BIOPHYSICALLY DETAILED MODELLING OF THE FUNCTIONAL IMPACT OF GENE MUTATIONS ASSOCIATED WITH THE ‘SHORT QT SYNDROME’

A thesis submitted to The University of Manchester for the degree of Doctor of Philosophy in the Faculty of Engineering and Physical Sciences

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# Contents

Abstract .................................................................................................................................................. 16

Declaration .............................................................................................................................................. 17

Copyright Statement .............................................................................................................................. 18

Acknowledgements ................................................................................................................................. 19

Dedication .................................................................................................................................................. 20

1 Ion Channels and the Action Potential ............................................................................................... 21
   1.1 The Cardiac Action Potential ........................................................................................................ 21
   1.2 Morphology of the Action Potential in different Parts of the Heart ........................................... 23
      1.2.1 Sinoatrial Node ......................................................................................................................... 23
      1.2.2 Atria ........................................................................................................................................... 25
      1.2.3 Atrioventricular Node .............................................................................................................. 25
      1.2.4 His Bundle Purkinje System .................................................................................................... 25
      1.2.5 Ventricle ................................................................................................................................... 25
   1.3 Ionic Basis of the Resting and Action Potential .............................................................................. 30
      1.3.1 The Uniformly Polarised Cell ..................................................................................................... 30
      1.3.2 Electrical Equivalent Circuit of a Cell ...................................................................................... 31
      1.3.3 Equilibrium Potential ................................................................................................................ 33
   1.4 Properties of Ion channels .......................................................................................................... 34
      1.4.1 Ion Permeation .......................................................................................................................... 35
      1.4.2 Gating .......................................................................................................................................... 35
   1.5 Sodium channels ............................................................................................................................. 36
   1.6 Calcium channels .......................................................................................................................... 37
      1.6.1 L-type Calcium Channel ........................................................................................................... 38
      1.6.2 T-type Calcium Channel ........................................................................................................... 38
   1.7 Potassium channels ....................................................................................................................... 39
      1.7.1 Voltage-dependent Potassium Channels .................................................................................... 39
      1.7.1.1 The Transient Outward Current ($I_o$) ................................................................................... 39
1.7.1.2 The Slowly-activated Delayed Outward Rectifier Current (I_{Ks}) .............................................................................................................. 40
1.7.1.3 The Rapidly-activated Delayed Outward Rectifier Current (I_{Kr}) .............................................................................................................. 41
1.7.3.4 The Ultrarapidly-activated Delayed Outward Rectifier Current (I_{Kur}) .............................................................................................................. 41
1.7.2 The Inward Rectifier Potassium Channels ........................................... 42
1.7.2.1 The Inward Rectifier K^+ Current (I_{K1}) ........................................ 42
1.7.2.2 Acetylcholine-activated K^+ Channel (I_{K,Ach}) .............................. 42
1.7.2.3 ATP-sensitive K^+ Channel (I_{K,ATP}) ............................................ 42
1.8 Stretch-activated Channels ..................................................................... 43
1.9 Cardiac Muscle Contraction .................................................................... 43
1.9.1 Cardiac Muscle Fibre ......................................................................... 43
1.9.1.1 Myofibrils; Actin and Myosin Filaments ........................................ 43
1.9.1.2 Sarcoplasm, Transverse Tubule and Sarcoplasmic Reticulum and the General Mechanism of Contraction .................................. 45
1.9.2 Molecular Mechanism of Contraction ............................................... 49
1.9.2.1 Myosin Filament ........................................................................ 49
1.9.2.2 Actin Filament ............................................................................ 49
1.9.2.3 Cross-Bridge Cycling or The “Walk-Along” Theory of Contraction ........................................................................................................ 50
1.9.2.4 The Source of Energy for Contraction: ATP ................................. 51

2 Potassium Channels Implicated in the Short QT Syndrome .................... 53
2.1 Rectification .......................................................................................... 53
2.2 The hERG/I_{Kr} Potassium Channel ...................................................... 55
2.2.1 hERG/I_{Kr} Potassium Channel Gating ............................................ 56
2.2.2 hERG/I_{Kr} Channel Structure ........................................................ 58
2.3 The KCNQ1-KCNE1/I_{Ks} Potassium Channel ....................................... 60
2.3.1 KCNQ1-KCNE1/I_{Ks} Potassium Channel Gating ............................ 61
2.3.2 KCNQ1-KCNE1/I_{Ks} Channel Structure ............................................ 62
2.4 The KCNJ2/I_{K1} Potassium Channel .................................................... 64
2.4.1 Kir2.1/I_{K1} Potassium Channel Gating ............................................ 66
2.4.2 KCNJ2/I_{K1} Channel Structure ......................................................... 68
2.5 Current Profiles during an Action Potential ......................................... 68
The Short QT Syndrome ................................................. 69
3.1 Introduction ................................................................ 69
3.2 SQT1 and KCNH2 ...................................................... 70
3.3 SQT2 and KCNQ1 ...................................................... 73
3.4 SQT3 and KCNJ2 ...................................................... 75
3.5 SQT4 and SQT5 ........................................................ 77
3.6 Current Treatment of SQT Patients ................................. 78

Model Development ......................................................... 82
4.1 Modelling Voltage-dependent Channels: Hodgkin-Huxley vs. Markov Chain Models ........................................ 82
  4.1.1 The Hodgkin-Huxley Formalism .................................. 83
    4.1.1.1 The Complete Hodgkin-Huxley Model ..................... 86
  4.1.2 Markov Chain Models ............................................. 87
4.2 Development of the SQT1 Markov Model ............................. 91
  4.2.1 The Base I_{Kr} Markov Model .................................... 91
  4.2.2 The SQT1 Markov Model .......................................... 93
4.3 Development of the SQT2 Markov Model ............................. 96
  4.3.1 The Base I_{Ks} Markov Model .................................... 96
  4.3.2 The SQT2 Markov Model .......................................... 98
4.4 Development of the SQT3 Hodgkin-Huxley Model ................... 101
  4.4.1 The Base I_{K1} Model ............................................. 101
  4.4.2 The SQT3 Hodgkin-Huxley Model ............................... 101

Methods, Experimental Protocols and Mathematical Preliminaries .... 103
5.1 Experimental Protocols ............................................... 103
  5.1.1 Action Potential Duration, Diastolic Interval and the Basic Cycle Length .............................................. 103
  5.1.2 S1-S2 Protocol ...................................................... 104
  5.1.3 Action Potential Duration restitution ............................ 106
  5.1.4 Effective Refractory Period Restitution ......................... 106
5.2 Governing Equations, Geometries and Associated Simulation Protocols .. 107
  5.2.1 Single Cell Model and AP Simulations ......................... 107
    5.2.1.1 Other I_{Kr} models used for comparison .................. 108
5.2.2 Heterogeneous transmural ventricular tissue model .......... 109
   5.2.2.1 Computation of the Diffusion Coefficient .......... 109
   5.2.2.2 Solving the Monodomain Equation ...................... 110
5.2.3 1D Heterogeneous Transmural Strand .................................................. 112
   5.2.3.1 Measurement of Conduction Velocity and Conduction
          Velocity Restitution .................................................. 112
   5.2.3.2 Measurement of the Temporal Vulnerability Window . 115
   5.2.3.3 Measurement of the Tissue Excitation Threshold ......... 116
5.2.4 2D Human Ventricle Geometry .................................................. 116
   5.2.4.1 Idealised 2D Geometry .................................. 116
   5.2.4.2 Realistic 2D Geometry ..................................... 116
5.2.5 3D Human Ventricle Geometry .................................................. 116
5.3 Other Simulation Protocols .......................................................... 117
   5.3.1 Computing the pseudo-ECG ..................................... 117
   5.3.2 Initiation of re-entry in 2D sheet .................................. 117
   5.3.3 Measurement of minimal size of S2 that sustains re-entry in 2D
          models .................................................................. 119
   5.3.4 Initiation of Re-entry in 2D Heart Cross-section .......... 119
   5.3.5 Initiation of re-entry in the 3D anatomical human ventricles ..... 121
5.4 Numerical Methods .......................................................... 121

6 Increased Vulnerability of the Human Ventricle to Re-entrant Excitation in
   hERG-linked SQT1 ........................................................................... 122
6.1 Introduction .................................................................................. 122
6.2 Simulation of Single Cell I_{Kr} under Control and SQT1 Conditions ........ 125
6.3 Simulation of the ECG with WT and N588K mutant I_{Kr} ................. 139
6.4 Simulation of the Spatial Gradient of the Membrane Potential .......... 142
6.5 Investigating the Arrhythmogenic Substrate in SQT1 – 1D simulations.... 144
6.6 Investigating the Arrhythmogenic Substrate in SQT1 – Idealised 2D
          geometry simulations .......................................................... 144
6.7 Investigating the Arrhythmogenic Substrate in SQT1 – 2D and 3D
          Simulations with Realistic Geometry ..................................... 146
   6.7.1 Simulations in Realistic 2D Geometry .............................. 148
   6.7.2 Simulations in Realistic 3D Geometry .............................. 148
6.8 Discussion and Conclusions .......................................................... 153
6.8.1 Summary of major findings ..............................................................153
6.8.2 Significance of the study .................................................................154
6.8.3 Relevance to previous studies ............................................................155

7 Mathematically Modelling the Functional Consequences of the SQT2
  Mutation .................................................................................................158
  7.1 Introduction ........................................................................................158
  7.2 Heterozygote Formulation ..................................................................160
  7.3 Simulation of Single Cell $I_{Ks}$ under Control and SQT2 Conditions ....160
  7.4 Simulation of the ECG with WT and SQT2 Mutant $I_{Ks}$ .....................168
  7.5 Investigating the Arrhythmogenic Substrate in SQT2 – 1D simulations....171
  7.6 Investigating the Arrhythmogenic Substrate in SQT1 – 2D and 3D
      Simulations with Realistic Geometry...................................................173
      7.6.1 Simulations in 2D Realistic Geometry........................................173
      7.6.2 Simulations in 3D Realistic Geometry........................................178
  7.7 Investigating Blockade of $I_{Ks}$ as a Potential Therapeutic Target in the
      SQT2 ..................................................................................................178
  7.8 Discussion and Conclusions................................................................180
      7.8.1 Summary of major findings .......................................................180
      7.8.2 Significance of the Study and Relevance to Previous Studies....184
      7.8.3 Arrhythmogenic mechanisms of the KCNQ1 V307L mutation ..185
  7.8.4 SQT2 Treatment..............................................................................186

8 Proarrhythmia in KCNJ2-linked Short QT Syndrome: Insights from
  Modelling ..................................................................................................187
  8.1 Introduction ........................................................................................187
  8.2 Simulation of Single Cell $I_{K1}$ under Control and SQT3 Conditions ....188
  8.3 Simulation of the ECG with WT, WT-D172N and D172N Mutant $I_{K1}$.....192
  8.4 Simulation of Transmural APD dispersion and Temporal Vulnerability ...195
  8.5 Investigating the Conduction Velocity in SQT3.................................197
  8.6 Investigating the Arrhythmogenic Substrate in SQT3 – 1D simulations....197
  8.7 Investigating the Arrhythmogenic Substrate in SQT3 – Idealised 2D
      geometry simulations ...........................................................................199
  8.8 Investigating the Arrhythmogenic Substrate in SQT3 – 2D and 3D
Simulations with Realistic Geometry .......................................................... 201
  8.8.1 Simulations in Realistic 2D Geometry ............................................ 201
  8.8.2 Simulations in Realistic 3D Geometry ............................................ 204
8.9 Discussion ................................................................................................. 204
  8.9.1 Pro-fibrillatory mechanisms of the Kir2.1 D172N mutation .......... 207
  8.9.2 Relevance to Previous Studies ............................................................ 208

9 Relationship between Electrical and Mechanical Systole in the Short QT Syndrome: Insights from Modelling ................................................................. 210
  9.1 Electromechanical Cardiac Myocyte Model ....................................... 210
  9.2 Myofilament Model .............................................................................. 210
  9.3 Coupling the Electrophysiology model with the Myofilament Mechanics Model .......................................................................................... 211
    9.3.1 Stretch-activated Channel .............................................................. 211
  9.4 Tissue Mechanics Model ................................................................. 212
  9.5 The Pole-Zero Strain Energy Function .............................................. 214
  9.6 Mechanical Feedback in the Electrophysiology Tissue Model ......... 217
  9.7 Numerical Methods ............................................................................ 217
    9.7.1 Meshes ...................................................................................... 218
    9.7.2 Electrophysiology Problem ......................................................... 218
    9.7.3 The Mechanics Problem ............................................................... 219
    9.7.4 Combining the Electrophysiology and Mechanics Problems ...... 220
  9.8 Single Cell Electromechanical Simulations without $I_{sac}$ .......... 221
    9.8.1 SQT1 ....................................................................................... 221
    9.8.2 SQT2 ....................................................................................... 221
    9.8.3 SQT3 ....................................................................................... 223
    9.8.4 Simulated AP Clamp ................................................................. 227
  9.9 Single Cell Electromechanical Simulations with $I_{sac}$ ............... 227
    9.9.1 SQT1 ....................................................................................... 230
      9.9.1.1 $P_{Na}:P_{K}:P_{Ca} = 1:1:0$ ...................................................... 230
      9.9.1.2 $P_{Na}:P_{K}:P_{Ca} = 1:1:1$ ...................................................... 234
    9.9.2 SQT2 ....................................................................................... 236
      9.9.2.1 $P_{Na}:P_{K}:P_{Ca} = 1:1:0$ ...................................................... 236
      9.9.2.2 $P_{Na}:P_{K}:P_{Ca} = 1:1:1$ ...................................................... 236
    9.9.3 SQT3 ....................................................................................... 239
9.9.3.1 $P_{Na}:P_{K}:P_{Ca} = 1:1:0$ ......................................................... 239
9.9.3.2 $P_{Na}:P_{K}:P_{Ca} = 1:1:1$ ......................................................... 241
9.10 Tissue Simulations ........................................................................... 241
9.11 Discussion ......................................................................................... 246
  9.11.1 Simulations without $I_{sac}$ .......................................................... 246
  9.11.2 Simulations with $I_{sac}$ ............................................................... 247

10 Discussion and Conclusion .................................................................. 249
  10.1 Summary of the Functional Consequences of the SQT1, SQT2 and SQT3 Variants .......................................................... 250
    10.1.1 SQT1 ....................................................................................... 250
    10.1.2 SQT2 ....................................................................................... 252
    10.1.3 SQT3 ....................................................................................... 253
  10.2 Common mechanisms between the SQT1-3 Variants ..................... 255
  10.3 Potential Limitations of the Simulations ......................................... 256
  10.4 Future Developments ..................................................................... 258
    10.4.1 Drug Actions .......................................................................... 258
    10.4.2 Mechanical Function ................................................................. 259
  10.5 Closing Words .............................................................................. 259
## List of Tables

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Voltage-gated K⁺ currents/channels in the human heart</td>
<td>40</td>
</tr>
<tr>
<td>3.1</td>
<td>Known SQTS ion channel gene mutations and their functional consequences</td>
<td>79</td>
</tr>
<tr>
<td>6.1</td>
<td>Computed APD₉₀ (ms) and ΔAPD₉₀ (ms) under WT and N588K conditions</td>
<td>133</td>
</tr>
<tr>
<td>7.1</td>
<td>Computed APD₉₀ (ms) and ΔAPD₉₀ (ms) under WT, WT-V307L and V307L conditions</td>
<td>164</td>
</tr>
<tr>
<td>8.1</td>
<td>Computed APD₉₀ (ms) and ΔAPD₉₀ (ms) under WT, WT-D172N and D172N conditions</td>
<td>192</td>
</tr>
<tr>
<td>9.1</td>
<td>SQT1 effects on sarcomere length and contractile force</td>
<td>226</td>
</tr>
<tr>
<td>9.2</td>
<td>SQT2 effects on sarcomere length and contractile force</td>
<td>226</td>
</tr>
<tr>
<td>9.3</td>
<td>SQT3 effects on sarcomere length and contractile force</td>
<td>226</td>
</tr>
<tr>
<td>9.4</td>
<td>WT Changes in APD due to $I_{sac}$ at $P_{Na} : P_{K} : P_{Ca} = 1:1:0$ and $P_{Na} : P_{K} : P_{Ca} = 1:1:1$</td>
<td>231</td>
</tr>
<tr>
<td>9.5</td>
<td>WT Changes in Caᵢ due to $I_{sac}$ at $P_{Na} : P_{K} : P_{Ca} = 1:1:0$ and $P_{Na} : P_{K} : P_{Ca} = 1:1:1$</td>
<td>231</td>
</tr>
<tr>
<td>9.6</td>
<td>WT Changes in sarcomere length due to $I_{sac}$ at $P_{Na} : P_{K} : P_{Ca} = 1:1:0$ and $P_{Na} : P_{K} : P_{Ca} = 1:1:1$</td>
<td>231</td>
</tr>
<tr>
<td>9.7</td>
<td>WT Changes in contractile force due to $I_{sac}$ at $P_{Na} : P_{K} : P_{Ca} = 1:1:0$ and $P_{Na} : P_{K} : P_{Ca} = 1:1:1$</td>
<td>231</td>
</tr>
<tr>
<td>9.8</td>
<td>SQT1, SQT2 and SQT3 changes in APD due to $I_{sac}$ at $P_{Na} : P_{K} : P_{Ca} = 1:1:0$ and $P_{Na} : P_{K} : P_{Ca} = 1:1:1$</td>
<td>232</td>
</tr>
<tr>
<td>9.9</td>
<td>SQT1 ($I_{sac}$ at $P_{Na} : P_{K} : P_{Ca} = 1:1:0$) effects on sarcomere length and contractile force</td>
<td>233</td>
</tr>
<tr>
<td>9.10</td>
<td>SQT1 ($I_{sac}$ at $P_{Na} : P_{K} : P_{Ca} = 1:1:1$) effects on sarcomere length and contractile force</td>
<td>235</td>
</tr>
<tr>
<td>9.11</td>
<td>SQT2 ($I_{sac}$ at $P_{Na} : P_{K} : P_{Ca} = 1:1:0$) effects on sarcomere length and contractile force</td>
<td>237</td>
</tr>
</tbody>
</table>
9.12 SQT2 ($I_{sat}$ at $P_{Na}:P_K : P_{Ca} = 1:1:1$) effects on sarcomere length and contractile force .................................................................238

9.13 SQT3 ($I_{sat}$ at $P_{Na}:P_K : P_{Ca} = 1:1:0$) effects on sarcomere length and contractile force ............................................................................................................................240

9.14 SQT3 ($I_{sat}$ at $P_{Na}:P_K : P_{Ca} = 1:1:1$) effects on sarcomere length and contractile force ............................................................................................................................242
# List of Figures

1.1 Phases of the cardiac action potential ................................................................. 22
1.2 Electrical conduction in the heart ........................................................................... 24
1.3 Action potential from a human sinoatrial node ..................................................... 26
1.4 Action potential in normal human atrium ............................................................. 26
1.5 Action potential in normal atrioventricular node .................................................. 27
1.6 Action potential in normal human purkinje fibre cell ........................................... 27
1.7 Action potential in a normal human ventricle ....................................................... 29
1.8 Cell membrane ...................................................................................................... 29
1.9 Electrical equivalent of the cell membrane ........................................................... 32
1.10 Passive membrane electrical circuit ...................................................................... 32
1.11 Equilibrium potential analogy ............................................................................ 33
1.12 Example state diagram for a channel showing activation and inactivation ........ 36
1.13 The structure of striated muscle .......................................................................... 44
1.14 The arrangement of the myofilaments in obliquely striated muscle ................... 46
1.15 A cross section of a muscle fibre ......................................................................... 46
1.16 Myofibril in relaxed and contracted states ........................................................... 47
1.17 Ultrastructure of a group of myofibrils .............................................................. 48
1.18 The structure of actin and myosin filaments ....................................................... 50
1.19 “Walk-along” mechanism for muscle contraction ............................................... 52
2.1 Schematic representation of the Current-voltage relationship for an ohmic channel and channels showing rectification ............................................................... 54
2.2 Representative current trace for hERG ................................................................. 57
2.3 Current-voltage relationship for $I_{\text{hERG}}/I_{Kr}$ ................................................. 57
2.4 Schematic diagram showing a representative hERG/$I_{Kr}$ $\alpha$-subunit .................. 59
2.5 Schematic diagram of the effect of depolarisation and repolarisation on ion channels .................................................................59
2.6 Representative superimposed current traces for KCNQ1 and KCNQ1+KCNE1 ..............................................................................62
2.7 Current-voltage relationship of $I_{Ks}$ tail current .................................................62
2.8 A structural representation of the $\alpha$ and $\beta$-subunits KCNQ1-KCNE1/I$_{Ks}$ ........63
2.9 Current-voltage relations of the KCNJ2-encoded Kir2.1 .................................65
2.10 Structure and current-voltage relationship of the Kir2.1/KCNJ2/I$_{K1}$ channel ...67
2.11 A schematic diagram of the current profiles of $I_{Kr}$, $I_{Ks}$ and $I_{K1}$ ionic currents during a ventricular action potential .................................................................68
3.1 Schematic representation and example clinical ECG of a normal and SQTS patient ........................................................................71
3.2 A schematic diagram of the current profiles of $I_{Kr}$, $I_{Ks}$ and $I_{K1}$ and $I_{Ca,L}$ ionic currents during a ventricular action potential .................................................................72
3.3 Current-Voltage relationships for hERG and N588K-hERG ...............................74
3.4 A schematic representation of the effect of the gain-of-function mutations of the $I_{Ks}$ channel (SQT1), $I_{Ks}$ channel (SQT2) and $I_{K1}$ channel (SQT3) on the normal action potential ........................................................................74
3.5 Tail current current-voltage relation for WT and V307L ....................................76
3.6 Current-voltage relationship for WT, WT/D172N .............................................76
4.1 A state-transition diagram of a two-state model of an ion channel ....................85
4.2 Free energy profile of conformational changes in ion channels .........................90
4.3 Markov model state diagram of cardiac $I_{Kr}$ .....................................................92
4.4 Markov model state transition diagram of the $I_{Ks}$ channel .............................97
4.5 A $K^+$ channel model that undergoes two transitions before channel opening ..97
5.1 The action potential duration, diastolic interval, basic cycle length, S1-S2 protocol and refractory periods .................................................................105
5.2 Geometry of the 1D strand of the transmural human ventricle wall ...............113
5.3 Geometry of the 2D realistic human ventricle cross-section .............................113
13

