0.3066	0.135	0.4541	0.010	0.04231	0.811
13	rs6314	13	rs6311	-0.09036	0.310	-0.1364	0.076	-0.04439	0.565
13	rs6314	17	rs1042173	-0.03133	0.728	-0.0294	0.706	-0.1255	0.107
13	rs6314	17	rs3794808	-0.05528	0.528	-0.03418	0.651	-0.1324	0.080
13	rs6314	17	rs140700	-0.06335	0.610	-0.0322	0.764	-0.05172	0.631
13	rs6314	17	rs2020942	-0.05922	0.549	-0.00981	0.909	0.06086	0.478
13	rs6314	17	rs6354	0.07291	0.469	0.0332	0.703	0.1088	0.213
13	rs6314	17	rs2020934	0.1612	0.071	0.02404	0.755	-0.01059	0.892
13	rs6314	17	rs25531	0.05116	0.747	0.0791	0.563	0.0558	0.685

13	rs6314	17	5-HTT_LPR	0.1286	0.147	0.01469	0.848	0.008302	0.914
13	rs9316232	13	rs2296972	0.04701	0.361	-0.04338	0.329	-0.01535	0.731
13	rs9316232	13	rs2770296	0.1243	0.016	0.1216	0.006	0.1348	0.003
13	rs9316232	13	rs1928040	-0.07512	0.106	-0.07275	0.070	-0.0824	0.039
13	rs9316232	13	rs731779	0.1933	0.002	0.1611	0.003	0.2079	0.000
13	rs9316232	13	rs985934	0.1236	0.009	0.1583	0.000	0.1278	0.002
13	rs9316232	13	rs6310	0.143	0.286	0.2194	0.056	0.2865	0.013
13	rs9316232	13	rs6311	-0.07362	0.138	-0.08085	0.060	-0.07751	0.075
13	rs9316232	17	rs1042173	-0.1225	0.007	-0.03997	0.315	-0.05499	0.167
13	rs9316232	17	rs3794808	-0.111	0.014	-0.0261	0.504	-0.0321	0.413
13	rs9316232	17	rs140700	0.05324	0.485	0.04876	0.460	-0.0954	0.150
13	rs9316232	17	rs2020942	0.07736	0.100	0.01029	0.801	0.03074	0.453
13	rs9316232	17	rs6354	0.08446	0.206	0.07207	0.211	0.05689	0.324
13	rs9316232	17	rs2020934	-0.02465	0.644	0.005645	0.903	0.01696	0.714
13	rs9316232	17	rs25531	-0.001137	0.989	-0.01856	0.801	-0.0695	0.346
13	rs9316232	17	5-HTT_LPR	-0.05855	0.199	-0.00254	0.949	0.02414	0.543
13	rs2296972	13	rs2770296	0.06954	0.187	0.06347	0.163	0.1002	0.028
13	rs2296972	13	rs1928040	-0.04194	0.380	-0.02082	0.613	-0.06661	0.105
13	rs2296972	13	rs731779	0.1526	0.018	0.1008	0.071	0.1693	0.003
13	rs2296972	13	rs985934	0.08484	0.085	0.1172	0.006	0.1229	0.004
13	rs2296972	13	rs6310	0.09944	0.488	0.1562	0.203	0.2865	0.020
13	rs2296972	13	rs6311	-0.05153	0.315	-0.0488	0.272	-0.06348	0.158
13	rs2296972	17	rs1042173	-0.1151	0.015	-0.03236	0.432	-0.05467	0.186
13	rs2296972	17	rs3794808	-0.1162	0.014	-0.02373	0.560	-0.042	0.305
13	rs2296972	17	rs140700	0.08389	0.277	0.06133	0.357	-0.07914	0.237
13	rs2296972	17	rs2020942	0.03964	0.415	-0.003399	0.936	0.04547	0.282
13	rs2296972	17	rs6354	0.1268	0.064	0.06446	0.275	0.06984	0.238
13	rs2296972	17	rs2020934	-0.03439	0.529	-0.01513	0.748	-0.03121	0.509
13	rs2296972	17	rs25531	0.02239	0.796	0.005441	0.942	-0.04736	0.529
13	rs2296972	17	5-HTT_LPR	-0.0532	0.260	-0.006685	0.870	-0.001306	0.975
13	rs2770296	13	rs1928040	0.2019	0.001	0.1853	0.000	0.09225	0.082
13	rs2770296	13	rs731779	-0.09581	0.178	-0.1099	0.075	-0.08264	0.184
13	rs2770296	13	rs985934	-0.09749	0.071	-0.1133	0.015	-0.107	0.022
13	rs2770296	13	rs6310	0.06878	0.679	-0.03015	0.833	-0.01261	0.930
13	rs2770296	13	rs6311	0.171	0.007	0.1513	0.006	0.099	0.075
13	rs2770296	17	rs1042173	-0.03629	0.446	-0.03383	0.414	0.01731	0.677
13	rs2770296	17	rs3794808	-0.05013	0.289	-0.06211	0.129	-0.005016	0.903