<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.4</td>
<td>Normal, unidirectional conduction block and bidirectional propagation in tissue</td>
</tr>
<tr>
<td>5.5</td>
<td>3D human ventricle geometry reconstructed by DT-MRI and fibre orientation</td>
</tr>
<tr>
<td>5.6</td>
<td>S1-S2 stimulation sites in the 2D human ventricle cross-section</td>
</tr>
<tr>
<td>5.7</td>
<td>S1-S2 stimulation sites in the 3D anatomical human ventricles</td>
</tr>
<tr>
<td>6.1</td>
<td>State transition diagrams of the full and reduced Markov chain models</td>
</tr>
<tr>
<td>6.2</td>
<td>Simulated Current-Voltage Relationships for ( I_{\text{HERG}} )</td>
</tr>
<tr>
<td>6.3</td>
<td>( I_{\text{HERG}}/I_{\text{Kr}} ) Current-Voltage relations during action potential clamp and effect of premature stimuli</td>
</tr>
<tr>
<td>6.4</td>
<td>Profile of ( I_{\text{HERG}} ) during ventricular AP voltage command under WT and N588K conditions obtained with the full Markov Chain model</td>
</tr>
<tr>
<td>6.5</td>
<td>Profile of ( I_{\text{HERG}} ) during ventricular AP voltage command under WT and N588K conditions obtained with the original TNNP ( I_{\text{Kr}} ) formulation</td>
</tr>
<tr>
<td>6.6</td>
<td>Simulation of ventricular action potential and ( I_{\text{Kr}} ) time courses</td>
</tr>
<tr>
<td>6.7</td>
<td>Rate-dependent APD restitution for WT and N588K conditions</td>
</tr>
<tr>
<td>6.8</td>
<td>Steady state APD rate dependence for the Full Markov Chain model</td>
</tr>
<tr>
<td>6.9</td>
<td>ERP restitution curves of four models of ventricular myocytes</td>
</tr>
<tr>
<td>6.10</td>
<td>Space-time plot of AP propagation along a 1D transmural ventricular tissue strand and computed pseudo-ECGs</td>
</tr>
<tr>
<td>6.11</td>
<td>Membrane potential heterogeneity (( \delta V ))</td>
</tr>
<tr>
<td>6.12</td>
<td>Transmural APD(_{90}) distribution and its spatial gradient along a 1D tissue strand</td>
</tr>
<tr>
<td>6.13</td>
<td>Measured width of the SQT1 vulnerable window along the 1D tissue strand</td>
</tr>
<tr>
<td>6.14</td>
<td>Snapshots of initiation and conduction of re-entry in a 2D idealised model of transmural ventricle</td>
</tr>
<tr>
<td>6.15</td>
<td>Snapshots of initiation and conduction of re-entry in realistic 2D model cross-section of ventricles</td>
</tr>
</tbody>
</table>
6.16 Snapshots of initiation and conduction of re-entry in a 3D anatomical model of human ventricles .................................................................................................................................................151

7.1 WT and V307L $I_{KCNQ1-KCNE1}$ experimental current traces under voltage clamp ..................................................................................................................................................................................161

7.2 Simulated Current-Voltage Relationships for $I_{Ks}$ ..................................................................................................................................................................................162

7.3 Simulation of action potential and $I_{Ks}$ time courses ........................................................................................................................................................................163

7.4 APD and ERP restitution curves for WT, WT-V307L and V307L ..............................................................................................................................................................165

7.5 WT, WT-V307L ad V307L state occupancy during the action potential ......167

7.6 Pseudo ECGs under the WT, WT-V307L and V307L conditions ..........169

7.7 Membrane potential heterogeneity ($\delta V$), transmural distribution and spatial gradient of APD$_{90}$ for WT, WT-V307L and V307L .................................................................................................170

7.8 Vulnerable window across the transmural 1D strand .........................172

7.9 Snapshots of initiation and conduction of re-entry in realistic 2D model cross-section of ventricles ........................................................................................................................................................................174

7.10 Snapshots of initiation and conduction of re-entry in a 3D anatomical model of human ventricles ........................................................................................................................................................................176

7.11 Blockade of $I_{Ks}$ in the single cell under the WT-V307L and V307L conditions .................................................................................................................................................................................................179

7.12 Termination of reentry by $I_{Ks}$ blockade .............................................181

8.1 SQT3 model fit to experimental data ......................................................189

8.2 Simulations of ventricular action potentials for WT, WT-D172N and D172N ........................................................................................................................................................................191

8.3 Rate-dependent APD and ERP restitution curves ..................................193

8.4 Space-time plot of AP propagation along a 1D transmural ventricular strand, computed pseudo-ECGs and conduction velocity .........................................................................................................................194

8.5 Transmural APD$_{90}$ distribution and its spatial gradient along a 1D transmural strand ........................................................................................................................................................................196

8.6 Excitation threshold plotted against stimulus intervals (SIs) for the WT, WT-D172N and D172N mutation conditions .................................................................................................................................................................198

8.7 The measured width of the temporal vulnerable window along the 1D strand 200
8.8 Snapshots of re-entry in realistic 2D model cross-section of ventricles ..........202
8.9 Reentry lifespan and dominant frequency in realistic 2D model cross-section of ventricles .................................................................203
8.10 Snapshots of re-entry in a 3D anatomical model of human ventricles ..........205
8.11 Reentry lifespan and dominant frequency in realistic 3D anatomical model of human ventricles .................................................................206
9.1 Action potentials from an electromechanically coupled cell ....................213
9.2 Single electromechanical effects of the SQT1 mutation without $I_{sac}$ ........222
9.3 Single electromechanical effects of the SQT2 mutation without $I_{sac}$ ........224
9.4 Single electromechanical effects of the SQT3 mutation without $I_{sac}$ ........225
9.5 Simulated AP clamp using the WT electromechanics model without $I_{sac}$ ......228
9.6 Effects of stretch-activated current on the WT electromechanics model ......229
9.7 Single cell electromechanical effects of the SQT1 mutation with $I_{sac}$ at $P_{Na} : P_K : P_{Ca} = 1:1:0$ ....................................................233
9.8 Single cell electromechanical effects of the SQT1 mutation with $I_{sac}$ at $P_{Na} : P_K : P_{Ca} = 1:1:1$ ....................................................235
9.9 Single cell electromechanical effects of the SQT2 mutation with $I_{sac}$ at $P_{Na} : P_K : P_{Ca} = 1:1:0$ ....................................................237
9.10 Single cell electromechanical effects of the SQT2 mutation with $I_{sac}$ at $P_{Na} : P_K : P_{Ca} = 1:1:1$ ....................................................238
9.11 Single cell electromechanical effects of the SQT3 mutation with $I_{sac}$ at $P_{Na} : P_K : P_{Ca} = 1:1:0$ ....................................................240
9.12 Single cell electromechanical effects of the SQT3 mutation with $I_{sac}$ at $P_{Na} : P_K : P_{Ca} = 1:1:1$ ....................................................242
9.13 Electromechanical coupling in 2D ventricular tissue under the SQTS mutations without $I_{sac}$ .................................................................244
9.14 Electromechanical coupling in 2D ventricular tissue under the SQTS mutations with $I_{sac}$ .................................................................245
10.1 Common mechanisms between SQT1-3 variants ..................................255
The recently identified genetic short QT syndrome is characterised by abbreviated QT intervals on the electrocardiogram, an increased risk of atrial and ventricular arrhythmias, and an increased risk of sudden death. Although the short QT syndrome has been suggested to provide a paradigm for increasing understanding of the role of potassium channels in ventricular fibrillation, the basis for arrhythmogenesis in the short QT syndrome is incompletely understood. There are no animal models that accurately reproduce a short QT phenotype, and whilst in vitro electrophysiology of short QT mutant channels provides a route to greater understanding of the effects of short QT mutants on action potential repolarisation, on its own, this approach is insufficient to explain how arrhythmias arise and are maintained at the tissue level. Consequently, this thesis is concerned with the use of the viable alternative; in silico (computational) modelling to elucidate how the short QT syndrome facilitates the genesis and maintenance of ventricular arrhythmias and its effects on ventricular contraction. Using extant biophysical data on changes induced by the short QT mutations and data from BHF-funded in vitro electrophysiology, three novel mathematical models of the first three variants of the short QT syndrome were developed; a Markov chain model for short QT variant 1, a Markov chain model for short QT variant 2 and a Hodgkin-Huxley model for short QT variant 3. These models were incorporated into single cell and anatomically detailed tissue and organ computer models to elucidate how these variants lead to ventricular arrhythmias. The developed short QT models were then incorporated into electromechanically coupled single cell and tissue models to investigate the effects of the short QT mutants on ventricular contraction. It was found that each short QT variant uniquely increased the transmural dispersion of action potential duration across the ventricular wall, increased the temporal window of tissue vulnerability to premature excitation stimulus, leading to increased susceptibility to re-entrant arrhythmia.
Declaration

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Dedication

To my mother, for her continued patience and support and my father, Lateef Ajani Adeniran, who passed away from heart disease in 1996.
Chapter 1

Ion Channels and the Cardiac Action Potential

Every cell in the body contains ion channels. These ion channels are proteins embedded in the cell membrane and regulate the electrical signaling of each cell via egress and ingress of various intracellular and extracellular ions (predominantly Na\(^+\), K\(^+\), Ca\(^{2+}\) and Cl\(^-\)). Defects to the genes encoding ion channel proteins result in the impairment of the functional properties of the ion channels. These ion channel defects lead to diseases (channelopathies) which can be life threatening [1–7]. Examples of such diseases are the short QT syndrome (SQTS) where a patient shows an abnormally short QT interval on the electrocardiogram (ECG) [8,9] and the long QT syndrome (LQTS) where a patient presents with an abnormally long QT interval on the ECG [10–12]. These usually result in arrhythmia, which is an improper rhythm in the beating of the heart.

Arrhythmias are one of the leading causes of death in the world. They are caused by several factors including mutations to ion channels [13], ischaemia with coronary occlusion [14,15], adverse reactions to therapeutic drugs targeting ion channel mutations [16] and steroids abuse by athletes [17]. In order to better understand the SQTS, which is the subject of this thesis, this chapter gives an overview of ion channels, particularly those with mutations that are associated with arrhythmia. This chapter will review their role in generating the cardiac action potential and the functional consequences of mutations to these channels.

1.1 The Cardiac Action Potential

The rhythmic and systematic contraction of cardiac myocytes in the heart is initiated by electrical events called action potentials (APs). An AP is an electrical impulse that reflects the time course of changes in the membrane potential of cardiac myocytes due to an elaborate sequence of openings and closings of the ion channels and electrogenic transporters within the myocyte cell membrane [18–20]. There are five phases of the
cardiac action potential [19,21,22] (Figure 1.1). Phase 0 is the depolarisation of the cell where the membrane potential is rapidly brought from a negative to a positive potential, while Phase 1 corresponds to the end of this upstroke. In some cell types, a partial repolarisation occurs (in which the membrane potential is brought from a positive to a more negative potential), resulting in a notch between phases 0 and 2. The membrane potential can be repolarised to about 0 mV and even more negative potentials.

Figure 1.1: Schematic diagram showing phases of the cardiac action potential. Phase 0: Upstroke. Phase 1: Early/Partial repolarisation. Phase 2: the plateau. Phase 3: Repolarisation. Phase 4: Resting period.
A plateau phase (phase 2) follows this partial repolarisation, which ends with a final repolarisation phase (phase 3). Phase 4 is the return of the membrane potential to a stable resting potential in atrial and ventricular cells. In spontaneously active cells such as those from the sinoatrial node (SAN; the heart’s primary pacemaker), phase 4 is a slow depolarisation that eventually causes spontaneous activity [19,21,22].

### 1.2 Morphology of the Action Potential in Different Parts of the Heart

The SAN – located at the junction of the superior vena cava and the right atrium – is responsible for the initiation of the heart’s spontaneous activity (Figure 1.2) [18,19,23,24]. Electrical activity from the SAN spreads to surrounding atrial tissue via gap junctions which couple cardiac cells electrotonically [18,19,23,24]. It then spreads to the atrioventricular node (AVN) from which it excites the His Bundle Purkinje system, which in turn conducts the electrical activity to the ventricular myocardium (Figure 1.2) [18,19,23,24].

The cells in the different regions of the heart can be grouped into slow and fast response cells based on the rate of depolarisation during the upstroke of the AP (phase 0) [19,24]. This difference in grouping is caused by the inward current that is predominantly responsible for depolarisation; Ca\(^{2+}\) for slow response cells and Na\(^{+}\) for fast response cells. The SAN and AVN cells are slow response cells while the atria, His Bundle, Purkinje and ventricular myocardial cells are fast response cells.

#### 1.2.1 Sinoatrial Node

The SAN contains slow response cells with a low upstroke velocity of about 1 - 10 V/s [24,25] in mammalian cells. Upstroke velocity is approximately 5 V/s in human [26,27]. It is characterised by a slowly rising diastolic depolarisation in phase 4 of the AP leading to spontaneous activity (Figure 1.3). Its diastolic potential ranges from -60 to -50 mV. SAN cells located peripherally have more negative diastolic potentials due to their electrical coupling with atrial cells (atrial cells have more negative resting
Figure 1.2: Electrical conduction in the heart in healthy individuals is controlled by pacemaker cells in the sinoatrial node. Electrical impulses are conducted from the sinoatrial node to the atrioventricular node and bundle of His, through the bundle branches, and into the ventricles [28].
potentials than the SAN). SAN cells have a diameter of about 5-10 µm, an action potential duration (APD) range of 100 - 200 ms and a propagation velocity that is less than 0.05 m/s [24,25].

1.2.2 Atria

Atrial cells have a resting potential of about -80 mV and unlike the SAN, this resting potential is stable with no spontaneous activity (Figure 1.4) [23,24,27]. In mammalian hearts, the APD is between 100-300 ms and is different in different regions of the atria [19,24]. It is shorter in the left atrium than in the right atrium [29,30]. There is a sharp upstroke with a velocity of 100-200 V/s [24,25,27] in mammalian hearts followed by a plateau phase. The cells return to the resting potential but there is no clear demarcation between the plateau (phase 2) and a final repolarisation phase (phase 3). Atrial cells have a diameter of about 10-15 µm and a propagation velocity between 0.3-0.4 m/s [19,24,25].

1.2.3 Atrioventricular Node

The atrioventricular node (AVN) lies at the bottom of the right atrium near the partition that divides the atria [24]. AVN cells have a diameter of about 5-10 µm and a resting potential of -60 to -70 mV [19,24,25]. Like the SAN, it has spontaneous activity but delays cardiac conduction from the atria to the ventricles, allowing the atria to contract and empty their contents first before relaying impulses to the His bundle [19,24]. Its rate of depolarisation is about 5-15 V/s with an action potential that propagates at about 0.1 m/s and a duration of 100 - 300 ms [19,24,25] in mammalian cells. An example AP is shown in Figure 1.5.

1.2.4 His bundle Purkinje System

The His bundle (also known as the AV bundle of His) proceeds from the AVN and penetrates the tissue separating the atria and ventricles. It penetrates the ventricles and divides into left and right bundle branches (also known as AV bundle branches), which
Figure 1.3: Action potential from a human sinoatrial node. Modified from Aslanidi et al. [31].

Figure 1.4: Action potential in normal human atrium. Modified from Courtemanche et al. [32].
Figure 1.5: Action potential in normal atrioventricular node. Modified from [33].

Figure 1.6: Action potential in normal human Purkinje fibre cell. Modified from [34].
end in a network of Purkinje fibres. This network of Purkinje fibres serves as the source of electrical activity to the ventricular myocardium as they form the only contact with the walls. The His bundle Purkinje system is the fast conduction pathway of the heart, propagating impulses rapidly towards the apex of the ventricles, ensuring their near uniform activation [19,24,25].

The cells have a diameter of 100 µm [25], a resting potential of -90 to -95 mV [19,24,25] and a fast upstroke of about 500 - 700 V/s [25]. The APD is about 319 ± 23 ms in human hearts [35] depending on the frequency of stimulation. The AP has propagation velocity of 2 to 5 m/s [19,24,25] and usually has all the phases of the action potential shown in Figure 1.1 (phase 1: the notch may sometimes be absent). Purkinje fibre cells also show slow diastolic depolarisation, which is not as marked as in SAN cells. At low frequencies, this may lead to spontaneous activity if not previously activated by normal sinus activity via electrotonic interactions with neighbouring cells [19,24]. An example AP is shown in Figure 1.6.

1.2.5 Ventricle

Ventricular myocardial cells show different characteristics in different regions of the ventricles. The APD is shorter in the right ventricle than in the left ventricle and also has a more markedly pronounced notch (phase 1 repolarisation) [19,24]. Transmurally, the ventricular myocardium has three cell types; the endocardium (ENDO), the mid-myocardium (MCELL) and the epicardium (EPI) [19,24,36,37]. The ENDO cell is the innermost layer while the EPI cell is the outermost layer. The MCELL has the longest APD (comparable to the purkinje fibre cells). Generally, the APD of the EPI cell is shorter than that of the ENDO cell across all species but there is some experimental data showing the opposite in human ventricular cells [37]. The EPI cell has a pronounced phase 1 repolarisation, as does the MCELL, which results in a pronounced notch, giving the AP a spike-dome appearance. In comparison, there is less phase 1 repolarisation in the ENDO cell and consequently a less pronounced notch [19,24].

Ventricular myocardial cells have a diameter of 10-20 µm [24,25], a resting potential of about -80 to -90 mV and an upstroke velocity of 100 - 200 V/s [24,25] in
Figure 1.7: Left ventricular epicardial action potential in a normal human ventricle. Modified from [38].

Figure 1.8: Intrinsic proteins penetrate and bind tightly to the lipid bilayer, which is made up largely of phospholipids and cholesterol and which typically is between 4 and 10 nanometers (nm; 1 nm = 10^{-9} metre) in thickness. Extrinsic proteins are loosely bound to the hydrophilic (polar) surfaces, which face the watery medium both inside and outside the cell. Some intrinsic proteins present sugar side chains on the cell’s outer surface [39].
mammalian cells. The AP propagates at about 0.3 - 1.0 m/s [19,24,25] and spreads from the endocardium near the apex to the epicardial wall at the base.

1.3 Ionic Basis of Resting and Action Potential

1.3.1 The Uniformly Polarised Cell

The cell membrane is electrically equivalent to a capacitor because it consists of a lipid bilayer that separates the intracellular medium from the extracellular medium (Figure 1.8). The lipid bilayer has a capacitance of about 1 µF/cm², is non-conducting and acts as a dielectric. It also contains proteins, some of which behave as ion permeation pathways through which ions enter or exit the cell membrane. The cell membrane is thus not a perfect isolator and can be regarded as a “leaky” capacitor. The electrical equivalent of the cell membrane is then a capacitor in parallel with a resistor (ion permeation pathways) (Figure 1.9 and 1.10).

When current passes through the membrane ($I_m$), some of it goes into charging the capacitor ($I_c$) while the rest ($I_{ion}$) flows through the resistive pathway (Figure 1.10). $I_c$ will cause a change in the amount of charge being separated and consequently, a change in the membrane potential. This change in potential will in turn cause an equivalent change in current. The total membrane current ($I_m$) can then be written as:

\[
I_m = I_c + I_{ion}
\]

\[
Q = C_m V_m
\]

\[
I_c = \frac{dQ}{dt}
\]

\[
I_m = \frac{dQ}{dt} + I_{ion}
\]

\[
I_m = C_m \frac{dV_m}{dt} + I_{ion}
\]

where $Q$ is the charge in coulomb, $C_m$ is the capacitance in farads and $V_m$ is the membrane potential in volts.
1.3.2 Electrical Equivalent Circuit of a Cell

The cell membrane contains many ion channels, each of which contribute to the net ionic current $I_{ion}$. The ion channels would then be represented as variable resistors in parallel (variable resistors because the channel opening and hence resistance may not be constant) [18–20,24]. For each ion, there is a concentration gradient across the cell membrane, which can be represented as a battery (electromotive force). Therefore, each ion channel is a variable resistor in series with a battery (Figure 1.9 and 1.10). The ionic current flowing through each ion channel can then be represented mathematically using a modification of Ohm’s law, which takes the ion concentration gradient into account:

$$I_{ion} = G_{ion} (V_m - E_{ion})$$

(2)

where $I_{ion}$ is the ionic current, $G_{ion}$ is the inverse of the resistance (conductance of the channel), $E_{ion}$ is the equilibrium potential of the ion and $V_m$ has the same meaning as in Equation (1). The equilibrium potential of the ion is the potential at which no net transport of the ion occurs. At this potential, the free energy due to the concentration gradient is equal and exactly opposite to the potential gradient (see Section 1.3.3).

According to standard convention, the direction of current flow is generally considered to be the direction of flow of the positive charges. Positive current flow is described as positive charges leaving the cell across the cell membrane, e.g., K$^+$ ions leaving the cell is outward current and Na$^+$ entering the cell is inward current. The membrane potential ($V_m$) is computed as the intracellular potential relative to the extracellular potential ($V_i$ - $V_e$) and at the normal resting potential, $V_m$ is negative [18,19,23,24]. A change to the potential that makes it more negative is referred to as hyperpolarisation while a change that makes it less negative (or, equivalently, more positive) is referred to as depolarisation. Following a depolarisation, the return of the membrane to its resting potential is known as repolarisation.
Figure 1.9: Electrical equivalent of the cell membrane.

Figure 1.10: Passive membrane electrical circuit. $V_m$ is the transmembrane potential, $I_m$ is the total membrane current, $I_{ion}$ is the ionic current, $I_c$ is capacitative current, $C_m$ is the membrane capacitance and $R_m$ is the membrane resistance.
1.3.3 Equilibrium Potential

As an analogy to a cell, let us assume there are two compartments, A and B separated by a membrane that is selectively permeable to one ion (for the sake of discussion, K$^+$; Figure 1.11). Let compartment A be the intracellular region and B the extracellular region. If K$^+$ has a higher intracellular concentration compared to its extracellular concentration (as it does in a cell), it would flow from compartment A to compartment B due to its concentration gradient. This will leave compartment A negatively charged, counteracting further efflux of K$^+$ ions.

Simultaneously, the efflux of K$^+$ ions will set up an electric potential and as more K$^+$ ions leave compartment A, the potential difference increases, also counteracting further K$^+$ efflux. The potential at which the gradient in electric potential exactly equals the opposite K$^+$ concentration gradient is the equilibrium potential of the ion. At the equilibrium potential, the system is in equilibrium and the net K$^+$ (ion) flux is zero.

The equilibrium potential for an ion, $E_{ion}$ is determined by the Nernst equation [40] developed by the German scientist, Walther Herman Nernst:

![Figure 1.11: Compartment with unequal concentrations of K$^+$ across a semipermeable membrane. Due to the concentration gradient, a net K$^+$ ion flux will occur setting up a potential difference across the membrane. Net K$^+$ ion flux will cease once the transmembrane potential equals the equilibrium potential of K$^+$.](image-url)
where $E_{\text{ion}}$ is equilibrium potential (as in Equation 2), $z$ is the valence of the ion, $R$ is the universal gas constant, $T$ is the temperature in degrees Kelvin, $F$ is Faraday’s constant, $[C]_o$ is the concentration of the ion outside the cell and $[C]_i$ is the concentration of the ion outside the cell.

### 1.4 Properties of Ion Channels

The movement of ions across the cell membrane via ion channels leads to the generation of action potentials. To understand the contribution of each ion channel to cardiac AP electrogenesis, it is necessary to understand the two basic characteristics of ion channels: ion permeation and gating [20]. Ion permeation refers to the selectivity of the channel for a particular ion while gating refers to the kinetics of opening and closing of the channel. Also of importance are the molecular structure of the channel and the density of the channel in the cell membrane.

In general, ion transport processes as well as the opening and closing of ion channels are functions of membrane potential. They can, however, be modulated by other processes such as agonists in the extracellular medium, binding of intracellular or membrane-bound ligands and mechanical forces [20,22,24]. These modulatory processes confer time dependence on the response of the channels, i.e., a conformational change of the channels is not instantaneous but takes time. To account for the effect of these modulatory processes, an additional kinetic factor (gating factor) is incorporated into the ionic current formulation given in equation (2). It is fractional; hence, it ranges from 0 – 1 and represents the fraction of channels that are open. The current for an ion X $(I_X)$ can then be written as:

$$I_X = \tilde{G}_X K_X (V_m - E_X)$$  \hspace{1cm} (4)
where $\overline{G}_x$ is the maximal conductance of the channel, $K_x$ is the kinetic factor representing the fraction of channels in the open state, $V_m$ is transmembrane voltage and $E_X$ is the equilibrium potential for the ion.

### 1.4.1 Ion Permeation

When the kinetic factor ($K_x$) from Equation (4) has a value of 1, all the channels are open and the equation is referred to as the fully activated current-voltage (I-V) relation. If the I-V relation behaves like an ohmic conductor, then the current is a linear function of the membrane potential. In general, however, the I-V relation for ion channels is nonlinear with the channels said to show rectification [20,24]. This is because they pass current inward or outward. If the channel passes current outwards more easily, it is called an outward rectifier otherwise it is an inward rectifier. With increasing depolarisation, the slope of an outwardly rectifying current increases whilst it decreases for an inwardly rectifying current.

### 1.4.2 Gating

Ion channels may be classified into different types according to the mechanism of gating (opening and closing): voltage-dependent, ligand-dependent and mechano-sensitive [20,22,24]. Voltage-dependent gated channels are the most abundant. Their conductance changes in response to a change in membrane potential. Most of the channels open in response to depolarisation and inactivate (if the channel shows inactivation) with further maintained depolarisation. A crude definition of inactivation is a ‘temporary’ blockade of the ion channel, which renders it non-conducting. To recover from inactivation, the channels need to close/deactivate at hyperpolarised potentials before they can be activated again. These channels can also inactivate directly from a closed state. Thus, these channels have two methods of closure: (1) closure from an open state and (2) closure from an inactivated state. Figure 1.12 shows a simple kinetic scheme for possible transitions between states.
In ligand-dependent gated channels, channel opening depends on the binding of a ligand to a receptor site. For example, the inward-rectifying acetylcholine-activated K\(^+\) channel (I\(_{K,Ach}\)), which is the best studied of these types of channels. Acetylcholine binds to the M-2 muscarinic receptor, which eventually leads to a release of G\(\beta\gamma\) subunit along a G-protein signaling pathway that activates I\(_{K,Ach}\) [20,22,24]. Another ligand-gated channel is the ATP-sensitive K\(^+\) channel (I\(_{K,ATP}\)). Its open probability is proportional to the ratio of the concentration of intracellular adenosine diphosphate (ADP) to adenosine triphosphate (ATP) ([ADP]/[ATP]). I\(_{K,ATP}\) therefore couples the metabolic state of the cell to the shape of the action potential [20,22,24].

The mechano-sensitive (stretch-activated) channels (see section 1.8) convert a physical input such as stretch into an electric signal by changing channel conductance. For example, if the heart experiences mechanical deformation at an appropriate time during the cardiac cycle (for example from a golf ball or base-ball impact on the chest), it is feasible that ventricular fibrillation may result [20,22,24].

1.5 Sodium Channels

The influx of Na\(^+\) depolarises the cell causing a rapid upstroke of the AP (phase 0). The AP thus generated propagates through the heart. With depolarisation to between -60 and 10 mV, the Na\(^+\) current (I\(_{Na}\)) magnitude increases but positive to 10 mV, it decreases
The current reverses at about 50 mV. $I_{Na}$ has properties of activation and inactivation. It activates at about -60 mV and recovers from inactivation very quickly (1 - 10 ms). This recovery rate increases with hyperpolarisation. $Na^+$ channels are abundant in the atria, ventricles and the His Bundle Purkinje system. $I_{Na}$ is also present in some cells from the SAN and AVN but plays little or no active role in central nodal cells due either/both to its absence or the low diastolic potential in these cells, which cause it to be inactivated [19,20,22,24]. $I_{Na}$ can contribute to cellular activity in peripheral nodal cells [41–43].

The gene encoding the major subunit of cardiac fast $Na^+$ channels is SCN5A [44]. Associated with mutations and defects of the channel are the congenital forms of the Long QT Syndrome (LQTS) [44,45], the Brugada syndrome [46], the primary cardiac conduction system disease (PCCP) [47,48] and dilated cardiomyopathy [49]. Variant 3 of the LQTS involves gain of function mutations that lead to a small but persistent $Na^+$ current contributing to the plateau phase of the action potential because of slow or incomplete inactivation of the channel [50,51]. Brugada syndrome involves loss of function mutations, which reduces the $Na^+$ current density and consequently shortens the APD [52–54]. Resulting clinical syndromes include AV block [54], atria standstill [55] and sinus node dysfunction [46]. The mechanisms that link $Na^+$ channel dysfunction to dilated cardiomyopathy are not well understood [56].

1.6 Calcium Channels

There are two types of Calcium (Ca$^{2+}$) channels: L-type (low threshold type) and T-type (transient type) calcium channels [22,57]. They differ in their electrophysiological and pharmacological characteristics. Their densities also vary in different parts of the heart. Mutations to the calcium channel result in disorders such as Timothy syndrome (a combination of hypoglycaemia, LQTS, cognitive abnormalities, immune deficiency and syndactyly [58]) and a sudden death syndrome that combines the Brugada syndrome with a short QT interval [59].
1.6.1 L-type Calcium Channel

The L-type \( \text{Ca}^{2+} \) current (\( I_{\text{Ca,L}} \)) is found in all cardiac cell types and is responsible for the generation of the action potential upstroke (phase 0) in SAN and AVN cells [22,24,57]. It activates rapidly upon depolarisation (but not as quickly as the sodium channel current, \( I_{\text{Na}} \)). In other cells, it contributes to the plateau phase and is responsible for the spike and dome appearance of the action potential. It also regulates excitation-contraction coupling by inducing \( \text{Ca}^{2+} \) release from the sarcoplasmic reticulum (SR). It has three modes of operation [24,57]. Mode 1 is characterised by bursts of short repetitive openings and closures, with long closures between bursts [24,57]. Mode two is characterised by longer opening times and occurs in the presence of dihydropyridine agonists or following \( \beta \)-reception stimulation [24,57]. Mode 3 corresponds to the inactivated state of the channel and has either no openings or opens infrequently [24,57].

The L-type \( \text{Ca}^{2+} \) channel shows two types of inactivation process: voltage-dependent inactivation and calcium-induced inactivation, which is dependent on the intracellular \( \text{Ca}^{2+} \) concentration [\( [\text{Ca}^{2+}]_i \)] [24,57]. \( \text{Ca}^{2+} \)-induced inactivation enhances the amplitude of [\( [\text{Ca}^{2+}]_i \)], which in turn increases the rate of inactivation. This then limits further \( \text{Ca}^{2+} \) influx thereby preventing \( \text{Ca}^{2+} \) overload of the cell. Recovery from inactivation is also both voltage-dependent and dependent on [\( [\text{Ca}^{2+}]_i \)] [57].

The channel shows a high selectivity for \( \text{Ca}^{2+} \) but a very low selectivity for \( \text{K}^+ \) [60–62]. Despite the low selectivity of \( \text{K}^+ \) by the channel, \( \text{K}^+ \) can still contribute a substantial amount of current through this channel. This is due to higher intracellular concentration of \( \text{K}^+ \) compared to \( \text{Ca}^{2+} \). This also explains the lower reversal potential of \( I_{\text{Ca,L}} \) compared to the theoretical equilibrium potential of \( \text{Ca}^{2+} \) ions [57].

1.6.2 T-type Calcium Channel

T-type calcium channel current (\( I_{\text{Ca,T}} \)) is found in the SAN, AVN, atrial and Purkinje cells [22,24,57]. Its threshold of activation is about -70 mV to -50 mV (lower than that for \( I_{\text{Ca,L}} \)). Like \( I_{\text{Ca,L}} \), it activates rapidly upon depolarisation (but not as quickly as \( I_{\text{Na}} \)) and it also shows faster inactivation than \( I_{\text{Ca,L}} \). It therefore contributes only to early
phases of the action potential. It is also thought to play a role in late pacemaker depolarisation [63,64].

1.7 Potassium Channels

Potassium (K\(^+\)) channels can be functionally subdivided into voltage-dependent (or voltage-activated) K\(^+\) channels (channels responsible for I\(_{to}\), I\(_{Kr}\), I\(_{Ks}\), I\(_{Kur}\)) and the inward-rectifying K\(^+\) channels (channels responsible for I\(_{K1}\), I\(_{K,ATP}\), I\(_{K,ACh}\)). I\(_{K,ATP}\) and I\(_{K,ACh}\) are ligand- gated or ligand-activated K\(^+\) channel currents [19,20,22,24,57]. Under normal physiological conditions, K\(^+\) channels play an important role in shaping the action potential, particularly the voltage-gated channels. This is because the cell membrane resting potential is greater than the equilibrium potential of K\(^+\) ions resulting in a net outward current, which serves to repolarise the cell membrane during an AP or (in the case of I\(_{K1}\)) help maintain a stable resting potential.

1.7.1 Voltage-dependent K\(^+\) Channels

Table 1.1 shows the voltage-gated K\(^+\) currents in the heart and some of their properties. The main channels are: the transient outward K\(^+\) currents (fast and slow), the rapid (I\(_{Kr}\)), slow (I\(_{Ks}\)) and ultra-rapid (I\(_{Kur}\))-delayed rectifier outward K\(^+\) currents. These currents play a significant role in the morphology of the action potential [19,20,22,24,57].

1.7.1.1 The Transient Outward Current (I\(_{to}\))

There are two types of transient outward current. I\(_{to}\) (also known as I\(_{to1}\)) is a K\(^+\) current while the second type, I\(_{to2}\) is a Ca\(^{2+}\)-activated Cl\(^-\) current [22,24,57]. I\(_{to1}\) has both fast I\(_{to,f}\) and slow I\(_{to,s}\) components with the fast component being the primary subtype expressed in the human atrium. Both components are expressed in the human ventricle. Henceforth, any reference to I\(_{to}\) is a reference to I\(_{to1}\). I\(_{to}\) is found in all cell types including the SAN and AVN. Its major contribution is to phase 1 (early repolarisation phase) of the action potential [19,20,22,24,57].
Different parts of the heart show different expression of $I_{to}$. Thus, $I_{to}$ shows a greater density in the right ventricle than the left ventricle [22,24,57,65,66]. Hence, the notch in phase 1 is more pronounced in the right ventricle [65–67]. There is also a difference in the transmural expression of $I_{to}$ across the ventricular myocardium; it has a greater density in the mid-myocardial cells than either the endocardial or epicardial cells [22,24,57].

Activation of $I_{to}$ occurs by depolarisation and is a fast process [68]. Inactivation and recovery from inactivation are also fast with recovery from inactivation being sensitive to voltage [68]. The greater the hyperpolarisation, the faster is the recovery from inactivation.

1.7.1.2 The Slowly-activated Delayed Outward Rectifier Current ($I_{Ks}$)

In the human heart, $I_{Ks}$ has been found in all cell types including the atrial and ventricular cells [22,24,57]. It is differentially expressed across the ventricular wall

<table>
<thead>
<tr>
<th>Current</th>
<th>Activation</th>
<th>Inactivation</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{to,f}$</td>
<td>Fast</td>
<td>Fast</td>
<td>Atrium, Ventricle, Purkinje fibres, SAN</td>
</tr>
<tr>
<td>$I_{to,s}$</td>
<td>Slow</td>
<td>Slow</td>
<td>Atrium, Ventricle, SAN</td>
</tr>
<tr>
<td>$I_{Kr}$</td>
<td>Moderate</td>
<td>Fast</td>
<td>Atrium, Ventricle, Purkinje fibres, SAN</td>
</tr>
<tr>
<td>$I_{Ks}$</td>
<td>Very slow</td>
<td>None</td>
<td>Atrium, Ventricle, Purkinje fibres, SAN</td>
</tr>
<tr>
<td>$I_{Kur}$</td>
<td>Fast</td>
<td>None</td>
<td>Atrium</td>
</tr>
</tbody>
</table>

Table 1.1: Voltage-gated $K^+$ currents/channels in the human heart.
with the lowest density being in the mid-myocardial cells [69–72]. Its density in the epicardial and endocardial cells far exceeds its density in the mid-myocardial cells [22,24,57]. It thus contributes to the transmural dispersion of the action potential duration (APD) across the ventricular wall. It shows activation but no inactivation and is important for regulating the APD. Activation is slow but deactivation is (comparatively) fast [70,71,73,74]. It contributes to phase 3 of the AP where it serves to repolarise the cell back to its resting potential following a depolarisation. The channel is very selective to K$^+$ ions (although less so than $I_{Ks}$) and is largely carried by K$^+$ ions [70,71,73,74]. The fully-activated current-voltage relation approaches linearity [70,71,73,74].

1.7.1.3 The Rapidly-activated Delayed Outward Rectifier Current ($I_{Kr}$)

In the human heart, $I_{Kr}$ has been found in all cell types including the atrial and ventricular cells [22,24,57]. It is differentially expressed across the ventricular wall with the highest density of its pore-forming transcript being in the epicardial cells [75]. Its transcript density in epicardial cells exceeds that in the mid-myocardium by approximately 60% [75]. It thus contributes to the transmural dispersion of the APD across the ventricular wall. It shows slow activation but fast and profound inactivation and is important for regulating the action potential duration [76–79]. Activation is moderately fast but deactivation is slow. It contributes to phase 3 of the AP where it serves to repolarise the cell back to its resting potential following a depolarisation. The channel is very selective to K$^+$ ions (more so than $I_{Ks}$ but less than $I_{K1}$) and is largely carried by K$^+$ ions.

1.7.1.4 The Ultrarapidly-activated Delayed Outward Rectifier Current ($I_{Kur}$)

In the human heart, $I_{Kur}$ is found in atrial cells but not in the ventricles [80–82] and contributes to the shorter APD in the atria, SAN and AVN cells [22,24,57]. It shows fast activation (faster than $I_{Kr}$ and $I_{Ks}$) and little inactivation [22,24,57]. Its fully-activated I-V relation shows outward rectification. Its recovery from inactivation is slow, and at high stimulation rates, its density is reduced [24].
1.7.2 Inward Rectifier Potassium Channels

1.7.2.1 The Inward Rectifier K⁺ Current (I_{K1})

I_{K1} is absent in the SAN but highly expressed in ventricular and purkinje cells but less so in atrial cells [22,24,57]. In the atria, it is still sufficiently expressed to produce a stable resting potential [22,24,57]. The channel is able to carry a substantial amount of current at negative potentials but little outward current at potentials more positive than ~-40 mV. It also shows strong inward rectification with a region of negative slope, allowing it to contribute to the maintenance of a stable resting potential (protection from pacemaker activity) without generating excessive repolarising current during the plateau phase or phases 0 and 1. The channel thus plays an important role during the plateau phase and in the final repolarisation portion of phase 3 [22,24,57]. Of all the K⁺ channels, it has the highest selectivity for K⁺ ions [24,57].

1.7.2.2 Acetylcholine-activated K⁺ Channel (I_{K,Ach})

I_{K,Ach} is highly expressed in the SAN, AVN and atria cells but has low expression in the ventricle [22,24,57]. Its activation by acetylcholine results in a weakly inward-rectifying K⁺ current (inward rectification is weaker than that for I_{K1}) that hyperpolarises the cell and shortens the APD. It is activated when acetylcholine binds to the M2 muscarinic receptor, which is coupled to the K⁺ channel via the guanine nucleotide binding protein (G protein) [22,24,57]. It deactivates on depolarisation but incompletely.

1.7.2.3 ATP-senstitive K⁺ Channel (I_{K,ATP})

I_{K,ATP} is expressed abundantly in all regions of the heart including ventricular, atrial, SAN and AVN cells [22,24,57]. It is activated according to the intracellular ratio of ADP to ATP, i.e., [ADP]:[ATP]. This ratio is increased during ischaemia by energy depletion. It consequently protects the heart by shortening the APD, reducing excitability and moving the membrane potential closer to the K⁺ equilibrium potential. Channels for I_{K,ATP} are very selective for K⁺ ions [22,24,57].
1.8 Stretch-activated Channels

A sequence of electrical excitation waves propagating in the heart trigger its mechanical contraction. This is known as excitation-contraction coupling [25,57]. In response to changes in volume load or contractile function, the heart is able to regulate its cellular electrical activity [83–86]. This is commonly referred to as mechano-electric feedback [85,87–89]. The stretch-related electrical activities that are regulated include prolongation [90–92] or shortening [93–96] of the AP, changes in AP morphology such as diastolic depolarisation [97,98], premature excitation [95,98,99] and after-depolarisation [89,95,100]. These changes occur via the activation of stretch-activated channels (SACs). Additionally, stretch induces a rise in the amplitude of the intracellular Ca\(^{2+}\) concentration [91,101–106]; a change that is related to contractile force in the heart.

1.9 Cardiac Muscle Contraction

1.9.1 Cardiac Muscle Fibre

Cardiac muscle just like skeletal and smooth muscles is composed of fibres, which in turn are composed of successively smaller subunits. The typical organisation of muscle fibres is shown in Figure 1.13. The cell membrane of the muscle fibres is called the sarcolemma. It consists of two parts; an inner plasma membrane (sometimes called the true membrane) and an outer membrane made up of numerous thin collagen fibrils [19,57,107].

1.9.1.1 Myofibrils; Actin and Myosin Filaments

Each muscle fibre (Figure 1.13B) consists of thousands of myofibrils clustered in groups (Figure 1.13C). Each myofibril in turn consists of thousands of thick myosin and thin actin filaments lying adjacent to each other (Figure 1.13D, E); these are responsible for muscle contraction. The ends of the actin filaments are embedded in a structure called the Z-line or Z-disc (Figure 1.13E) from which the actin filaments extend in both
Figure 1.13 The structure of striated muscle

Striated muscle tissue, such as the tissue of the human heart and biceps muscle, consists of long, fine fibres (A,B), each of which is in effect a bundle of finer myofibrils (C,D). Within each myofibril are filaments of the proteins myosin and actin (E); these filaments slide past one another as the muscle contracts and expands. On each myofibril, regularly occurring dark bands, called Z lines, can be seen where actin and myosin filaments overlap. The region between two Z lines is called a sarcomere; sarcomeres can be considered the primary structural and functional unit of muscle tissue [108].
directions, interlocking with the myosin filaments. The part of the myofibril that lies between two Z-lines is called the sarcomere.

Projecting from the sides of the myosin filaments are cross-bridges (Figure 1.14). It is the interaction between the crossbridges and the actin filaments that causes contraction [19,25,107]. The Z-disc or Z-line traverses the myofibril crosswise and also traverses crosswise from myofibril to myofibril and in doing so, attaches each myofibril to the other, hence, the clustered groups of myofibrils seen in Figure 1.13B and C. The oblique traversal of the Z-line across the muscle fibre results in light and dark bands, which gives cardiac and skeletal muscle a striated appearance [19,25,107] (Figure 1.14).

Figure 1.13E shows that the actin and myosin filaments overlap and interlock. This gives rise to light and dark bands on the myofibril just as the Z-line results in light and dark bands across the muscle fibre. The light bands contain only actin filaments and due to being isotropic to polarised light are called I bands [19,25,107] (Figure 1.15). The dark bands are called A bands because they are anisotropic to polarised light [19,25,107]. They consist of myosin filaments and the ends where the actin filaments overlap the myosin filaments. The H zone is the portion of the A band where the actin and myosin filaments do not overlap [19,25,107].

Figure 1.16 shows a myofibril in a relaxed and contracted state. On contraction, the sarcomere length (the portion between two successive Z-lines) is approximately 2µm [25,107]. At this length, the thin actin filaments are drawn much closer than shown in the bottom figure of Figure 1.16. The maximum contractile force is also generated at this length [25,107].

1.9.1.2 Sarcoplasm, Transverse Tubule and Sarcoplasmic Reticulum and the General Mechanism of Contraction

The intracellular space between the thousands of myofibrils making up a muscle fibre is filled with the fluid called sarcoplasm; it consists of large amounts of potassium, magnesium, phosphate and protein enzymes [19,25,107]. Mitochondria, which align themselves parallel to the fibre are also present in abundant amounts. These serve
Figure 1.14: The arrangement of the myofilaments in obliquely striated muscle [109].