13	rs2770296	17	rs140700	0.01944	0.816	0.03092	0.670	-0.05378	0.461
13	rs2770296	17	rs2020942	0.03718	0.460	0.05706	0.191	0.02761	0.530
13	rs2770296	17	rs6354	-0.01486	0.836	-0.02184	0.725	-0.05269	0.398
13	rs2770296	17	rs2020934	-0.1264	0.021	-0.06192	0.190	-0.01509	0.751
13	rs2770296	17	rs25531	0.05147	0.549	-0.02983	0.689	-0.101	0.178
13	rs2770296	17	5-HTT_LPR	-0.08443	0.081	-0.03257	0.437	0.0153	0.717
13	rs1928040	13	rs731779	0.2091	0.006	0.2057	0.002	0.1089	0.102
13	rs1928040	13	rs985934	0.09951	0.038	0.05884	0.156	0.004881	0.906
13	rs1928040	13	rs6310	-0.1242	0.499	-0.1271	0.420	-0.2432	0.120
13	rs1928040	13	rs6311	-0.1355	0.007	-0.1038	0.017	-0.09489	0.030
13	rs1928040	17	rs1042173	0.05496	0.210	0.005044	0.894	-0.05057	0.181
13	rs1928040	17	rs3794808	0.04693	0.281	0.01229	0.744	-0.04123	0.272
13	rs1928040	17	rs140700	-0.006208	0.935	0.006864	0.917	0.07897	0.227
13	rs1928040	17	rs2020942	-0.06315	0.166	-0.05177	0.189	-0.02023	0.607
13	rs1928040	17	rs6354	0.04861	0.454	0.07933	0.156	0.1233	0.026
13	rs1928040	17	rs2020934	0.07918	0.116	0.0559	0.198	0.006661	0.877
13	rs1928040	17	rs25531	0.05955	0.484	0.0785	0.288	0.06594	0.369
13	rs1928040	17	5-HTT_LPR	0.00484	0.915	-0.004286	0.913	-0.02954	0.448
13	rs731779	13	rs985934	-0.06564	0.360	-0.08308	0.182	-0.06329	0.311
13	rs731779	13	rs6310	0.2456	0.198	0.1585	0.333	0.2241	0.171
13	rs731779	13	rs6311	0.1824	0.018	0.1796	0.007	0.0735	0.277
13	rs731779	17	rs1042173	-0.007171	0.897	-0.03136	0.516	-0.003702	0.939
13	rs731779	17	rs3794808	-0.04401	0.423	-0.05849	0.220	-0.007818	0.871
13	rs731779	17	rs140700	0.1291	0.205	0.1262	0.154	0.07536	0.397
13	rs731779	17	rs2020942	-0.03396	0.567	-0.004337	0.933	-0.02833	0.586
13	rs731779	17	rs6354	0.05997	0.479	0.1063	0.146	0.05677	0.438
13	rs731779	17	rs2020934	-0.05278	0.390	-0.003703	0.945	0.02799	0.599
13	rs731779	17	rs25531	0.008801	0.931	-0.05338	0.547	-0.1283	0.149
13	rs731779	17	5-HTT_LPR	-0.06432	0.255	-0.03424	0.486	0.006029	0.903
13	rs985934	13	rs6310	-0.3114	0.088	-0.2401	0.125	-0.2421	0.121
13	rs985934	13	rs6311	0.08218	0.142	0.07095	0.145	0.03754	0.443
13	rs985934	17	rs1042173	-0.04915	0.278	-0.0167	0.671	0.03376	0.391
13	rs985934	17	rs3794808	-0.04143	0.357	-0.02242	0.565	0.02472	0.527
13	rs985934	17	rs140700	0.03933	0.607	0.02422	0.715	-0.03004	0.652
13	rs985934	17	rs2020942	0.04682	0.329	0.05249	0.206	-0.009002	0.829
13	rs985934	17	rs6354	-0.01508	0.819	-0.05967	0.294	-0.0235	0.680
13	rs985934	17	rs2020934	-0.1042	0.047	-0.04789	0.291	-0.0117	0.796

13	rs985934	17	rs25531	0.03008	0.717	0.03611	0.617	-0.003797	0.958
13	rs985934	17	5-HTT_LPR	-0.03611	0.429	-0.03358	0.396	0.009271	0.816
13	rs6310	13	rs6311	-0.03548	0.846	-0.01159	0.941	-0.1651	0.293
13	rs6310	17	rs1042173	0.171	0.193	0.1989	0.078	0.1894	0.093
13	rs6310	17	rs3794808	0.1846	0.142	0.2015	0.061	0.1314	0.224
13	rs6310	17	rs140700	0.06533	0.777	-0.066	0.738	-0.02917	0.883
13	rs6310	17	rs2020942	-0.1861	0.192	-0.05438	0.658	-0.08793	0.475
13	rs6310	17	rs6354	-0.09004	0.598	-0.3791	0.010	-0.2169	0.140
13	rs6310	17	rs2020934	0.1597	0.213	0.0771	0.483	0.1279	0.246
13	rs6310	17	rs25531	-0.358	0.166	-0.2896	0.192	-0.1695	0.446
13	rs6310	17	5-HTT_LPR	0.193	0.162	0.08895	0.452	0.04318	0.716
13	rs6311	17	rs1042173	0.0629	0.176	-0.009595	0.812	-0.0293	0.473
13	rs6311	17	rs3794808	0.03601	0.438	-0.01872	0.642	-0.04607	0.257
13	rs6311	17	rs140700	-0.04322	0.580	-0.03562	0.599	0.02753	0.688
13	rs6311	17	rs2020942	-0.07938	0.102	-0.04391	0.298	-7.46E-05	0.999
13	rs6311	17	rs6354	0.0441	0.483	0.06645	0.222	0.05264	0.334
13	rs6311	17	rs2020934	0.107	0.033	0.05573	0.200	0.02	0.647
13	rs6311	17	rs25531	0.02094	0.810	-0.005031	0.947	-0.007799	0.919
13	rs6311	17	5-HTT_LPR	0.03368	0.480	0.04575	0.267	0.0007182	0.986
17	rs1042173	17	rs3794808	0.008777	0.843	0.01204	0.753	-0.02173	0.571
17	rs1042173	17	rs140700	0.008936	0.930	-0.01634	0.853	-0.04826	0.584
17	rs1042173	17	rs2020942	0.009655	0.838	0.01951	0.634	0.05137	0.212
17	rs1042173	17	rs6354	-0.05301	0.500	-0.04445	0.512	-0.0285	0.674
17	rs1042173	17	rs2020934	-0.02225	0.656	-0.03897	0.368	-0.04943	0.254
17	rs1042173	17	rs25531	0.04399	0.625	0.008996	0.909	0.01548	0.844
17	rs1042173	17	5-HTT_LPR	0.02922	0.502	-0.02249	0.552	0.01386	0.715
17	rs3794808	17	rs140700	0.04338	0.672	-0.01185	0.894	-0.0005936	0.995
17	rs3794808	17	rs2020942	0.01913	0.709	-0.003714	0.933	0.0228	0.609
17	rs3794808	17	rs6354	-0.05077	0.479	-0.04463	0.471	-0.03966	0.523
17	rs3794808	17	rs2020934	0.0106	0.832	-0.003618	0.933	-0.03069	0.479
17	rs3794808	17	rs25531	0.01045	0.908	-0.01608	0.838	0.01681	0.832
17	rs3794808	17	5-HTT_LPR	0.03992	0.356	0.006417	0.864	0.02381	0.527
17	rs140700	17	rs2020942	-0.03095	0.763	0.04711	0.597	-0.0375	0.676
17	rs140700	17	rs6354	0.05686	0.630	-0.02889	0.777	0.08771	0.389
17	rs140700	17	rs2020934	0.001221	0.991	-0.01752	0.848	-0.08113	0.377
17	rs140700	17	rs25531	0.07919	0.441	0.05433	0.543	0.126	0.160