Figure 1.15: A cross section of a muscle fibre [110].
Figure 1.16: Myofibril in relaxed and contracted states. The thin filaments (actin) in a relaxed non-contracted state (top) slide and overlap the thick myosin filaments (bottom) pulling the Z-lines towards each other. The thin actin filaments are able to completely overlap the thick myosin filaments [111].

the purpose of providing energy for the contracting myofibrils via the formation of adenosine triphosphate (ATP) [19,25,107].

The transverse tubule (T-tubule) originates from the cell membrane and traverses across the myofibrils from one side all the way across to the other [19,25,107] (Figure 1.17). From its starting point at the cell membrane, it is open to the same extracellular fluid as the cell membrane, implying that the T-tubule is an extension of the cell membrane [19,25,107]. Thus an action potential spreading over a muscle fibre membrane causes potential changes that also spread through the T-tubule to the interior of the muscle fibre. The resulting electrical excitation is what initiates muscle contraction [19,25,107].

The sarcoplasmic reticulum is shown in yellow in Figure 1.17. It completely covers the surface of the myofibrils. It contains an excess of calcium ions. When the action potentials from the cell membrane reach the T-tubules, it causes the opening of the
calcium ion channels of the sarcoplasmic reticulum terminals, which touch the T-tubules. The spread of electrical excitation consequently activates nearby calcium channels of the other parts of the sarcoplasmic reticulum. The T-tubules themselves contain calcium ions, which are also released into the sarcoplasm [19,25,107]. Calcium ions thus flow out of the sarcoplasmic reticulum into the sarcoplasm, diffuse into the myofibrils and catalyse the reactions that cause the sliding of the actin and myosin filaments along one another and cause muscle contraction [19,25,107].

As long as calcium ions remain in high concentration in the sarcoplasm, muscle contraction persists. However, pumps in the walls of the sarcoplasmic reticulum pump calcium ions back into it and in addition, calsequestrin, which is a protein in the sarcoplasmic reticulum also binds to some of the calcium ions. The end of phase 3 of the action potential (plateau phase) is approximately when the flow of calcium ions into

![Figure 1.17: Ultrastructure of a group of myofibrils, showing the sarcoplasmic reticulum and transverse tubules, which constitute the two membrane systems within a muscle fibre [112].](image)

...
the sarcoplasm ceases and is rapidly pumped back into the sarcoplasmic reticulum and the extracellular fluid space of the T-tubules. These mechanisms ensure that muscle contraction terminates and only restarts at the commencement of a new action potential.

1.9.2 Molecular Mechanism of Contraction

1.9.2.1 Myosin Filament

The thick myosin filament consists of multiple myosin molecules. Each myosin molecule (Figure 1.18) consists of two heavy chains and four light chains. The two heavy chains wrap around each other in a double helix called the tail or rod. The end of each heavy chain is folded into a globular structure called a head. Thus, the myosin molecule consists of two heads. Each head contains two light chains making up the four aforementioned light chains [19,25,107].

The collection of multiples of the myosin molecules forms the myosin filament shown in the middle of Figure 1.18. The heads of the myosin molecules protrude from the body of the myosin filament via rope-like arms. The arms together with the protruding heads are called cross-bridges [19,25,107]. The arms impart the flexibility that allows the heads to be extended far away from the body of the myosin filament or drawn closer to it. The point where the arm attaches to the head also allows movement of the head enabling it to participate in the contraction process [19,25,107].

1.9.2.2 Actin Filament

Figure 1.18 shows the myosin filament interacting on both sides with two actin filaments. The actin filament consists of three protein components: actin, tropomyosin and troponin [19,25,107]. The actin protein molecule consists of other proteins that enable it to carry one molecule of adenosine diphosphate (ADP), which are believed to be the sites on the actin filament with which the cross-bridges on the myosin filament interact to cause contraction [19,25,107]. These ADP sites are staggered across the actin filament.
Figure 1.18: The structure of actin and myosin filaments [113].

The tropomyosin constituent (shown in blue in Figure 1.18) of the actin filament wraps around the actin protein constituent. It covers the active ADP sites of the actin filament in the resting state, so that interaction between the actin and myosin filaments cannot occur, consequently hindering contraction [19,25,107].

Attached at intermittent intervals to the tropomyosin protein is troponin, which itself is a complex of three loosely bound protein subunits; Troponin I which binds strongly with actin, Troponin T with a high affinity for tropomyosin and Troponin C which binds strongly with calcium ions [19,25,107]. The troponin complex attaches tropomyosin to actin and its strong binding with calcium ions initiates the contraction process [19,25,107].

1.9.2.3 Cross-Bridge Cycling or The “Walk-Along” Theory of Contraction

In the presence of the troponin-tropomyosin complex, contraction is inhibited because the tropomyosin protein covers the active ADP sites on the actin filament. The role of the calcium ions is to remove the inhibitory effect of the troponin-tropomyosin complex [25,107]. It is believed that the binding of troponin C with calcium ions makes the troponin complex undergo a conformational change which drags the tropomyosin
deeper into the recesses between the actin strands, thus uncovering the active ADP sites [25,107]. Once uncovered, the actin filament binds strongly with the cross-bridges on the myosin filament permitting contraction to proceed.

The precise mechanism by which the binding of the actin filament with the myosin filament leads to contraction is still a subject of much research but a hypothesis with considerable evidence is “cross-bridge cycling” or the “walk-along” or “ratchet” theory of contraction [25,107]. This idea is depicted in Figure 1.19 with two myosin heads attaching to and disengaging from the active ADP sites of an actin filament. It is postulated that when a myosin head attaches to an active site, profound changes in the intermolecular forces between the myosin head and its arm occur (i.e., within the cross-bridge). These forces cause the myosin head to incline towards the active site, attach to it and with the force of inclination, to drag the actin filament forwards. This tilt of the head is known as the power stroke. Once complete, the myosin head unfastens from the active site and extends back to its original angle, whence it attaches again to the next active site farther down the actin filament and drags that forward with another power stroke. As this cycle continues, the ends of two successive actin filaments are moved towards the center of the myosin filament. The cross-bridges are thought to work independently of one another in carrying out this power stroke cycle implying that the greater the number of cross-bridges attached to the actin filament active sites, the greater, theoretically, the force of contraction [25,107].

**1.9.2.4 The Source of Energy for Contraction: ATP**

Muscle contraction requires energy and ATP is the source of this energy. When contraction occurs, ATP is broken down into ADP and phosphate ions. The greater the amount of work carried out by the muscle, the greater the amount of ATP that is broken down. This phenomenon is known as the Fenn Effect [107,114,115].

The process involved in obtaining the energy for contraction is thought to be as follows:

1. An additional characteristic of the myosin head is that it acts as an ATPase enzyme, which breaks down ATP into ADP and a phosphate ion. It does this prior to the commencement of contraction when the cross-bridges bind to ATP.
The ADP and phosphate ion are left on the myosin head, which extends towards the active site on the actin filament but does not yet bind to it [25,107].

2. When calcium ions are released into the sarcoplasm from the sarcoplasmic reticulum and the T-tubules, the troponin-tropomyosin complex binds to the calcium ions leaving the active sites on the actin filament uncovered. This allows the myosin head to attach to the active site [25,107].

3. The energy stored from the breakdown of ATP in step 1 is then used by the head of the bound cross-bridge to generate the power stroke that allows the myosin head to move the actin filament towards the center of the myosin filament [25,107] (see Section 1.9.2.3).

4. Once the myosin head tilts and drags the actin filament forward, the ADP and phosphate ion are released from it. Another ATP molecule then attaches to the now empty ADP site. This ATP binding causes the detachment of the myosin head from the actin filament [25,107].

Steps 1-4 are then repeated again and again until the Z-line is pulled up against the ends of the myosin filament or until the load on the muscle becomes too great for further pulling to occur [25,107].

Figure 1.19: “Walk-along” mechanism for muscle contraction
Chapter 2

Potassium Channels Implicated in the Short QT Syndrome

The work presented in this thesis centres on the *in silico* investigation of arrhythmia substrates in an inherited cardiac condition: the short QT syndrome (SQTS). Chapter 3 provides detailed background information on this syndrome (which consequently will be discussed only briefly here). The three variants of the SQTS examined in this thesis – SQT1, SQT2 and SQT3 – involve gene mutations that affect proteins forming different potassium channels [8,116–120]. SQT1 affects the *hERG* channel, which is responsible for the rapid-delayed outward rectifier potassium current (*I*$_{Kr}$) [121–123]. SQT2 affects the *KCNQ1* gene, which encodes the 6-subunit of channels mediating slow-delayed outward rectifier potassium channel (*I*$_{Ks}$) [124]. SQT3 affects *KCNJ2*, which encodes the Kir2.1 protein that contributes to inwardly-rectifying potassium channel current (*I*$_{K1}$) [125]. Building upon the brief descriptions of these channels in Chapter 1, this chapter gives a detailed consideration of their structure and characteristics.

2.1 Rectification

In section 1.3, it was established that the lipid bilayer of the cell membrane can be thought of as a capacitor that separates charges in the intracellular and extracellular regions. The charges separated are the ions in intracellular/extracellular fluid that can flow across the membrane through ion channels, when these are in a conducting state. The ion channels are thus resistors. Simple resistors follow Ohm’s law:

\[ V = IR \]  

where *V* is the voltage (membrane potential), *I* is the current and *R* is the resistance. To describe the biophysical properties of ion channels during voltage clamp experiments,
Figure 2.1: Schematic representation of the I-V relationship for an ohmic channel and channels showing rectification. The black dashed line represents an ohmic channel with a linear I-V relationship. The green line shows the I-V relationship for $I_{Kr}$, which shows outward rectification. The blue line represents the I-V relationship for $I_{Ks}$, which also shows outward rectification and the red line represents $I_{K1}$, which is an inwardly rectifying current because it passes current preferentially in the inward direction. Note that currents are not drawn to scale.
the conductance of the channels (inverse of resistance) is normally employed. If an ion channel has a linear current-voltage (I-V) relationship, i.e., the slope (conductance) is linear, the channel is said to show ‘ohmic’ behaviour (Figure 2.1).

When the I-V relationship of the channel is nonlinear, i.e., channel conductance changes with voltage, the channel is said to show voltage-dependent rectification (Figure 2.1). In this event, the channel passes current preferentially in one direction over another. For example, Figure 2.1 shows a schematic representation of the I-V relationships for four channels: an ohmic channel, hERG/I_{Kr} (outward rectifier), KCNQ1-KCNE1/I_{Ks} (outward rectifier) and KCNJ2/I_{K1} (inward rectifier). hERG/I_{Kr} and KCNQ1-KCNE1/I_{Ks} pass current preferentially in the outward direction while KCNJ2/I_{K1} passes current preferentially in the inward direction over the outward direction.

### 2.2 The hERG/I_{Kr} Potassium Channel

The significance of the hERG/I_{Kr} channel for normal human cardiac electrical activity was discovered when inherited gene mutations to hERG resulted in long QT syndrome (LQTS) [126–128]; a cardiac repolarisation disorder. In the LQTS, patients have a lengthened QT interval on the ECG and become susceptible to the potentially fatal arrhythmia *torsades de pointes* [10,129,130]. hERG is also now known to be responsible for pharmacologically induced (“acquired”) Long QT syndrome [129,131–133]. This is due to structural features of the channel that have been established to render it particularly susceptible to pharmacological blockade [10,129,134,135]. The consideration of hERG here focuses on its electrophysiological properties and for detailed consideration of the basis of its pharmacological promiscuity the reader is referred to [10,129,134,135]. Some antihistamines and antibiotics have also been known to cause arrhythmia and sudden death through blockade of the hERG/I_{Kr} channel [136].

hERG/I_{Kr} is expressed in several tissue and cell types including cardiac [127], neurons, neuroendocrine glands [137,138], smooth muscle [139] and tumour cells [140,141]. Its expression is greatest in cardiac cells and this is the region that has received the greatest research focus and from which its properties and function are best understood. Heterologous expression studies have shown that hERG encodes the α-subunit of the I_{Kr} channel [127,128,142–144].
2.2.1 hERG/I_{Kr} Potassium Channel Gating

hERG/I_{Kr} plays a significant role in cardiac action potential repolarisation; it is the channel that is largely responsible for the early and middle stages of ventricular action potential repolarisation (phase 3; Figure 1.1) after which I_{K1} (being responsible for terminal repolarisation) takes over, bringing the membrane potential back to its normal resting value (phase 4; Figure 1.1) [127,128]. It is characterised by slow activation but fast and profound voltage-dependent inactivation [79,128,142,145–147]. Its inactivation (a non-conducting state) is considerably faster than its activation.

Figure 2.2 shows characteristic features of I_{hERG}. With depolarisation to more positive membrane potentials, outward I_{hERG} is elicited, which increases rapidly and then plateaus for the duration of the pulse. A step repolarisation of the membrane potential (to a negative potential) results in a resurgent increase in I_{hERG} (tail current); this increase in I_{hERG} is despite the decrease in the driving force for K^+ out of the cell membrane. This is due to the rapid recovery of I_{hERG} from inactivation [127,128,145]. Eventually, via channel deactivation, I_{hERG} decreases bi-exponentially [148].

Further insight can be gleaned from the current-voltage (I-V) relation of I_{hERG}/I_{Kr} (Figure 2.3). At the membrane resting potential (~80 mV), hERG/I_{Kr} channels are closed and in that state conduct no current. As the membrane is depolarised to more positive membrane potentials greater than ~-60 mV, the channels get activated (open) and conduct current (Figure 2.3); K^+ ions flow out of the cell across the cell membrane according to their electrochemical gradient. At the same time, the channel begins to inactivate. As hERG/I_{Kr} inactivation with progressive depolarisation is faster than its activation, channel inactivation with increasing depolarisation eventually overtakes channel activation resulting in the channel entering the non-conducting, inactivated state. This gives the current-voltage relationship a region of negative slope (Figure 2.3). This helps to prolong phase 2 of the action potential. Eventually, the channel begins to deactivate (close) and also begins to recover from inactivation. However, recovery from inactivation for hERG/I_{Kr} is faster than deactivation, thus allowing the channel to again
Figure 2.2: Representative current trace for hERG elicited by a standard voltage protocol from a hERG-expressing Chinese Hamster Ovary (CHO) cell (recording made at 37°C). Outward $I_{\text{hERG}}/I_{\text{Kr}}$ current is elicited when the membrane potential is stepped to 20 mV from a holding potential of -80 mV. On stepping the membrane potential to -40 mV, $I_{\text{hERG}}/I_{\text{Kr}}$ (tail current) of greater amplitude than that seen at the 20 mV are produced. Modified from [147].

Figure 2.3: Current-voltage (I-V) relationship for $I_{\text{hERG}}/I_{\text{Kr}}$ (recorded in Xenopus oocytes). Depolarisation activates $I_{\text{hERG}}/I_{\text{Kr}}$ causing an increase in outward current. At around 0 mV, $I_{\text{hERG}}/I_{\text{Kr}}$ reaches a maximum then declines in amplitude with further depolarisation (negative slope region). Modified from [127].
conduct current, which repolarises the cell membrane (phase 3 of the action potential), returning it to its resting state (phase 4).

### 2.2.2 hERG/I\textsubscript{Kr} Channel Structure

The hERG/I\textsubscript{Kr} K\textsuperscript{+} channel is a comprised of a protein tetramer consisting of four identical \(\alpha\)-subunits [142,149–151]. Each subunit contains six \(\alpha\)-helical transmembrane domains (S1-S6). Functionally, each \(\alpha\)-subunit can be divided into two parts: a voltage-sensing region and a pore-forming K\textsuperscript{+}-selective filter (Figure 2.4). Segments S1-S4 form the transmembrane potential sensor region with S4 in particular having positive charges, which allow it to react to a change in membrane potential by moving its position [72,152–154]. Voltage-sensor movement leads to conformational changes that open, inactivate or close the channel (Figure 2.5). Segments S5-S6 form the ion permeation pathway and consist of the P-loop (S5) that penetrates the membrane and creates a pore through which ions enter or leave. Together, these traits confer voltage dependence and ion selectivity on the channel [72,152–154].

Below the P-loop is a water-filled cavity that is lined by S6 \(\alpha\)-helices. In a conducting (open) state, i.e., in response to membrane depolarisation, all four S6 \(\alpha\)-helices are spread out allowing the passage of K\textsuperscript{+} ions. They crisscross in the closed state, forming a narrow cleft that blocks entry of K\textsuperscript{+} ions [152]. hERG also has a pair of intracellular termini (an N terminus and a C terminus), which contribute to its function. The N-terminus is responsible for channel deactivation following membrane depolarisation while mutations to the C-terminus affect trafficking and disrupt the processing of hERG channels [155,156].

Voltage-gated K\textsuperscript{+} channels can inactivate via two different mechanisms: rapid ‘N-type’ inactivation (also known as “ball and chain” type) and slow ‘C-type’ inactivation. N-type inactivation occurs when a ball-like structure (an intracellular protein segment) anchored to the channel’s N-terminus blocks the channel pore [145] whereas ‘C-type’ inactivation has been suggested to occur via a slight narrowing of the K\textsuperscript{+}-selectivity filter [157] at the extracellular mouth. Deletion of the N-terminus removes ‘N-type’ inactivation but has no significant effect on hERG inactivation [145,158] suggesting
Figure 2.4: Schematic diagram showing a representative hERG/I\textsubscript{K\textalpha}, \(\alpha\)-subunit. hERG channels consist of four identical \(\alpha\)-subunits. Each subunit contains six transmembrane segments S1-S6. S4 has positively charged amino acids and acts as the main voltage sensor for transmembrane potential changes. The blue cylinder indicates the pore of the P-loop that acts as a \(K^{+}\) selectivity filter by blocking or unblocking the pore. Intracellularly, the \(\alpha\)-subunit has N and C termini.

Figure 2.5: Membrane depolarisation to voltages more positive than ~60 mV activates (opens) the channel slowly. With greater depolarisation, the channel rapidly inactivates (it undergoes C-type inactivation – a slight constriction of the \(K^{+}\) selectivity filter). Repolarisation reverses the whole process.
that hERG inactivates via ‘C-type’ inactivation. This was confirmed by removing ‘C-type’ inactivation via the Ser631 Val mutation [159], which resulted in the elimination hERG inactivation. In addition, application of extracellular tetraethylammonium (TEA) blockade but not intracellular TEA blockade has been shown to slow inactivation suggesting that the process involves changes towards the channel exterior [160].

In addition to the four α-subunits, hERG also interacts with a β-subunit, MiRP1 (minK-related protein 1) in heterologous expression systems. The expression of MiRP1 in atrial or ventricular tissue is quite low but it is highly expressed in Purkinje fibres and the atrial pacemaking cells [161,162]. The effect of this co-expression of the hERG α-subunit and MiRP1 is to reduce the trafficking of the channel to the cell membrane surface, reduce channel conductance and to increase channel deactivation [163]. As MiRP1 expression is low outside of the conduction system [164], it has been suggested that it may not function as hERG β-subunit in other regions and comparison of hERG with and without MiRP1 has shown that when hERG is heterologously expressed in mammalian instead of amphibian cells (Xenopus oocytes), it adequately recapitulates I_{Kr} [165]. Also, MiRP1 can co-assemble with a variety of other cardiac ion channels [164,166], and so may exhibit broad interactions rather than being specific for hERG.

hERG is heteromeric and consists of two isoforms: hERG 1a (the major isoform) and hERG 1b, which differ by a truncated N-terminus in the latter. This heteromeric form of hERG (hERG 1a/1b) has recently been proposed to recapitulate native I_{Kr} more accurately than hERG 1a expressed alone [167,168].

### 2.3 The KCNQ1-KCNE1/I_{Ks} Potassium Channel

Similar to the hERG/I_{Kr} potassium channel, the KCNQ1 potassium channel also plays a significant role in cardiac repolarisation. The KCNQ1 α-subunit co-expresses with the KCNE1 β-subunit (minK) [72,169,170], the result of which is a protein complex that is responsible for the slow-delayed rectifier potassium channel I_{Ks}, which is partially responsible for action potential repolarisation (phase 3) along with hERG/I_{Kr} [72,169,170]. In the event of an impairment to I_{Kr}, e.g., channel block or gene mutation, I_{Ks} is a key component of repolarisation reserve [71,72,171]. A *loss-of-function*
mutation to the channel results in the first variant of the long QT syndrome LQTS1 [172–174].

In the ventricular wall, KCNQ1-KCNE1/I_{Ks} is heterogeneously distributed with its expression being very low in the mid-myocardial cells [71,175–177]. Consequently, mid-myocardial cells have the longest APD and repolarise later than epicardial and endocardial cells. This leads to a transmural dispersion of repolarisation across the ventricular wall [70,178,179] (meaning repolarisation occurs at different rates in the different cell types that make up the wall of the myocardium), which is a substrate for re-entry. Re-entry is the continuous (usually several cycles) re-excitation of a cardiac tissue region by a single electrical signal and it usually results in arrhythmias.

In addition to being expressed in cardiac tissue, the \textit{KCNQ1} protein is also expressed in epithelial tissue of different organs: stomach, cochlea, lungs, intestine and kidney. Its function in these tissue types is the transport of salt and water [175]. In humans, impairment to \textit{KCNQ1} expression in epithelial tissue has been known to cause deafness [175,180,181], while in knockout mice, it causes deafness, balance problems and morphological abnormalities in the gastrointestinal tract and inner ear [175,182,183].

\subsection*{2.3.1 KCNQ1-KCNE1/I_{Ks} Potassium Channel Gating}

Similar to hERG, KCNQ1 is a voltage-gated channel, though its kinetic properties differ considerably from those of hERG (Figure 2.8). Depolarisation activates (opens) KCNQ1 channels but very slowly. Upon further depolarisation, a fraction of the open channels inactivates [71,72,184–186] (Figure 2.6). The channel is also characterised by slow deactivation kinetics [71,72,184–186]. Recovery from inactivation, however, is faster than deactivation and is seen as a hook on the current trace during the repolarisation (Figure 2.6). On co-expression with KCNE1, thus reproducing I_{Ks}, the current is enhanced (Figure 2.6) because KCNE1 increases the single channel conductance causing a positive shift in voltage activation threshold, i.e., KCNE1 slows activation (Figure 2.6) considerably and inactivation is completely eliminated [71,142,185]. Figure 2.7 shows the tail current I-V relationship for KCNQ1-KCNE1/I_{Ks} measured from peak tail current to complete deactivation.
Figure 2.6: Representative superimposed current traces for KCNQ1 (purple) and KCNQ1+KCNE1 (black) elicited by a standard voltage protocol from KCNQ1-expressing oocytes (recording made at 36°C). The left arrow shows the slower activation of KCNQ1 when co-expressed with KCNE1 when the membrane potential is stepped to 60 mV from a holding potential of -80 mV. On stepping the membrane potential to -60 mV, KCNQ1-KCNE1/I_{Ks} (tail current) deactivates more slowly than KCNQ1 (right arrow). Modified from [74].

Figure 2.7: I-V relationship of I_{Ks} tail current (recording in HEK 293 cells at 36°C) measured from peak tail current to complete deactivation. Depolarisation activates KCNQ1-KCNE1/I_{Ks} causing an increase in outward current. Modified from [187].
Figure 2.8: A structural representation of the α and β-subunits KCNQ1-KCNE1/I_{Ks}. KCNQ1 channels consist of four identical α-subunits. Each subunit contains six transmembrane segments S1-S6. S4 has positively charged amino acids and acts as the main voltage sensor for transmembrane potential changes. The blue cylinder indicates the pore of the P-loop that acts as a K⁺ selectivity filter by blocking or unblocking the pore. KCNQ1 has intracellular N and C termini.
2.3.2 KCNQ1-KCNE1/I_Ks Channel Structure

Structurally, KCNQ1 is a voltage-gated potassium channel like hERG described in section 2.2.2 above. It consists of four identical subunits, each of which is comprised of six α-helical transmembrane domains (S1-S6). Segments S1-S4 form the voltage sensor while segments S5 and S6 form the ion permeation pathway with a pore loop and K⁺ selectivity filter [169,170,175,188] (Figure 2.8). Each α-subunit also has intracellular ‘N’ and ‘C’ terminals. A major difference from hERG is KCNQ1’s co-assembly with KCNE1 β-subunit, which together encode the human I_Ks. A representation of the KCNQ1-KCNE1 structure is shown in Figure 2.8. The effect of the KCNE1 subunit is to stabilise the open state of the channel by altering the interaction between the pore loop, the K⁺ selectivity filter and the S5/S6 segment [189]. However, the stoichiometry of KCNQ1:KCNE1 is still a matter of debate because there is no extant crystal structure for KCNQ1 with or without KCNE1 [73,190]. Stoichiometries of 4:4 [191], 4:2 [192] and other forms [186,190] have been proposed. A recent review can be found in [190].

2.4 The KCNJ2/I_K1 Potassium Channel

The KCNJ2-encoded Kir2.1 protein belongs to the family of inwardly rectifying potassium (Kir) channels [193,194]. Specifically, it belongs to the Kir2.x channel family. It is expressed in skeletal muscle, blood vessels, neurons and richly in cardiac tissue, where it is expressed in Purkinje fibres, atrial and ventricular tissues [194–199]. This family of channels allows K⁺ to flow more easily into the cell than out of it. They therefore, preferentially pass current in the inward direction over the outward direction. A schematic representation of the I-V relation for I_K1 is shown in Figure 2.1 (red line) and an experimentally observed I-V relation is shown in Figure 2.9.

Unlike voltage-gated K⁺ channels such as I_Kr and I_Ks, the behaviour of Kir channels depends not only on the membrane potential but also predominantly on the electrochemical gradient of K⁺ in the cell, i.e., the difference between the membrane potential and the K⁺ reversal potential (E_K). They therefore have greater K⁺ conductance at potentials negative to E_K and pass comparatively little current at depolarised membrane potentials, i.e., potentials positive to E_K (see red line in Figure
Figure 2.9: Current-voltage (I-V) relations of the KCNJ2-encoded Kir2.1 channel expressed in HEK cells elicited by the ascending voltage ramp command shown inset. Modified from [200].
2.1 and Figure 2.9) [193–195,201–204]. As membrane depolarisation increases further, the outward $I_{K1}$ current decreases due to its inward rectification.

These characteristics make myocardial cells expressing $I_{K1}$ have resting potentials close to $E_K$ whereas cells without $I_{K1}$ or those with an insignificant expression of $I_{K1}$ have depolarised resting potentials and tend to show spontaneous activity, e.g., the SAN [205]. This is because with little $I_{K1}$ present, the cells have high membrane resistances at negative voltages and so small changes in current can produce substantial changes in voltage (i.e. membrane potential is more ‘labile’ in the absence of $I_{K1}$). Consequently, in cardiac cells, $I_{K1}$ plays a role in stabilizing the resting potential of the cell and in the duration of the action potential [193–195,201–204]. As $I_{K1}$ channels pass little outward current at depolarised potentials, there is little $K^+$ efflux through $I_{K1}$ channels during phase 2 (the plateau phase) of the ventricular action potential [206] (Figure 2.10B); therefore, the rectification property of $I_{K1}$ channels also serves to maintain membrane depolarisation, thereby facilitating prolonged action potential duration. When phase 3 repolarisation commences (via $I_{Kr}$ and $I_{Ks}$) and the membrane becomes more and more hyperpolarised, relatively large outward $I_{K1}$ current is generated. This serves to accelerate terminal repolarisation [207–210]. Figure 2.10B shows the I-V relationship of $I_{K1}$ and relates it to the action potential, which is drawn sideways to give a better indication of the current amplitude at different phases of the action potential.

### 2.4.1 Kir2.1/$I_{K1}$ Potassium Channel Gating

The inward rectification of $I_{K1}$ and Kir2.x channels is due to blockade of outward $K^+$ movement through the channel pore by intracellular $Mg^{2+}$ ions and polyamines [194,204,211–214]. At depolarised membrane potentials, the $Mg^{2+}$ ions and polyamines such as spermine and spermidine, which are present in sub-micromolar quantities within the cell, reduce outward $K^+$ current. On hyperpolarisation, the $Mg^{2+}$ ions and polyamines unblock the pore, thereby leading to increased current. This inward current first increases time-independently due to the unblocking of $Mg^{2+}$ ions and then increases time-dependently due to unblocking of the polyamines. The unblocking of the $Mg^{2+}$ ions is fast while the polyamine unblocking is slow [194,215].
Figure 2.10. A: Structure of the Kir2.1/KCNJ2/I_{K1} channel. Each of its four α-subunits consists of two transmembrane segments (TM1 and TM2), a pore-forming loop (H5) and intracellular N and C terminals. B: I_{K1} current-voltage relationship related to the membrane action potential. The action potential is drawn sideways to give a better understanding of the contribution of I_{K1} during the action potential. The reader should focus on the repolarisation phase and resting potential (the non-dashed parts of the action potential).
2.4.2 KCNJ2/I\textsubscript{K\textsubscript{1}} Channel Structure

Functional Kir2.x channels (similarly to hERG/I\textsubscript{Kr} and KCNQ1/I\textsubscript{Ks}) also have four \(\alpha\)-subunits but each subunit consists of only two transmembrane segments, TM1 and TM2 (Figure 2.10A), which are highly homologous to the S5 and S6 domains of the K\textsuperscript{+} channels discussed previously [194,216,217]. These two segments are linked by the extracellular pore-forming loop or P-loop (H5), which acts as the K\textsuperscript{+} selectivity filter. The intracellular ‘N’ and ‘C’ termini are also present. Unlike hERG/I\textsubscript{Kr} and KCNQ1/I\textsubscript{Ks}, there is no voltage sensor region or S4 segment; hence the channel is not truly voltage-gated in a traditional sense, with voltage-dependence instead occurring through voltage-dependent channel block/unblock by Mg\textsuperscript{2+} and polyamines (as described above).

2.5 Current Profiles during an Action Potential

Figure 2.11 shows a schematic diagram of the current profiles of each potassium channel currents discussed in this chapter during a ventricular action potential. I\textsubscript{Kr} contributes during most of phase 3 repolarisation, I\textsubscript{K\textsubscript{1}} contributes later in phase 3 than I\textsubscript{Kr} while I\textsubscript{K\textsubscript{s}} contributes earlier during phase 3 and some part of phase 2.

![Figure 2.11: A schematic diagram of the current profiles of I_{Kr}, I_{Ks} and I_{K1} ionic currents during a ventricular action potential. Also shown are the major gene candidates responsible for these currents. Modified from [218].](Image)
Chapter 3

The Short QT Syndrome

3.1 Introduction

The Long QT Syndrome (LQTS) is well-established as a distinct pathological, clinical entity [51,127,128,219] as is its association with increased susceptibility to the polymorphic ventricular arrhythmia torsade de pointes [133,220,221]. It has a congenital form and an acquired form. The congenital form is due to genetic channelopathies while the acquired form occurs as a result of ion channel-blocking effects of cardiac-related or non-cardiac drugs [10,133–135]. It is characterised by an abnormally long QT interval on the ECG; rate-corrected QT (QTc) of >440-460 ms.

In contrast to LQTS, there are circumstances in which the QT interval can become abnormally short and in some instances, this is associated with an increased arrhythmia risk [222–225]. It is now established that QT interval shortening can either be ‘acquired’ or associated with cardiac ion channel gene defects [119,120,223–226]. Acquired QT interval shortening can be caused by several chemical agents, for example, exposure to glycosides increases intracellular Na$^+$ and Ca$^{2+}$, which increase outward Na-Ca exchange current during the phase 2 (plateau phase) of the action potential [227] leading to a shortening of the action potential. Clinically, this can be observed as digitalis-induced QT interval shortening in some patients [228–230]. Another acquired form of QT interval shortening is that associated with anabolic steroid use [231–233] and its abuse by bodybuilders and strength athletes [232,234]. In that instance, it is possible that androgen associated modulation of ion channels involved in ventricular repolarisation may account for the observed QT interval shortening seen in some strength athletes who abuse androgenic steroids [232,234].

SQTS associated with ion channel gene defects is a relatively new clinical entity when compared to the LQTS. In 2000, SQTS was first recognised as a distinct clinical syndrome by Gussak et al. [223] who identified three members of the same family with

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1 Portions of this chapter appear in [8].
QTc <300 ms. The youngest presented with syncope and paroxysmal atrial fibrillation (AF). A patient from an unrelated family underwent sudden death [223]. Thereafter, six members from two different families were identified with QTc intervals of 300 ms or less, shortened atrial and ventricular refractory periods and easily induced ventricular tachyarrhythmias [5]. The SQTS is characterised by an abnormally short QT interval on the ECG with QTc intervals that are typically less than 320 ms [116,235–237], tall and peaked T-waves and a wider than normal Tpeak-Tend duration (Figure 3.1), patients usually have structurally normal hearts and have shortened atrial and ventricular refractory periods, poor heart rate-adaptation, and exhibit increased susceptibility to atrial and ventricular arrhythmia and sudden death [5,9,117,118,238].

To date, five different forms of the SQTS have been identified: SQT1 to SQT5. A mutation to the KCNH2 (hERG) gene responsible for the SQT1 variant was the first to be identified in 2004 [121,122], followed by a mutation for KCNQ1 responsible for the SQT2 variant [124,239]. The SQT3 variant is due to a mutation in the KCNJ2 gene [125]. SQT4 and SQT5 are the most recently identified variants and are due to mutations to the CACNB2b and CACNA1C genes [59]. These mutations (SQT4 and SQT5) are linked to a mixed short QT/Brugada syndrome phenotype because in addition to shortening the QT interval, ST-segment elevation on the ECG is observed [59]. Figure 3.2 shows a schematic of the current profiles for the channels involving the KCNH2-, KCNQ1-, KCNJ2- and CACNB2b + CACNA1C-encoded subunits.

3.2 SQT1 and KCNH2

Mutations to the KCNH2 [137] gene are responsible for the first variant of the SQTS. The first and main of these was discovered during the genetic screening of two unrelated families, members of which exhibited the same hERG mutation. Both families manifested distinct substitutions at nucleotide 1764 of KCNH2. In one family, there was a C → G substitution while the other displayed a C → A substitution. Both substitutions led to a common amino-acid substitution in the KCNH2 product of the hERG K+ channel (Kv11.1), which was an asparagine to lysine exchange (N588K) at position 588 in the hERG channel protein [121]. Another independent study identified a third family with the same asparagine to lysine N588K amino-acid substitution [122]. Affected
Figure 3.1: Schematic representation (Ai, Bi) and example clinical ECG (Aii, Bii) of a normal and an SQTS patient. Patient A shows a normal QT interval on the ECG (Ai, Aii). Patient B has short QT syndrome (SQTS) and displays a shorter QT interval on the ECG including a taller and peaked T-wave (Aii, Bii). Modified from [237].
Figure 3.2: A schematic diagram of the current profiles of $I_{Kr}$, $I_{Ks}$ and $I_{K1}$ and $I_{Ca,L}$ ionic currents during a ventricular action potential. Also shown are the major gene candidates responsible for these currents. Modified from [218].

individuals displayed shortened rate corrected QT ($QT_C$) intervals (225 – 240 ms over the normal heart range), shortened atrial and ventricular refractory periods, atrial fibrillation (AF), easily induced ventricular fibrillation (VF) during programmed electrical stimulation, syncope and sudden death [121].

As noted in Chapter 2, fast and profound voltage-dependent inactivation causes the current elicited in membrane depolarisation to decline at voltages positive to about 0 mV [127,128,145,158] (and see Figure 2.3). This kinetic property limits the amount of current produced early during the AP [76,77,148]. $I_{hERG}/I_{Kr}$ reaches a maximum towards phase 3 of the ventricular AP. The S5 Pore (S5P) linker in which residue N588 resides plays a part in conferring inactivation to the channel [240–242], as do conformational changes in the mouth of the channel [144,146,158,243].

In SQT1, the N588K-hERG mutation attenuates inactivation of the channel (seen as non-rectification of the current on the I-V curve in Figure 3.3), leading to excessive current being generated earlier during the ventricular AP (more precisely during the
plateau phase), thereby shortening the AP [79,121,244]. AP clamp measurements conducted at 37°C have shown that this enhances hERG current early during atrial, ventricular and purkinje APs [245]. This is consistent with shortened APD, QT interval and increased incidence of AF in SQT1 patients [121,122]. Figure 3.3 shows the excessive current generated by the N588K-hERG mutation on the I-V relation while Figure 3.4 shows a schematic representation of APD shortening by the mutation.

The initial study performed by Brugada et al. [121] suggested that the N588K mutation might eliminate entirely inactivation of the channel with the I-V relation showing no rectification [121] (Figure 3.3). However, subsequent detailed kinetic studies conducted at both ambient [244] and physiological [79] temperatures show that inactivation is not eliminated by the N588K mutation but is attenuated and shifted to more positive voltages by ~ +60 mV to +90 mV. Accompanying this is a modest increase in Na+/K+ permeability ratio [79,244].

Recently, a second mutation in the KCNH2 gene was discovered in a 34-year old man [246]. It involved a single base pair G → A substitution, which resulted in an amino-acid substitution of histidine for arginine at codon 1135 (R1135H) in the hERG channel protein [246]. The R1135H mutation alters hERG gating kinetics by significantly attenuating deactivation resulting in a gain-of-function in the I_Kr channel [246].

A third mutation in the KCNH2 gene was also very recently discovered in a Chinese family [123]. It involved a single base pair C → T substitution at nucleotide 1853 (C1853T) of the KCNH2 gene, which resulted in an amino-acid substitution (threonine to isoleucine exchange) at position 618 (T618I) in the hERG channel protein [123]. The T618I mutation alters hERG gating kinetics by attenuating inactivation and increasing the rate of recovery from inactivation [123].

3.3 SQT2 and KCNQ1

Mutations to the KCNQ1 gene are responsible for variant 2 of the short QT syndrome (SQT2) [124,239]. The first clinical case of SQT2 was that of a 70-year-old male who
Figure 3.3: Current-Voltage (I-V) relationships for hERG and N588K-hERG. The N588K-hERG mutation attenuates the rectification of hERG. Modified from [121].

Figure 3.4: A schematic representation of the effect of the gain-of-function mutations of the $I_{Kr}$ channel (SQT1), $I_{Ks}$ channel (SQT2) and $I_{K1}$ channel (SQT3) on the normal action potential. The mutations shorten the action potential (green) and the QT interval. Modified from [247].
was resuscitated from VF [124]. He had a short QTc interval of 302 ms. Genetic screening revealed no abnormalities to KCNH2, KCNE2 or KCNE1 but showed a G919 → C base transition, which resulted in an amino acid substitution (V307L) in the pore helix of the KCNQ1 channel protein [124]. The mutation was found to shift negatively the voltage dependence of activation of the mutant KCNQ1 channels [124] and to accelerate the time course of current activation in comparison to wild type (WT) channels [124] (Figure 3.5). When these changes were incorporated into a human ventricular single cell model, the AP and QT intervals were shortened [124]. Subsequent detailed cell and tissue simulations involving the SQT2 mutation also showed marked ERP shortening and significant effects on the transmural distribution of the AP across the ventricular wall [248].

The second clinical case of SQT2 was found in a baby girl who had bradycardia and irregular heart rhythm in utero, which persisted after she was born (in 38 weeks) [239]. Her ECG showed no P or F wave and had a QT interval of 280 ms [239]. Genetic screening revealed a G → A substitution on codon 421 of the KCNQ1 gene, which resulted in an amino acid substitution (V141M) in the S1 domain of the KCNQ1 channel protein. The mutation was de novo as it was not present in her parents [239]. In a human ventricular cell model, the mutation shortened the AP while it arrested spontaneous activity in a rabbit SAN cell model [239]. Both mutations have recently been suggested to result in a marked negative shift of the voltage dependence of I_{Ks} channels and to a strong deceleration of current deactivation [249].

3.4 SQT3 and KCNJ2

In 2005, during a routine clinical examination, a 5-year-old child showed a QTc interval of 315 ms and a tall, narrow and peaked T-wave on the ECG [125]. Her father, who had a history of pre-syncopal events and palpitations also had a QTc interval of 320 ms and similar ECG T-wave characteristics [125]. Her mother exhibited a normal ECG [125]. On genetic analysis, a single base pair substitution (G514A) in KCNJ2 was discovered in both the child and her father. This led to an aspartate to asparagine (D → N; D172N) amino acid substitution in codon 172 of the Kir 2.1 K⁺ channel protein [125]. This case gave rise to the “SQT3” variant of the SQTS.
Figure 3.5: Tail current I-V relation for WT and V307L (recorded in COS-7 cells). The half-activation voltage ($V_{0.5}$) for V307L is more negative compared to WT implying that the V307L mutation accelerates channel activation. Modified from [124].

Figure 3.6: I-V relationship (recorded in CHO cells) for WT, WT/D172N (heterozygous mutant) and D172N (homozygote) mutant showing the increased outward current by the mutations compared to WT. Modified from [125].
The D172N residue lies in the ion conduction pathway of Kir 2.1 where it binds to polyamines and Mg$^{2+}$ ions [250]. This binding is responsible for the inward rectification of the current, which the D172N mutation impairs giving rise to an increased outward current and a modest rightward voltage-shift of the peak outward current [125,250,251] (Figure 3.6). The proband – the 5-year-old child – had a heterozygote mutation, i.e., a co-expression of WT and D172N channels which, when replicated in vitro, resulted in an outward current that is intermediate between WT and pure D172N expressed alone [125,251] (Figure 3.6).

In an AP model, the kinetic changes due to the Kir 2.1 D172N mutation led to AP shortening and steeper AP duration restitution [125]. The ECG T-wave characteristics of the mutation seen in the proband were also replicated using 1D tissue [125]. Results from in vitro action potential voltage clamp experiments have also shown that the heterozygote mutation possessed by the proband leads to increased I_K1 during terminal repolarisation of ventricular action potentials [251]; these experiments also showed that the mutation has the potential to affect the contribution of I_K1 to atrial repolarisation [251].

Very recently, a second heterozygous mutation in the KCNJ2 gene was discovered in an 8-year old girl [252]. It involved a single base pair C → T substitution at nucleotide 902 (c.902T>A) of the KCNJ2 gene, which resulted in an amino-acid substitution (methionine to lysine exchange) at position 301 (M301K) in the Kir2.1 K$^+$ channel [252]. The M301K mutation impaired inward rectification and consequently resulted in larger outward currents compared to WT [252].

**3.5 SQT4 and SQT5**

The SQT4 and SQT5 variants of the SQTS were discovered during genetic screening of patients with Brugada syndrome [59]. The classic feature of the Brugada syndrome phenotype is ST segment elevation in leads V1 to V3 on the ECG, which descends with upward convexity into an inverted T-wave [52,53,253]. SQT4 and SQT5 are therefore associated with a mixed Brugada-SQT phenotype. While SQT1, SQT2 and SQT3 affect repolarising K$^+$ currents (Figure 3.2 and Figure 3.4) and involve gain-of-function mutations, SQT4 and SQT5 affect the depolarising L-type Ca$^{2+}$ current (I_{ca,L}) (Figure
3.2) and involve loss-of-function mutations to channel subunits. SQT4 results from a loss-of-function mutation to \( CACNA1C \) (which is responsible for the \( \alpha_1 \) subunit of L-type channels) while SQT5 results from a loss-of-function mutation to \( CACNB2b \) (which is responsible for the \( \beta_{2b} \) subunit of L-type channels) [59]. Eighty-two probands with Brugada syndrome were genetically screened. Seven of these had mutations to the genes responsible for the \( \alpha_1 \) and \( \beta_{2b} \) subunits of the L-type \( \text{Ca}^{2+} \) channel and of these seven, three had moderately short QT \(_C\) intervals (<360 ms).

The first patient studied was a 25-year old male with a QT\(_C\) interval of 330 ms. He presented with aborted sudden cardiac death (SCD). His brother was also symptomatic and his relatives had tall and peaked T-waves on the ECG. Genetic analysis showed that the proband had a serine → leucine substitution at position 481 (S481L) of the \( \beta_{2b} \) subunit due to a heterozygous C1442T change in \( CACNAB2b \).