17	rs140700	17	5-HTT_LPR	-0.1417	0.087	-0.07178	0.318	-0.08448	0.241
17	rs2020942	17	rs6354	-0.007423	0.930	0.02543	0.729	-0.0009951	0.989
17	rs2020942	17	rs2020934	0.05183	0.324	0.0617	0.174	0.06121	0.179
17	rs2020942	17	rs25531	-0.05055	0.569	-0.0203	0.793	-0.08317	0.284
17	rs2020942	17	5-HTT_LPR	0.03303	0.467	0.0439	0.264	0.007715	0.845
17	rs6354	17	rs2020934	-0.06634	0.277	-0.06178	0.241	-0.02873	0.586
17	rs6354	17	rs25531	-0.03486	0.738	0.0213	0.812	0.04497	0.617
17	rs6354	17	5-HTT_LPR	-0.1337	0.034	-0.1022	0.060	-0.08239	0.130
17	rs2020934	17	rs25531	-0.05469	0.648	0.07865	0.446	-0.1218	0.240
17	rs2020934	17	5-HTT_LPR	0.02682	0.603	-0.01306	0.769	0.01475	0.741
17	rs25531	17	5-HTT_LPR	-0.1338	0.195	0.02324	0.795	-0.1371	0.128

CHR1: chromosome of SNP1
CHR2: chromosome of SNP2
P values are uncorrected

Supplementary Table 4: Change of tagging SNPs between 2005 and 2010

4a New Mood taggers and current taggers of the *SLC6A4* gene

New Mood taggers	**Current taggers**	**Position**
rs1042173		25549136
	rs9913038	25549802
	rs11080121	25552967
rs3794808	rs3794808	25555918
	rs10438799	25562664
rs140700		25567514
rs2020942		25571039
rs6354		25574023
	rs3783593	25576967
	rs9896947	25578193
rs2020934		25585585
	rs9903062	25586449
rs25531		25588471

4b. New Mood taggers and current taggers of the *HTR2A* gene

New Mood taggers	Current taggers	Position
rs3125	rs3125	46306851
rs6314	rs6314	46307034
	rs7994746	46314676
	rs3742278	46317577
	rs6561333	46318312
	rs9567736	46318983
	rs9567738	46319706
	rs7330636	46321592
	rs7338257	46322896
rs9316232	rs9316232	46324722
	rs658101	46325676
rs2296972	rs2296972	46326471
	rs9534495	46327228
	rs1885884	46328276
	rs2760351	46328315
	rs2760350	46328366
	rs17359763	46328602
	rs4942578	46330610
	rs6561335	46331497
	rs2405863	46332793
	rs4942580	46335317
	rs2760345	46336574
	rs2760346	46336738
rs2770296		46338560
	rs9567743	46338800
	rs2146672	46341868
	rs582854	46343877
	rs677702	46344498
	rs666693	46344916
rs1928040		46345236
	rs1410657	46346661
rs731779	rs731779	46350038
	rs1928038	46351730
rs985934		46353725
	rs927544	46354051
	rs17069089	46354999
	rs2760348	46355358
	rs17288723	46355693
	rs1328683	46356874
	rs660358	46358465
	rs9534505	46358744
	rs9534508	46361623
	rs12584920	46363037
	rs2296973	46364781

	rs1885882	46365461
	rs1805055	46367968
rs6310		46368352
rs6311		46369478