The second patient was a 41-year old male (from a different family to patient 1) with a QT\(_C\) interval of 300 ms. He presented with AF and his brother died suddenly at the age of 45. Genetic analysis showed that the proband had an A → G substitution (A1468G) that led to a glycine → arginine substitution at position 490 (G490R) of the \( CACNA1C \) protein. This mutation was also found in his two daughters.

The third identified case was a 48-year old male with a QT\(_C\) interval of 360 ms and a prominent ST-segment elevation on the ECG. His father had no known symptoms but his mother had undergone SCD at the age of 48. Genetic analysis showed that the proband had an alanine → valine amino acid substitution (A39V) due to a heterozygous C → T transition in position 116 of \( CACNA1C \). Co-expression of WT and the pertinent \( \alpha/\beta \) subunits for each mutant resulted in significantly reduced current through the channel. Table 3.1 summarises the currently identified SQTS variants, their associated ion channels and functional consequences.

### 3.6 Current Treatment of SQT Patients

Due to the risk of sudden death in SQTS, the prevailing treatment is the use of an implantable cardioverter defibrillator device (ICD; [117,119,254,255]).
<table>
<thead>
<tr>
<th>SQT Variant</th>
<th>Gene/gene Product</th>
<th>Channel (Subunit)</th>
<th>Mutation (Amino Acid Change)</th>
<th>Principal alterations to channel function/activity</th>
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<td>SQT1</td>
<td>KCNH2 (hERG)</td>
<td>(I_{Kr} (\alpha \text{ [pore-forming] subunit}))</td>
<td>N588K</td>
<td>Reduced inactivation</td>
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<td></td>
<td></td>
<td></td>
<td>R1135H</td>
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<td>T618I</td>
<td>Reduced inactivation</td>
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<tr>
<td>SQT2</td>
<td>KCNQ1 (KCNQ1/KvLQT1)</td>
<td>(I_{Ks} (\alpha \text{ subunit}))</td>
<td>V307L</td>
<td>Enhanced/accelerated activation</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>V141M</td>
<td>Constitutively open/shifted activation and delayed deactivation</td>
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<tr>
<td>SQT3</td>
<td>KCNJ2 (Kir 2.1)</td>
<td>(I_{K1})</td>
<td>D172N</td>
<td>Preferential increase in outward current</td>
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<td>M301K</td>
<td>Preferential increase in outward current</td>
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<tr>
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<td>CACNA1C (Ca(_v)1.2)</td>
<td>(I_{Ca,L} (\alpha \text{ subunit}))</td>
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<td>Reduced trafficking/current</td>
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<td>G490R</td>
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<td>SQT5</td>
<td>CACNB2b</td>
<td>(I_{Ca,L} (\beta_{2b} \text{ subunit}))</td>
<td>S481L</td>
<td>Reduced current</td>
</tr>
</tbody>
</table>

Table 3.1: Known SQTS ion channel gene mutations and their functional consequences. Modified from [8].
However, this is not an ideal, sole solution because of the possibility of an inappropriate shock by the ICD due to T-wave over-sensing (due to the pronounced tall, peaked T-waves in many SQT patients) [9,117,119,254]. This risk can be lessened by ICD reprogramming [254]. ICDs do not restore the QT interval to its normal duration and are not suitable for all patients (e.g., infants). These drawbacks to ICDs make it important also to have adjunct (or alternative – for patients who refuse ICDs) pharmacological treatments that may help restore the normal QT interval duration and protect against arrhythmia [117,119,256,257].

Historically, the first drug to be investigated as a pharmacological treatment for the SQTS was sotalol (a class III antiarrhythmic drug) by Brugada et al. [121]. They found that the N588K mutation reduced the blocking potency of sotalol and the QTc interval was unchanged on its administration [121]. A subsequent study investigated the effects of a number of drugs on six SQTS patients [258]. The drugs were flecainide (a class Ic antiarrhythmic drug), hydroquinidine (a class Ia agent) and sotalol and ibutilide (both class III agents). Only hydroquinidine successfully produced a significant lengthening of the QT interval and prolongation of the ERP [258]. It was also found to protect against VF. A subsequent study compared quinidine and sotalol in vitro [259]. It found that sotalol’s IC$_{50}$ for I$_{hERG}$/I$_{Kr}$ current was raised 20-fold by the N588K mutation but only by 5-fold for quinidine. The IC$_{50}$ is the concentration of a drug that produces a half-maximal effect [260]. In SQT1 patients, quinidine – which was less affected in vitro by the N588K mutation than was sotalol – restored the heart rate adaption of the QT interval towards normal [259].

In 2006, McPate et al. [261] reported effects of the N588K hERG mutation on the I$_{hERG}$ blocking potency of disopyramide (which, like quinidine, is a class Ia antiarrhythmic agent) and the high affinity class III inhibitor E-4031. For disopyramide, they found that its I$_{hERG}$ blocking potency was comparatively little affected by the N588K mutation, i.e., its IC$_{50}$ was 1.5-fold that of WT-hERG compared to 3.5-fold that for quinidine. For E-4031, its I$_{hERG}$ blocking potency was changed significantly (it had a >11-fold change in IC$_{50}$). Subsequently, disopyramide was used in a pilot study involving two SQT1 patients [262] and was found to extend the QT interval, rate dependence and ventricular ERP. Mizobuchi et al. have also reported beneficial effects of disopyramide in an SQTS patient of unknown genotype [263].
In a subsequent study [147], McPate et al identified amiodarone and propafenone as effective N588K-hERG inhibitors. This study was *in vitro* and the efficacy of amiodarone on SQT1 *in vivo* is currently unknown although it has been found to be beneficial in an SQT patient of unknown genotype [120]. Propafenone on the other hand has been reported to prevent AF in an SQT1 patient but without affecting the QT interval [122].

Compared to SQT1, there is very little available on the *in vitro* pharmacology of SQT2 – SQT5. Lerche et al. [264] showed that the V307L-KCNQ1 mutation reduced the KCNQ1/I\(_{Ks}\) blocking potency of chromanol 293B. Recently, El Harchi et al. [265] identified mefloquine as an effective pharmacological inhibitor of recombinant I\(_{Ks}\) channels incorporating the V307L-KCNQ1 mutation. These findings are relevant to SQT2, though neither compound has been tested on patients *in vivo*.

Chloroquine has recently been reported to be an effective inhibitor of the D172N-KCNJ2 SQT3 mutation [251,266] *in vitro*, but its efficacy in SQT3 patients is as yet unknown.

In a study involving the long-term prognosis of a large cohort of SQT patients [267], hydroquinidine was found to be effective in preventing the induction of ventricular arrhythmias with patients receiving hydroquinidine having no arrhythmic events [267]. Nevertheless, due to the scarcity of data on the *in vitro* pharmacology of SQT2 –SQT5, *in silico* characterisation provides an alternative means to characterise and quantify the effects of drugs targeting these mutations.
Chapter 4

Model Development

In the absence of phenotypically accurate experimental models of \( \text{K}^+ \) channel mutation linked SQTS variants (SQT1 – SQT3), *in silico* models offer the best complementary method to *in vivo* and *in vitro* electrophysiology for investigating the functional consequences of these and other gene mutations. These models can be used for investigations at the single cell, tissue and organ levels. Importantly, explorations to determine the effect of a gene mutation can be performed from the single cell to the tissue and organ levels or in reverse; from the organ to the tissue and single cell levels. Thus, the *in silico* approach offers a means both of recapitulating kinetic changes to ion channel function and of exploring with specificity, the links between single gene mutations and effects at the single cell, tissue and organ levels. This chapter elucidates on the development of the *in silico* biophysically detailed and physiologically accurate models used to investigate the functional consequences of SQT1, SQT2 and SQT3.

4.1 Modelling Voltage-dependent Channels: Hodgkin-Huxley vs. Markov Chain Models

Alan Lloyd Hodgkin and Andrew Fielding Huxley developed the first model of cellular excitability in 1952 [268] for which they were awarded a Nobel prize. The model, which reproduced their experimental data on the currents from the \( \text{Na}^+ \) and \( \text{K}^+ \) channels of the Squid Giant Axon was based on biophysical characterisation of the conductance of these channels which generate the axon’s action potential. Today, Hodgkin-Huxley (HH) models still form the basis of most ionic channel model development because estimating the parameters necessary to reproduce experimental data is relatively straight-forward with modern computational methods.

Hodgkin and Huxley’s work preceded the identification of ion channel proteins or single channel current recording. They postulated that the electrical currents flowing
through the cell membrane was due to freely moving gating particles within it. Consequently, they produced equations to describe macroscopic ionic current flow, incorporating concepts of gating [268], but no identification of ion channel proteins as their work preceded this and single channel measurements. Molecularly, it is now known that proteins within the membrane form a pore through which ions flow. These pores are selectively permeable to different ions. Their permeability is mediated by several factors including sensitivity to voltage and ligand binding [20,269].

From single channel recordings of ion channel activity, we now know that ion channels undergo rapid and stochastic transitions between conducting (open) or non-conducting (closed) states [270]. Occupancy of these states is governed by conformational changes of the underlying protein structure of these channels. With Markov chain models, these conformational changes can be represented accurately with state diagrams. Markov chain models therefore capture ion channel kinetics more precisely than HH models [271]. Their only limitation in comparison with HH models is the greater difficulty in estimating the necessary parameters from experimental data.

In this thesis, the SQT1 and SQT2 variants of the SQTS are modeled using the Markov chain model formalism while the SQT3 variant uses the Hodgkin-Huxley formalism. The reasons for using a HH model for SQT3 will be discussed below. As will be shown, the three models developed can reproduce accurately experimental data and reproduce electrophysiological behaviour.

4.1.1 The Hodgkin-Huxley Formalism

In 1952, Sir Alan Lloyd Hodgkin and Sir Andrew Fielding Huxley discovered that membrane ionic conductance was modulated by membrane potential [268]. They described a mathematical model of the cell, which was able to reproduce the action potential of a Squid Giant Axon [268]. They studied the time course of the ionic current when subjected to different voltage steps and investigated the relationship between membrane potential and membrane current. From their experiments, they were able to separate the ionic current into three components – an inward Na⁺ current (I_{Na}), an outward K⁺ current (I_{K}) and a leakage current (I_{L}) carried by other unspecified ions. Thus, the ionic current can be written as:
They described each ionic current component as a product of two factors:

1. an instantaneous function of potential and
2. a continuous function of voltage and time.

The current and membrane potential relationship (I-V relationship) for each ion was linear and passed through the equilibrium potential of that ion. Consequently, it could be described using Ohm’s law with an accounting for the equilibrium potential of the particular ion. The instantaneous function of potential is thus driving force and is equivalent to the difference between the membrane potential and the equilibrium potential of the specific ion \((V_m - E_x)\).

The second factor (continuous function of voltage and time) is the conductance of the ion obtained by dividing the current from that ion by the driving force: \(G_x = I_x / (V_m - E_x)\). It describes a gradual change in the membrane permeability for that ion. Each current in the model can then be written as:

\[
I_{\text{ion}} = I_{\text{Na}} + I_{\text{K}} + I_{\text{L}}
\]

\[
I_{\text{Na}} = G_{\text{Na}} (V_m - E_{\text{Na}})
\]

\[
I_{\text{K}} = G_{\text{K}} (V_m - E_{\text{K}})
\]

\[
I_{\text{L}} = G_{\text{L}} (V_m - E_{\text{L}})
\]

Hodgkin and Huxley proposed a gating mechanism to describe the kinetics of each ionic current. Each gating particle could be on either side of the membrane and carries with it a net charge and consequently a membrane potential, which allows it to switch position from one side of the membrane to the other. The gate or gating particle could then be in an open or closed state depending on the voltage thereby modulating the movement of ions through the membrane. A kinetic representation is shown in Figure 4.1:
Figure 4.1: A state-transition diagram of a two-state model of an ion channel.

C is the closed state, O is the open state, $\alpha$ and $\beta$ are voltage-dependent transition rate constants governing the rate of transition from one state to the other.

If $N$ represents the fraction of gates in the open state, then following a change in membrane potential, the net rate of change in the fraction of open gates can be calculated using the state diagram in Figure 4.1 as:

$$\frac{dN}{dt} = \alpha(1 - N) - \beta N$$  \hspace{1cm} (8)

At steady state, $dN/dt = 0$ and the steady state fraction of open gates ($N_\infty$) is:

$$N_\infty = \frac{\alpha}{\alpha + \beta}$$  \hspace{1cm} (9)

A change in membrane potential will cause a deviation from steady state and consequently, a change in the fraction of open gates. The time it takes for the channel to return to steady state is the time constant and is given by:

$$\tau_n = \frac{1}{\alpha + \beta}$$  \hspace{1cm} (10)

Taking into account the fraction of open gates, Hodgkin and Huxley represented the conductance of a channel by:

$$G_x = \bar{G}_x N$$  \hspace{1cm} (11)
where $\bar{G}_X$ is the maximal conductance of the ion channel and $N$ is the fraction of gates in the open state.

From their experiments, Hodgkin and Huxley were able to describe the properties of each ionic current using one or more gates, acting independently of each other. These gates controlled the kinetic properties of the channel and for the channel to conduct ions, all the gates must be open. They discovered that the $K^+$ channel could be represented using four identical, independent activation gates ($n$) governed by a first-order voltage-dependent process. Consequently, its conductance is given by:

$$G_K = \bar{G}_K n^4$$  \hspace{1cm} (12)

where $\bar{G}_K$ is the maximal potassium conductance. The $Na^+$ channel displayed activation and inactivation behavior; inactivation being the state where the channel is open but non-conducting or blocked and hence, cannot conduct current. The kinetics of the channel is represented using three independent activation gates ($m$) and one independent inactivation gate ($h$) written as:

$$G_{Na} = \bar{G}_{Na} m^3 h$$  \hspace{1cm} (13)

where $\bar{G}_{Na}$ is the maximal sodium conductance, $m$ is the fraction of open activation gates and $h$ is the fraction of open inactivation gates.

For each channel to conduct current, all the gates must be in the open state. Depolarisation causes the $Na^+$ and $K^+$ channels’ $m$ and $n$ gates to open. This is referred to as activation. The $h$ gate of the $Na^+$ channel however is closed by depolarisation (referred to as inactivation), which would result in the closure of the whole channel as all the gates must be open for the channel to conduct current.

**4.1.1.1 The Complete Hodgkin-Huxley Model**

The complete Hodgkin-Huxley (HH) model can then be written as:
\[ I_{Na} = G_{Na} m^3 h (V_m - E_{Na}) \]
\[ I_K = G_K n^4 (V_m - E_K) \]
\[ I_L = G_L (V_m - E_L) \]  

(14)

### 4.1.2 Markov Chain Models

While the Hodgkin-Huxley model was forward-looking and closely reproduced macroscopic current behaviour, it suffers from the assumption of independent gating. Experiments since its conception show that this is not accurate. For example, for the Na\(^+\) channel to inactivate, it must first be in an activated state, i.e., activation and inactivation are coupled and not independent processes [272,273]. Aldrich *et al.* [274] also showed that Na\(^+\) channels might also show inactivation that is not voltage-dependent but state-dependent.

In contrast, Markov chain models use state diagrams to express conformational changes of the ion channel protein and assume that transitions from one state to another is dependent only on the present state. Thus, they can be mapped structurally to the molecular representation of the ion channel. HH models can be considered to be a subset of Markov chain models so that any HH model can be written as a Markov chain model scheme but the converse does not apply [72,275]. An example of a Markov chain model state diagram is shown below where \( S_i \) represent the distinct conformational states which the channel can occupy.

\[ S_1 \leftrightarrow S_2 \leftrightarrow \cdots \leftrightarrow S_n \]  

(15)

If \( P(S_i, t) \) represents the probability of the channel being in state \( S_i \) at time \( t \) and \( P(S_i \to S_j) \) represent the probability of a transition from state \( S_i \) to \( S_j \), where \( i,j \) run from 1 to \( n \), we can write:

\[ P(S_i \to S_j) \]
\[ P(S_j \to S_i) \]

(16)
From Equation (15), we can obtain an expression for the time evolution of the states of the ion channels as:

\[
\frac{dP(S_i,t)}{dt} = \sum_{j=1}^{n} P(S_j,t)P(S_j \rightarrow S_i) - \sum_{j=1}^{n} P(S_i,t)P(S_i \rightarrow S_j)
\]  

Equation (17) is called the master equation [276,277]. The term on the left of the minus sign is the source term and represents the transitions entering state \(S_i\) while the term on the right of the minus sign represents the sink term and represents all contributions leaving state \(S_i\). The time evolution depends only on the present state of the system, and is defined entirely by knowledge of the set of transition probabilities (Markovian system) [278–280].

A small patch of cell membrane will contain of a large number of identical channels. Therefore, in the limit, the master equation can be replaced by its macroscopic interpretation. Equation (15) can then be replaced by:

\[
S_i \Leftrightarrow S_j
\]

where \(S_i\) and \(S_j\) are now the probabilities of the channel being in these states and \(r_{ij}\) and \(r_{ji}\) become the transition rate constants. The time rate of evolution of the occupancy of different states by the channel (Equation 17) can then be rewritten as:

\[
\frac{dS_i}{dt} = \sum_{j=1}^{n} s_j r_{ji} - \sum_{j=1}^{n} s_i r_{ij}
\]

The rate constants, \(r\) in Equation (18) can be voltage-dependent. This voltage-dependence is due to the sensitivity of the channel conformational changes to membrane potential changes. Determination or extraction of the rate constants for a Markov chain model is quite difficult and is one of its limitations.

Changes in the membrane potential set up a transmembrane electric field, which influences formal charges or induced charges (dipoles) within the protein, thus setting up energy barriers. Conformational changes of the channel protein will then only occur
when the rate constants surmount these energy barriers [281,282]. According to reaction rate theory [281,282], the rate constants depend exponentially on the free energy barrier between two states:

$$r(V) = r_0 e^{-\frac{\Delta G(V)}{RT}}$$  \hspace{1cm} (20)

where $r_0$ is a constant, $\Delta G(V)$ is the free energy barrier, $R$ is the thermodynamic gas constant and $T$ is the temperature. The free energy barrier can be expressed as:

$$\Delta G(V) = G^*(V) - G_0(V)$$  \hspace{1cm} (21)

where $G^*(V)$ is the free energy of an intermediate state (activated complex) and $G_0(V)$ is the free energy of the initial state (Figure 4.2). To make a transition from an initial state to a final state, ion channels have to overcome the free energy barrier, which is the difference in energy between $G_0(V)$ and $G^*(V)$. The smaller this free energy barrier is, the faster are the kinetics of the channel and the more ion channels that can make the transition [283].

The free energy can be expanded with a Taylor series as:

$$G_i(V) = A_i + B_i V + C_i V^2$$  \hspace{1cm} (22)

$A_i$, $B_i$ and $C_i$ describe the potential energy barriers between each conformational state of the ion channel. $A_i$ represents the energy barrier height in the absence of an electrical field, $B_i$ represents the energy barrier height due to interactions between isolated charges and dipoles [277,283–286] and $C_i$ represents total distortion polarisation and pressure induced by $V$ [277,283–286] or mechanical restrictions on the charges by the channel protein’s structure [283].

Making use of Equations (21) and (22), Equation (20) can then be rewritten as:
Figure 4.2: Free energy profile of conformational changes in ion channels. The figure shows the free energy involved during the transition from one state to another. $G_0$ is the initial state, $G^*$ is the intermediate state (activated complex) and $G_1$ is the final state. $\Delta G(V)$ is the free energy barrier which the ion channel has to overcome to make the transition from $G_0$ to $G_1$. A smaller energy barrier means faster kinetics, as more ion channels will have the required energy to make the transition. Figure modified from [283]).
\[ r(V) = r_0 e^{-\frac{\left[ (A + B'V + C'V^2 + \cdots) - (A_0 + B_0V + C_0V^2 + \cdots) \right]}{RT}} \]

\[ r(V) = r_0 e^{-\frac{\left[ (A + BV + CV^2 + \cdots) \right]}{RT}} \]  

(23)

In the limiting case of small transmembrane voltages, the nonlinear terms \((V^2\) and higher) may be insignificant and Equation (23) can be simplified to:

\[ r(V) = r_0 e^{-\frac{\left[ (A + BV) \right]}{RT}} \]  

(24)

which is the commonly used form.

### 4.2 Development of the SQT1 Markov Model

#### 4.2.1 The Base \( I_{Kr} \) Markov Model

To develop a Markov model that reflected the kinetic and structural changes induced by the SQT1 mutation on hERG, an existing cardiac \( I_{Kr} \) Markov model was modified. This initial base Markov model (Figure 4.3) was based on the hERG/\( I_{Kr} \) Markov model formulation of Kiehn et al. [78], Clancy and Rudy [287], Lu et al. [288] and Wang et al. [289]. The macroscopic current density of hERG/\( I_{Kr} \) is calculated as:

\[ I_{ikr} = G_{ikr} \cdot P_{O_{ikr}} \cdot (V_m - E_{rev}) \]  

(25)

where \( P_{O_{ikr}} \) is the sum of all \( I_{Kr} \) channel open probabilities, \( V_m \) is the membrane potential, \( E_K \) is the potassium reversal potential and \( G_{ikr} \) is the maximum membrane conductance of \( I_{Kr} \) and is given by:

\[ G_{ikr} = \sigma \cdot g_{ikr} \]  

(26)

with \( \sigma \) being the channel density and \( g_{ikr} \) the unitary channel conductance. The state probabilities are described by first order differential equations as described in Equation...
Figure 4.3: Markov model state diagram of cardiac $I_{Kr}$ [78,287,288,289].

(19). The parameters of the model were fit to measured experimental data for activation, inactivation and deactivation [287].

The gating scheme was originally produced by the study of Kiehn et al. [78] following the study by Wang et al. [289]. The transition rates of the model were then modified (as described in Section 4.2.2) to account for the proper behaviour of macroscopic currents relative to data obtained at physiological temperatures and ionic concentrations from guinea pig ventricular myocytes [287].

The model consists of three closed states (C1, C2 and C3), an open state (O) and an inactivated state (I). Inactivation can occur from the open or the closed state but does so preferentially from the open state [71,287]. The transition ($\alpha_1$, $\beta_1$) between states C2 and C3 is voltage independent [287,289]. The transitions from states C3 to I (C3→I) and from C3 to O (C3→O) are the same [78]. The model also includes dependencies on the extracellular K$^+$ concentration [K$^+$]$_{out}$; the transition between O and I as well as the maximal $I_{Kr}$ conductance. Increasing [K$^+$]$_{out}$ has the effect of decreasing the transition rate between O and I.
4.2.2 The SQT1 Markov Model

The base \( I_{Kr} \) Markov model described above (section 4.2.1) was modified to incorporate the experimentally observed kinetic properties of WT and N588K-mutated hERG/\( I_{Kr} \) channel. These kinetic properties include:

1. The profound (> +60 mV) shift in the voltage dependence of inactivation of N588K-hERG that alters rectification of \( I_{hERG} \) [79,121,147,245];
2. The substantial increase of \( I_{hERG} \) early during the ventricular action potential (AP) waveform [79,121,147,245]; and
3. The generation of rapid, transient, outward currents in response to premature, depolarizing stimuli under ‘paired stimuli’ experiments [245].

To obtain the transition rates of the Markov chain model that reproduced the experimentally observed kinetic properties of WT and N588K-hERG/\( I_{Kr} \), experimental current-voltage (I-V) relationships for WT and N588K-hERG/\( I_{Kr} \) [245] were simulated using the voltage clamp protocol in [79,245]. In order to simulate the experimental I-V relationship, the original \( I_{Kr} \) transition rate equations were modified. First, a simulated voltage clamp as described in [245] above was set up: the membrane potential was held at -80 mV and then depolarised briefly to -40 mV (to evaluate instantaneous current), followed by 2s depolarisations to a range of potentials from -40 mV to +60 mV (in 10 mV increments); finally, ‘tail’ currents were elicited by repolarisation to -40 mV for 4000 ms. The currents at the end of the 2000 ms depolarising steps were normalised and compared to the experimental data.

To obtain a good agreement with the experimental data [79,245], variables that modified each transition rate were introduced. The values of these variables were calculated by minimising the least squared difference between the experimental data and the simulation result. The BFGS (Broyden-Fletcher-Goldfarb-Shano) method [292] and a cubic spline interpolation algorithm [293] were used for the minimisation. The variables that produced the best fit and behaviour of macroscopic currents relative to the experimental data were selected [294]. Figure 6.2Aiiii-Biii (the full Markov Chain model) shows the simulated I-V relationship for both the WT and N588K mutation compared to the experimental data, which is the end pulse currents produced by the
model and experimental data for both conditions. The SQT1 Markov model reproduces quite closely the experimental data in both the WT and N588K mutation conditions.

The Markov chain model formulations were validated by comparing simulated results from different voltage clamp protocols – premature stimuli (Figure 6.3Biii) and AP Clamp (Figure 6.3Bi and Bii) – to experimentally obtained data. To simulate the AP clamp, the same digitised ventricular AP used to generate the experimental data [245] was used to elicit the I_{hERG} in the simulation. For paired premature stimuli simulations, the protocol utilising paired ventricular AP waveform commands was applied [245]. Following an initial ventricular AP command, a second, premature AP command waveform was superimposed 100 ms before the APD_{90} (action potential duration at 90% repolarization) of the first command. The premature stimulus was then applied in 10 ms increments for subsequent sweeps [245]. The modified model rate transition equations and parameters of the I_{Kr} Markov model for WT and N588K conditions are presented below.

\begin{equation}
I_{Kr} = G_{Kr} \cdot O_{Kr} \cdot (V_m - E_{Kr})
\end{equation}

(27)

\(G_{Kr}\) is the channel membrane conductance, \(O_{Kr}\) is the channel open probability, \(V_m\) is the membrane potential and \(E_{Kr}\) is the potassium reversal potential.

\begin{equation}
G_{Kr} = 0.0243 \times \left[ K^+ \right]_o^{0.59}
\end{equation}

(28)

\(\left[ K^+ \right]_o\) is the extracellular potassium concentration.

\begin{equation}
E_{Kr} = \frac{RT}{F} \log \left( \frac{\left[ K^+ \right]_o}{\left[ K^+ \right]_i} \right)
\end{equation}

(29)

\(R\) is the universal gas constant, \(T\) is the temperature, \(F\) is Faraday’s constant, \(\left[ K^+ \right]_o\) and \(\left[ K^+ \right]_i\) are the extracellular and intracellular potassium concentrations respectively.
\[ \alpha_i = 2.172 \]  
\[ \beta_i = 1.077 \]

\[ \alpha_2 = 0.00655 \times e^{0.02735765 \nu_n - 36} \]  
\[ \beta_2 = 0.001908205 \times e^{-0.01489021 \nu_n} \]

\[ \alpha_i = 0.04829 \times e^{-0.039984 \nu_n + 25} \times \left( \frac{4.5}{K_O} \right) \]

\[ \beta_i = 0.2624 \times e^{0.0009421 \nu_n} \times \left( \frac{4.5}{K_O} \right)^{0.3} \]

\[ \alpha = 0.00555 \times e^{0.0554715 \nu_n - 12} \]
\[ \beta = 0.002357 \times e^{-0.036588 \nu_n} \]

\[ \mu = \frac{\alpha_i \beta_2}{\beta_i} \]

\[ \alpha_i, \beta_i, \alpha_2, \beta_2, \alpha_i, \beta, \alpha, \beta, \mu \] are state transition rates.
4.3 Development of the SQT2 Markov Model

4.3.1 The Base $I_{\text{Ks}}$ Markov Model

The initial base Markov chain model (Figure 4.4) used in developing the SQT2 WT and V307L mutant Markov models was based on the $I_{\text{Ks}}$ Markov chain model formulation of Koren et al. [295], Zagotta et al. [296] and Silva and Rudy [71]. The Markov state transition diagram, for this base model is shown in Figure 4.4. It is developed based on experimental data at physiological temperature (37°C) including data from human ventricular myocytes representing human $I_{\text{Ks}}$ activation kinetics [297] and deactivation kinetics [298].

As described in section 4.1.2, channel activation and inactivation are dependent. In 1994, Zagotta et al. [296,299] suggested that channel activation also consists of dependent transitions. They proposed a model for Shaker $K^+$ channels with four subunits, each with identical activation rates. Each subunit was suggested to undergo two conformal transitions; say R1 (rest state) and R2 (intermediate state) prior to entering the activated state (A). Figure 4.5 shows one of the four subunits with the three states, R1, R2 and A. Silverman et al. [300] provided experimental data in 2003 validating Zagotta et al’s two-stage voltage-sensor transition. They found that arginine residues in the voltage sensor (S4) of the Shaker $K^+$ channel interact sequentially with acidic residues in S2, thus providing a mechanism for the two-stage voltage-sensor transition.

The base $I_{\text{Ks}}$ Markov model (Figure 4.4) is based on this two-stage voltage sensor transition principle. It shows all the possible conformations of the channel’s four subunits with a unique combination of their voltage sensor positions. It consists of 15 closed states (C1 to C15) and two open states (O1 and O2) [71,72]. It contains three zones: an activated (open) zone which is encoded in red; zone 2 (green) consists of all those closed states where the voltage sensors are yet to complete the first transition; and zone 1 (blue) consists of those closed states where the voltage sensors have made this first transition [71,72].
Figure 4.4: Markov model state transition diagram of the $I_{Ks}$ channel. There are three zones: zone 2 (green) consists of all closed states with voltage sensors that are yet to complete the first transition; zone 1 (blue) consists of the closed states where all four voltage sensors have completed the first transition; and the open zone (red).

Figure 4.5: A model of $K^+$ channel that undergoes two transitions before channel opening. R1 is the rest state, R2 is the intermediate state and A is the activated (open) state. Modified from [71,72].
Each left to right transition represents the movement of a voltage sensor from its rest state (R1) to the intermediate state (R2) while each top to bottom transition represents a voltage sensor movement from the intermediate state (R2) to the activated (A) state. For example, C_2 has three sensors in R1 and one in R2; C_8 has one voltage sensor in R1, two in R2 and one in A; C_{13} has one voltage sensor in R2 and three in A. All such combinations are represented by the 15 closed states and when all the voltage sensors are activated (C_{15}), a cooperative voltage-independent transition to the open state O_1 occurs before a final cooperative voltage-dependent transition to the open state O_2.

The first open state (O_1) models a cooperative voltage-independent transition opening to reproduce steady state activation measurements [301] according to the work of Loussouarn et al. [302]. On application of Phosphatidylinositol-4,5-bisphosphate (PIP_2) to I_{Ks} channels on excised patches of cell membrane, they discovered that it affected their open probability markedly but had no effect on the voltage-dependent properties of the channel. Koren et al. [295] also observed this in Shaker K^+ channels. When the I_{Ks} channel is probed with rubidium, a second open state (O_2) responsible for two experimentally observed exponential components of deactivation is evident [303]. The macroscopic current density of I_{Ks} is calculated similar to Equations (25)-(26). A full description of the derivation of the initial base Markov model can be found in Silva and Rudy [71].

4.3.2 The SQT2 Markov Model

Experimental current-voltage (I-V) relationships for I_{Ks} [265] were simulated using the voltage clamp protocol in [265]. In order to simulate the experimental I-V relationship, the original I_{Ks} transition rate equations were modified. First, a simulated voltage clamp as described in [265] above was set up (Figure 7.2Aii-Bii): the membrane potential was held at -80 mV and then depolarised briefly to -40 mV for 50 ms (to evaluate instantaneous current), followed by 3s depolarisations to a range of potentials from -70 mV to +60 mV (in 10 mV increments); finally, ‘tail’ currents were elicited by repolarisation to -40 mV for 5s. The currents at the end of the 3s depolarising steps were normalised and compared to the experimental data.
To obtain a good agreement with the experimental data, variables that modified each transition rate were introduced. The values of these variables were calculated by minimising the least squared difference between the experimental data and the simulation result. The Nelder-Mead Simplex algorithm [304] was used for the minimisation. The variables that produced the best fit and behaviour of macroscopic currents relative to the experimental data were selected [294]. Figure 7.2Aiii and 7.2Biii show the simulated I-V relationship for both the WT and V307L mutation compared to the experimental data. The SQT2 Markov model reproduces quite closely the experimental data in both the WT and V307L mutation conditions. The modified model rate transition equations and parameters of the $I_{Ks}$ Markov model for WT and V30L conditions are presented below.

\[ I_{Ks} = G_{Ks} \cdot O_{Ks} \cdot (V_m - E_{Ks}) \]  

(48)

\[ G_{Ks} = 0.779 \cdot \left( \frac{0.6}{1 + \left( \frac{3.8 \cdot 10^{-5}}{Ca^{2+}} \right)^4} \right) \]  

(49)

\[ O_{Ks} = O_1 + O_2 \]  

(50)

\[ E_{Ks} = \frac{R \cdot T}{F} \cdot \log \left( \frac{K^+}{K^+} + P_{Na/K} \cdot \left[ Na^+ \right] \right) \]  

(51)

**WT**

\[ \alpha = 5.56 \cdot 10^{-5} \cdot \exp \left\{ 3.61 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T} \right\} \]  

(52)

\[ \beta = 8.25 \cdot 10^{-6} \cdot \exp \left\{ -9.23 \cdot 10^{-2} \cdot \frac{V_m \cdot F}{R \cdot T} \right\} \]  

(53)

\[ \gamma = 3.78 \cdot 10^{-4} \cdot \exp \left\{ 8.68 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T} \right\} \]  

(54)

\[ \delta = 1.32 \cdot 10^{-3} \cdot \exp \left\{ -3.30 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T} \right\} \]  

(55)

\[ \theta = 6.10 \cdot 10^{-4} \]  

(56)
\[
\eta = 1.95 \cdot 10^{-3} \cdot \exp\left\{-4.81 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T}\right\} \tag{57}
\]
\[
\psi = 6.25 \cdot 10^{-4} \cdot \exp\left\{1.27 \cdot 10^{-6} \cdot \frac{V_m \cdot F}{R \cdot T}\right\} \tag{58}
\]
\[
\omega = 3.50 \cdot 10^{-4} \cdot \exp\left\{-6.79 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T}\right\} \tag{59}
\]

\section*{V307L}
\[
\alpha = 2.52 \cdot 10^{-5} \cdot \exp\left\{3.61 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T}\right\} \tag{60}
\]
\[
\beta = 2.51 \cdot 10^{-3} \cdot \exp\left\{-9.23 \cdot 10^{-2} \cdot \frac{V_m \cdot F}{R \cdot T}\right\} \tag{61}
\]
\[
\gamma = 1.71 \cdot 10^{-3} \cdot \exp\left\{8.68 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T}\right\} \tag{62}
\]
\[
\delta = 6.45 \cdot 10^{-4} \cdot \exp\left\{-3.30 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T}\right\} \tag{63}
\]
\[
\theta = 3.40 \cdot 10^{-3} \tag{64}
\]
\[
\eta = 4.75 \cdot 10^{-4} \cdot \exp\left\{-4.81 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T}\right\} \tag{65}
\]
\[
\psi = 1.93 \cdot 10^{-3} \cdot \exp\left\{1.27 \cdot 10^{0} \cdot \frac{V_m \cdot F}{R \cdot T}\right\} \tag{66}
\]
\[
\omega = 4.52 \cdot 10^{-4} \cdot \exp\left\{-6.79 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T}\right\} \tag{67}
\]

where $G_{Ks}$ is the membrane channel conductance, $\left[Ca^{2+}\right]$ is the intracellular calcium concentration, $O_{Ks}$ is the channel open probability, $O_1$ and $O_2$ are the probabilities of being in these pen states respectively, $E_{Ks}$ is the potassium reversal potential, $R$ is the universal gas constant, $T$ is the temperature, $F$ is Faraday’s constant, $\left[K^+\right]_o$ and $\left[K^+\right]_i$ are the extracellular and intracellular potassium concentrations respectively, $\left[Na^+\right]_o$ and $\left[Na^+\right]_i$ are the extracellular and intracellular sodium concentrations respectively, $P_{Na/K}$ is the Na$^+$:K$^+$ permeability ratio, $V_m$ is the membrane potential and $\alpha, \beta, \gamma, \delta, \theta, \eta, \psi, \omega$ are state transition rates (see Figure 4.4).
4.4 Development of the SQT3 Hodgkin-Huxley Model

4.4.1 The Base $I_{K1}$ Model

The base $I_{K1}$ model used in the formulation of the SQT3 WT, WT-D172N and D172N formulations was that of ten Tusscher et al. [38,305,306]. Their formulation was based on that used in the Priebe-Beuckelmann et al. model [307]. The formulation is:

$$I_{K1} = G_{K1} \frac{K_o}{5.4} x_{K1\infty} (V - E_K) \quad (68)$$

where $E_K$ is the potassium reversal potential, $V$ is the membrane potential, $G_{K1}$ is the membrane conductance, $x_{K1\infty}$ is a rectification factor that is time-independent and a function of voltage. It describes the open probability of the channel. $\sqrt{K_o / 5.4}$ represents the $K_o$ dependence of $I_{K1}$ and ten Tusscher et al. [38,305,306] assumed it to be similar to that of animal myocytes due to a lack of data on the $K_o$ dependence of human myocytes. Fortuitously, Sakmann and Trube [203] have described this square root dependence in mammalian ventricle while Bailly et al. [308] (and others) have confirmed this in human ventricular myocytes.

This is a Hodgkin-Huxley type model and not a Markov model as was used for the SQT1 and SQT2 formulations. Yan and Ishihara [309] have developed an $I_{K1}$ Markov model but in order to more accurately represent the quasi-instantaneous dynamics of $I_{K1}$ (it maintains its steady state values during applied membrane potential changes), they applied a quasi-steady state approach in the development of their Markov model. The Markov model also does not represent the square root dependence of the conductance on $K_o$. A Hodgkin-Huxley formulation for $I_{K1}$ was thus adopted for formulating the SQT3 models. The formulation in Equation (68) assumes that the gating kinetics are sufficiently fast to be considered instantaneous.

4.4.2 The SQT3 Hodgkin-Huxley Model

The base $I_{K1}$ model was modified to incorporate the experimentally observed kinetic
properties of the WT, WT-D172N and D172N-mutant I_{Kir,2.1}. These kinetic properties include:

1. the marked augmentation of outward Kir2.1 current through D172N channels [125,251]; and
2. the rightward voltage-shift of peak repolarising current during both ventricular and action potential (AP) clamp commands [125,251].

To obtain the model parameters that reproduced the experimentally-observed kinetic properties of WT, WT-D172N and D172N Kir2.1 current (I_{Kir,2.1}), experimental current-voltage (I-V) relationships for WT, WT-D172N and D172N I_{Kir,2.1} were simulated using the voltage clamp protocol from El Harchi et al. [251]. Variable values for the modified model equations were obtained by minimising the least-squared difference between the experimental data and the simulation. The variables that produced the best fit and behaviour of macroscopic currents relative to the experimental data were selected (see Equations (69)-(77). The minimisation was performed using the Broyden-Fletcher-Goldfarb-Shanno (BFGS) algorithm [292]. The SQT3 model reproduced quite closely the experimental data in all the three conditions.

The WT conductance was adjusted to show the same peak current density as the original ten Tusscher et al. [38,305,306] I_{K1} formulation current density during the I-V relation, thus maintaining the overall current densities, APD90 and dynamic properties of the ten Tusscher et al. human ventricular model [38,305,306]. Relative current proportions for WT, heterozygous (WT-D172N) and homozygous (D172N) conditions were then scaled using relative proportions of peak I_{Kir,2.1} obtained previously from AP clamp experiments [251] (V_{hold} of -80 mV and E_{rev} of ~-88 to -89 mV). Peak outward D172N and WT-D172N I_{K1} was respectively ~4.6-fold and ~2.2-fold that for WT I_{Kir,2.1}. The simulated AP clamp data (Figure 8.1Ci-Ciii) matched closely prior experimental observations [251]. The modified model equations and parameters of I_{K1} for WT, WT-D172N and D172N conditions are presented below.

\[ \alpha_{K1} = \frac{0.07}{1 + e^{0.017(V-E_{rev}-200.2)}} \]  

(69)
\[ \beta_{K1} = \frac{3e^{0.0002(V-E_K+100.2)} + e^{0.08(V-E_K-8.7)}}{1 + e^{-0.024(V-E_K)}} \]  
(70)

\[ G_{K1} = 4.8 \frac{nS}{pF} \]  
(71)

**WT-D172N**

\[ \alpha_{K1} = \frac{0.1}{1 + e^{0.023(V-E_K-199.9)}} \]  
(72)

\[ \beta_{K1} = \frac{3e^{0.0002(V-E_K+100.4)} + e^{0.07(V-E_K-9.8)}}{1 + e^{-0.021(V-E_K)}} \]  
(73)

\[ G_{K1} = 6.27 \frac{nS}{pF} \]  
(74)

**D172N**

\[ \alpha_{K1} = \frac{0.1}{1 + e^{0.05(V-E_K-199.9)}} \]  
(75)

\[ \beta_{K1} = \frac{3e^{0.0002(V-E_K+100.1)} + e^{0.08(V-E_K-10.3)}}{1 + e^{-0.006(V-E_K)}} \]  
(76)

\[ G_{K1} = 11.32 \frac{nS}{pF} \]  
(77)
Chapter 5  Methods, Experimental Protocols and Mathematical Preliminaries

5.1 Experimental Protocols

5.1.1 Action Potential Duration, Diastolic Interval and the Basic Cycle Length

Figure 5.1A illustrates schematically a number of basic measures of action potential intervals and their associated terminology. Action potential duration (APD) is the time interval between the start of membrane depolarisation (initiated by the influx of Na\(^+\) ions (phase 0)) and the end of repolarisation (largely mediated by K\(^+\) ion efflux; (The diastolic interval (DI) is the time interval between the end of repolarisation and the next membrane depolarisation, i.e., the quiescent period preceding the subsequent APD. The basic cycle length (BCL) is the total duration consisting of the APD and DI (Figure 5.1A). It is the duration of one heartbeat and encompasses all events occurring from the commencement of one heartbeat to the next. A commonly used measure of the duration of the action potential is APD\(_{90}\), which is the time interval between the start of membrane depolarisation and 90% repolarisation; APD\(_{90}\) therefore reflects well ventricular repolarisation time and also its dispersion has been observed experimentally to correlate well with QT interval dispersion [310].

5.1.2 S1-S2 Protocol

The S1-S2 protocol is one of two commonly used protocols for elucidating the restitution properties of cardiac tissue [311–313]. In simulations, a BCL is chosen (e.g., 1000 ms) and the tissue model is paced at this cycle length until steady state is attained. Then, after a time delay (and utilising a variable length of the time delay, DI), a premature stimulus (S2) is applied (Figure 5.1B). Repeatedly performing this procedure at different DIs permits the construction of an APD restitution curve, which shows the APD plotted as a function of the DI [311–313].
Figure 5.1. A: The action potential duration, diastolic interval and basic cycle length shown for an action potential. B: A train of S1 stimuli that evoke action potentials and an S2 stimulus applied after the 10th S1 stimulus. C: During the absolute refractory period, an action potential cannot be generated regardless of the strength of applied stimulus whereas a slowly rising action potential can be evoked during the relative refractory period. The effective refractory period is the duration that encompasses both the absolute and relative refractory periods.
5.1.3 Action Potential Duration Restitution

For this study, action potential duration (APD) was defined as the action potential duration at 90% repolarization (APD\textsubscript{90}). APs were elicited with an S1-S2 protocol consisting of 10 S1 stimuli and an S2 stimulus (Figure 5.1B). The S1 stimuli were applied at a frequency of 1 Hz and at twice the strength of the threshold value. The S2 stimulus was applied at some diastolic interval (DI) after the AP evoked by the last S1 stimulus. The Action Potential Duration Restitution (APD-R) curve was generated by decreasing the DIs and plotting the APD\textsubscript{90} evoked by the S2 stimulus against the DIs. Steady-state rate dependence of the APD curve was determined by pacing single cell models at different basic cycle lengths (BCL) and plotting the APD\textsubscript{90} against the BCLs.

5.1.4 Effective Refractory Period Restitution

Applying a stimulus prematurely (S2) before the cell membrane potential has recovered from a previous depolarisation (S1) could result in either an action potential that rises slowly, or in no activity at all. This period of depressed excitability is known as the refractory period.

The refractory period consists of two phases (Figure 5.1C). The first is an absolute refractory period, during which no magnitude of S2 stimulus can evoke an action potential. The second is the relative refractory period during which only S2 stimuli exceeding the normal threshold can evoke an action potential.

At varying BCLs, the “Effective Refractory Period” (ERP) was measured as the smallest DI for which the overshoot of the AP evoked by the S2 stimulus reached 80% of the AP evoked by the 10th S1 stimulus at each BCL. The Effective Refractory Period restitution (ERP-R) curve was generated by plotting the measured ERP against the BCLs [314].
5.2 Governing Equations, Geometries and Associated Simulation Protocols

5.2.1 Single Cell Model and AP Simulations

The SQT1-SQT3 model ionic current formulations developed in Chapter 4 were incorporated into the 2006 version of the ten Tusscher, Noble, Noble and Panfilov (TNNP) human ventricular cell model [38].

The model reproduces human ventricular cell and membrane channel properties and reproduces transmural heterogeneity of the AP [38,305] across the ventricular wall. It has also been suggested to be well-suited to the study of re-entrant arrhythmias in human ventricular tissue [38,305]. In 2006, based on newly available experimental data, Xia et al [315] updated and modified the TNNP model; their modifications were also employed in the present study.

In the single cell model, the cell membrane is modelled as a capacitor connected in parallel with variable resistances and batteries representing the different ionic channels, exchange and pump currents. Hence, the electrophysiological behaviour of a cell can be described with the following differential equation:

\[
\frac{dV}{dt} = \frac{-I_{ion} + I_{stim}}{C_m}
\] (78)

where \(V\) is voltage, \(t\) is time, \(I_{ion}\) is the sum of all transmembrane ionic currents, \(I_{stim}\) is the externally applied stimulus current and \(C_m\) is the cell capacitance per unit surface area.

Equation (78) was integrated using the forward Euler method with a time step of 0.02 ms. The Hodgkin-Huxley-type equations for the gating variables of the various time-dependent currents in the TNNP model were integrated using the Rush and Larsen scheme [316]. The \(I_{Kr}\) Markov chain model (SQT1) was integrated with the forward Euler method. Due to the stiffness of the system of Ordinary Differential Equations (ODEs) comprising the \(I_{ks}\) Markov chain model (SQT2), it was integrated using the
'explicit method based on the 4th order Merson’s method and the first order multistage method of up to and including nine stages with stability control'. This ODE algorithm is available via the Intel Ordinary Differential Equations Solver Library [317]. The I_{K1} Hodgkin-Huxley model (SQT3) was integrated using the Rush and Larsen scheme [316].

5.2.1.1 Other I_{Kr} models used for comparison

Results from the I_{Kr} Markov chain model (SQT1) were compared to three other I_{Kr} models, including (i) a reduced Markov model (r-MC); (ii) the original HH I_{Kr} formulation of the TNNP model; and (iii) the HH I_{Kr} formulation from the Luo Rudy model [69,318]. The r-MC model was obtained by removing the transitions between the closed (C3) and inactivated (I) states in Figure 4.3.

This comparative approach is similar to and complements recent work from Bett et al. [319] who have recently compared WT Markov and HH formulations for wild-type hERG, describing qualitative and quantitative differences that influence the predictive properties of the different models studied [319].

The TNNP I_{Kr} formulation [38,305] is described by:

\[ I_{Kr} = G_{Kr} \sqrt{\frac{K_{O}}{5.4}} x_{r1} x_{r2} (V - E_{K}) \] (79)

where \( x_{r1} \) is an activation gating variable and \( x_{r2} \) is an inactivation gating variable. \( G_{Kr} \) is the maximal conductance of I_{Kr} and is set to 0.153 nS pF^{-1} for both WT and N588K-hERG, \( K_{O} \) is the extracellular K^+ concentration, \( \sqrt{K_{O}/5.4} \) represents the \( K_{O} \) dependence of the current, \( V \) is the membrane potential and \( E_{K} \) is the K^+ reversal potential given by the Nernst equation. To enable the TNNP formulation to reproduce our N588K-hERG experimental data, the steady state value of the activation gating variable was doubled. The original formulation, without modification, served as the WT formulation.

The Luo-Rudy I_{Kr} formulation is described by:
\[ I_{Kr} = G_{Kr} \cdot X_r \cdot R \cdot (V - E_{Kr}) \]  \hspace{1cm} (80)

where \( X_r \) is a time-dependent activation gate and \( R \) is a time-dependent inactivation gate. \( G_{Kr} \) is the maximal conductance of \( I_{Kr} \) and is modelled as \( 8.6 \times 10^{-3} \sqrt{K_o} / 5.4 \) nS pF\(^{-1}\) for both WT and N588K-hERG. \( V \) is the membrane potential and \( E_{Kr} \) is the \( K^+ \) reversal potential given by the Nernst equation. To enable the Luo-Rudy formulation to reproduce our N588K-hERG experimental data, ‘\( R \)’ was set to a value of 1, mimicking defective inactivation caused by the mutation. The original formulation, without modification served as the WT formulation.

### 5.2.2 Heterogeneous transmural ventricular tissue model

Initiation and conduction of action potentials in multicellular tissue models was modelled with the monodomain equation [320–322]:

\[ C_m \frac{dV}{dt} = -(I_{ion} + I_{stim}) + \nabla \cdot (D \nabla V) \]  \hspace{1cm} (81)

where \( D \) is the diffusion coefficient describing the tissue conductivity, \( I_{ion} \) is the sum of all transmembrane ionic currents, \( I_{stim} \) is the externally applied stimulus current and \( C_m \) is the cell capacitance per unit surface area.

#### 5.2.2.1 Computation of the Diffusion Coefficient

The diffusion coefficient \( (D) \) in Equation (81) is defined as [321,323–326]:

\[ D = \Lambda \Omega \Lambda^T \]  \hspace{1cm} (82)

where \( \Lambda \) is a matrix of perpendicular unit vectors as columns (\( \alpha_f \) in the fibre direction, \( \alpha_s \) in the sheet direction and \( \alpha_c \) in the cross-sheet direction). \( \Omega \) is the conductivity tensor expressed in the basis formed by these three perpendicular unit vectors.
where \( \sigma_f \) is the conductivity in the fibre direction, \( \sigma_s \) is the conductivity in the sheet direction and \( \sigma_c \) is the conductivity in the cross-sheet direction. Therefore, an entry in \( D \) can be written as:

\[
D_{ij} = \alpha'_f \alpha'_f \sigma_f + \alpha'_f \alpha'_s \sigma_s + \alpha'_s \alpha'_c \sigma_c
\]  

(84)

for \( i,j = 0,1,2 \).

5.2.2.2 Solving the Monodomain Equation

The operator splitting method as proposed by Qu and Garfinkel [327] was used to solve the monodomain equation representation of cardiac tissue (Equation 40). Specifically, Strang splitting [324] was employed.

From Equation (81), two operators can be defined:

\[
L_1 V = -\frac{(I_{ion} + I_{stim})}{C_m}
\]  

(85)

\[
L_2 V = \frac{1}{C_m} \nabla \cdot (D \nabla V)
\]  

(86)

These two operators define two sub-problems:

\[
C_m \frac{dV}{dt} = -(I_{ion} + I_{stim})
\]  

(87)

\[
C_m \frac{dV}{dt} = \nabla \cdot (D \nabla V)
\]  

(88)

The quasi-nonlinear Monodomain equation (Equation 81) has now become a linear partial differential equation (PDE) (Equation 88) and a set of nonlinear ODEs and differential-algebraic equations (DAEs) (Equation 87), which is equivalent to Equation
(78) and therefore was solved using the methods outlined in section 5.2.1. A time step using the Strang splitting algorithm [324] now proceeds as follows:

1. Solve the system of ODEs and DAEs of Equation (87) for half a time step, i.e., for \( t_n < t \leq t_n + \frac{1}{2} \Delta t \). The solution is denoted by \( V_{0.5}^n \).

2. Solve the linear PDE (Equation 88) for a full time step, i.e., for \( t_n < t \leq t_n + \Delta t \) with \( V(t_n) = V_{0.5}^n \). The solution is denoted by \( V_{0.5}^{n+1} \).

3. Solve the system of ODEs and DAEs of Equation (87) for the remaining half a time step, i.e., for \( t_n + \frac{1}{2} \Delta t < t \leq t_n + \Delta t \) to obtain the approximate solution \( V^{n+1} \) at \( t = t_n + \Delta t \).

To solve the PDE in step 2, Equation (88) is discretised in time using the Crank-Nicholson scheme [328], which is a specific form of the \( \theta \)-rule [328] commonly used for discretising time-dependent PDEs. Using the \( \theta \)-rule, Equation (88) discretised in time becomes:

\[
C_m \frac{V^{n+1} - V^n}{\Delta t} = \theta \left( \nabla \cdot (D \nabla V^{n+1}) \right) + \left( (1 - \theta) \nabla \cdot (D \nabla V^n) \right)
\]  

(89)

The choice of \( \theta = \frac{1}{2} \) gives the Crank-Nicholson scheme. It is unconditionally stable, second-order accurate and importantly, matches the accuracy of the Strang splitting scheme, which is also second order accurate.

The PDE in step 2, i.e., Equation (88) is discretised in space using the Finite Element Method [328–331]. It is multiplied by a test function, \( \phi \) and integrated over the entire domain, \( \Omega \). The resulting weak formulation for Equation (88) is:

\[
\int_{\Omega} V^{n+1} \phi \, dx + \theta \lambda \int_{\Omega} D \nabla V^{n+1} \cdot \nabla \phi \, dx = \int_{\Omega} V^n \phi \, dx - (1 - \theta) \lambda \int_{\Omega} D \nabla V^n \cdot \nabla \phi \, dx
\]  

(90)

for all \( \phi \in \mathcal{V} \) where \( \mathcal{V} \) is test function space. For convenience, \( \lambda = \frac{\Delta t}{C_m} \).
5.2.3 1D Heterogeneous Transmural Strand

The 1D mesh used for the simulations was a 15 mm long single fibre, with 100 nodes spaced 0.15 mm apart. Each node represents a 150-µm cylindrical cell (Figure 5.2). This total strand length of 15 mm is close to the normal range of human transmural ventricle width; ~4.0 – 14.0 mm [37,332,333]. The strand comprises 25 endocardial cells (ENDO), 35 middle cells (MCELL) and 40 epicardial cells (EPI) representing 3.75 mm, 5.25 mm and 6.00 mm in the ENDO, MCELL and EPI regions respectively. The chosen proportion for each region is similar to those used in other studies [248,334–336].

The diffusion coefficient, ‘D’ was set at 0.001 cm²/ms giving a planar conduction velocity of 65 cm/s through the strand. This is close to the 70 cm/s velocity of conduction along the fibre direction in human myocardium [337]. At the EPI-MCELL border, there is a 5-fold decrease in ‘D’. This is similar to the work of Gima and Rudy [335] who based this on the experimental work of Yan et al. [333] who reported a sharp transition in the tissue resistance in a left ventricular wedge model. Drouin et al. [37] also reported a sharp APD transition in this region in their experimental work characterising MCELLs in the normal human heart. At the ENDO end of the strand, a supra-threshold stimulus (amplitude: -52 pA/pF, duration: 2ms) was applied to initiate a conducting excitation wave.

5.2.3.1 Measurement of Conduction Velocity and Conduction Velocity Restitution

The conduction velocity was calculated from nodes one-quarter and three-quarters of the way along the strand. The activation time of each point was defined to be the time at which the maximum upstroke velocity occurred.

Following the application of a sequence of 10 conditioning pulses at 1Hz, conduction velocity (CV) was measured in the 1D strand by calculating the time ΔT for the wavefront to propagate from \( x - \Delta x \) to \( x + \Delta x \) and defining \( CV = \frac{2\Delta x}{\Delta T} \). To obtain CV restitution curves, CV was plotted against varying BCLs.
Figure 5.2: Geometry of the 1D strand of the transmural human ventricle wall used for simulations. The strand is transmurally heterogeneous consisting of 25 endocardial cells (red), 35 mid-myocardial cells (blue) and 40 epicardial (green) cells making a total of 100 cells. The length of the strand is 15 mm.

Figure 5.3: Geometry of the 2D realistic human ventricle cross-section used for simulations (left) and the fibre orientation (right).
Figure 5.4: Space-Time plot of a propagating condition wave in a 1D strand and response of the tissue to a premature test stimulus at a local part of the tissue at various time delays after a previous conditioning wave. Space runs horizontally from the ENDO end (0 mm) to the EPI end (15 mm). Time runs vertically from bottom to top. The membrane potential of 1D strand cells are mapped onto a colour spectrum ranging from blue (-86 mV) to red (+50 mV).

A: Initiated conditioning excitation wave. Also, bidirectional conduction block.

B: Antegrade propagation following the application of a test stimulus after a previous conditioning excitation wave.

C: Retrograde propagation following the application of a test stimulus after a previous conditioning excitation wave.

D: Bidirectional propagation following the application of a test stimulus after a previous conditioning excitation wave.
5.2.3.2 Measurement of the Temporal Vulnerable window

In the 1D strand (Figure 5.2), a conditioning excitation wave is initiated at the ENDO end by applying a stimulus (amplitude: -52 pA/pF; duration: 1 ms). This wave propagates from the ENDO end towards the EPI end (Figure 5.4A). Following the propagating wavefront in cardiac tissue is a refractory tail. If a test stimulus is applied too late after this time period, another excitation wave that propagates in both directions along the strand will develop (bi-directional conduction, Figure 5.4D) as the tissue surrounding this stimulus site is sufficiently recovered from the previous excitation wave. Applying this test stimulus too early after the refractory tail results in conduction block in both directions along the strand as the tissue around the test stimulus is still refractory (i.e., excited) and would not have recovered from the previous excitation wave. Between these two extremes is a time period, known as the vulnerable window (VW) during which an applied test stimulus produces a solitary wave that propagates in either the antegrade (Figure 5.4B) or retrograde (Figure 5.4C) direction but not in both (i.e., there is a unidirectional conduction block). In Figure 5.4B, the solitary wave from the test stimulus propagates in the antegrade direction because the tissue in the retrograde (backward) direction is still refractory and hence conduction in the retrograde direction is blocked. The situation is reversed in Figure 5.4C. This unidirectional conduction block makes the tissue susceptible to re-entry [248,338,339]. The propagation direction depends on the region in the tissue to which the test stimulus is applied.

A sequence of 10 S1 stimuli at 1Hz was applied at one end of the 1D transmural strand (spatial size: 0.4 mm, amplitude: -52 pA/pF, duration: 1 ms) to evoke a propagating wavefront. Following a time delay (ΔT) after the 10th S1 stimulus, a second stimulus (S2) with the same duration and amplitude as the S1 stimulus was applied to a 0.4 mm region of the strand. During the time window (T1, T2), where T1 and T2 denote the maximal and minimal value of ΔT respectively, the excitation wave evoked by the S2 stimulus propagated unidirectionally in the strand. The width (T1-T2) provides a measure of the temporal vulnerability of the tissue. This procedure was carried out for all regions across the strand.
5.2.3.3 Measurement of Tissue Excitation Threshold

The tissue excitation threshold is the minimal stimulus that evokes a propagating action potential in cardiac tissue [339]. This was measured using a standard S1-S2 protocol (Figure 5.1B) in the 1D strand. The S1 stimulus was applied at one end of the strand with an amplitude of -52 pA/pF, spatial size of 0.4 mm and for a duration of 2 ms. The S2 stimulus was applied in the middle of the strand with the same spatial size and duration as the S1 stimulus. The tissue excitation threshold was calculated as the minimal S2 amplitude that evoked an action potential that propagated in the strand.

5.2.4 2D Human Ventricle Geometry

5.2.4.1 Idealised 2D Geometry

The idealised geometry is a simple sheet of tissue measuring 15 mm by 50 mm. It was modelled by expanding the 1D transmural strand in Figure 5.2 (length of 15 mm in the x-direction) into a sheet with a width of 50 m in the y-direction.

5.2.4.2 Realistic 2D Geometry

The realistic geometry is a transverse cross-sectional slice taken from the middle of a 3D ventricular geometry reconstructed by DT-MRI [340] with a spatial resolution of 0.2 mm (Figure 5.3). It was segmented into distinctive regions of ENDO (25%), MCELL (35%) and EPI (40%) layers with similar contiguous proportions transmurally as the 1D strand model (Figure 5.2). Anisotropic fibre orientations is implemented as used in the work of [340]. The intracellular conductivities in the fibre and cross-fibre directions were set to 0.3 and 0.1 mS mm$^{-1}$ respectively.

5.2.5 3D Human Ventricle Geometry

In three-dimensions (3D), simulations were performed using an anatomical human ventricle geometry of a healthy 30 year-old male that was reconstructed via DT-MRI. Its spatial resolution is 0.2 mm with approximately 24.2 million nodes in total and
includes anisotropic fibre orientation (Figure 5.5). The tissue was segmented into distinct ENDO, MIDDLE and EPI regions in both the left and right ventricles [341] with similar contiguous proportions transmurally as the 1D strand model. The conditioning activation sites were determined empirically across the ventricular wall, and were validated by reproducing the activation sequence and the QRS complex in the measured 64-channel ECG [341] of the 30 year-old male. The intercellular conductivities in the fibre, cross-fibre and sheet directions were set to 3.0, 0.1 and 0.3 mS mm\(^{-1}\) respectively.

### 5.3 Other Simulation Protocols

#### 5.3.1 Computing the pseudo-ECG

The pseudo-ECG was computed following the method of Gima and Rudy [335]. At the extracellular space located at position \((x', y', z')\), a far-field unipolar potential can be computed as an integral of the spatial gradient of membrane potential at position \((x,y,z)\) on the strand by:

\[
\phi_e(x', y', z') = \frac{\alpha^2 \sigma_i}{4\sigma_e} \int \frac{(-\nabla V_m)}{r} dx
\]

\[
r = \sqrt{(x-x')^2 + (y-y')^2 + (z-z')^2}
\]

where \(\sigma_e\) and \(\sigma_i\) are the extracellular and intracellular conductivities respectively, \(\alpha\) is the radius of the strand, \(r\) the distance from a source point \((x,y,z)\) to a field point \((x',y',z')\). The pseudo-ECG was computed as \(\phi_e\) at a position 2.0 cm away from the epicardial end of the strand.

#### 5.3.2 Initiation of re-entry in 2D sheet

Re-entry was initiated by a standard S1-S2 protocol in both the idealised and realistic geometries. In the idealised 2D sheet, a plane wave was initiated at the ENDO end by an S1 stimulus. During the vulnerable window of the tissue, an S2 stimulus was applied.
Figure 5.5: 3D human ventricle geometry reconstructed by DT-MRI and fibre orientation.
A, C: Anterior and cross-section view of the human left and right ventricle geometry.
B, D: Fibre orientations of the geometry.
to a local tissue area in the EPI region to evoke unidirectional propagation that can lead to re-entry.

5.3.3 Measurement of minimal size of S2 that sustains re-entry in 2D models

On application of an S2 stimulus, unidirectional conduction of the S2-evoked excitation wave leads to formation of a pair of re-entrant excitation waves, with their counter-rotating tips that move towards each other. If the distance between both tips is sufficiently long, each will have ample space to complete its pathway, and consequently, the paired re-entrant excitation waves will be sustained. Otherwise, the two tips collide and the re-entrant excitation wave is terminated. To provide an adequate re-entrant pathway, a sufficient S2 size is required, which is dependent on the wavelength of the spiral wave. In order to evaluate the critical size of re-entrant pathway of tissue, the minimal spatial S2 length that supports the formation of re-entrant spiral waves under control and mutant conditions was estimated. This minimal length of S2 gives an indication of the susceptibility of the tissue to re-entry, i.e., the larger the minimal length, the harder the initiation of re-entry.

5.3.4 Initiation of Re-entry in 2D Heart Cross-section

In the 2D realistic model (cross-sectional slice), multiple stimulus sites (Figure 5.6A) were chosen in an effort to recreate the activation pattern in a human heart observed by Durrer et al.[342]. To initiate re-entry, an S2 stimulus was applied in the endocardium of the left ventricle (see Figure 5.6B) partly during the repolarisation phase of a conditioning wave and partly within fully recovered tissue. The S2-evoked excitation wave propagated uni-directionally, leading to the formation of re-entrant excitation wave within the transmural wall.
Figure 5.6: S1-S2 stimulation sites in the 2D human ventricle cross-section.
A: S1 stimulation sites (red).
B: S2 stimulus site (red).

Figure 5.7: S1-S2 stimulation sites in the 3D anatomical human ventricles.
A: S1 stimulation sites (gold dots).
B: S2 stimulus site (red).
5.3.5 Initiation of re-entry in the 3D anatomical human ventricles

3D scroll waves were initiated by using an S1-S2 protocol. The S1 stimulus was applied to multi-stimulation sites in the endocardium of the ventricles (Figure 5.7A). These stimulation sites were generated to produce the activation timing sequence across the ventricles as seen experimentally. The S2 stimulus was applied over a small epicardial region consisting mainly of the left ventricle and a fraction of the right-ventricular outflow tract (Figure 5.7B) (amplitude: -124 mV; duration: 2 ms) during the refractory tail of the S1 stimulus.

5.4 Numerical methods

For the 1D, 2D and 3D simulations, Equation (81) was solved using a Strang splitting scheme [324] and a Crank-Nicholson time-stepping scheme in the temporal direction, together with Lagrangian Q1 finite elements in the spatial direction using the deal.II adaptive finite element library [343]. The Strang splitting scheme is second-order accurate and the Crank-Nicholson time-stepping scheme is unconditionally stable and second-order accurate with respect to time [328]. The resulting computed solution is therefore second-order accurate.

The system of linear algebraic equations resulting from the discretisation of the monodomain equation was solved using the preconditioned Conjugate Gradient method with the Symmetric Successive OverRelaxation (SSOR) method as the preconditioner [344].

The TNNP single cell model was converted to CUDA/C++ via the Thrust CUDA library [345]. The cell kinetics are represented by a system of ODEs ($I_{ion}$ in Equation 81). In the 1D, 2D and 3D simulations, the collection of systems of ODEs ($I_{ion}$) for all the cells was solved on a Tesla C2050 “Fermi” GPU with 448 CUDA cores. The host system for the Tesla GPU is a Dell Precision T7500 with 12 Intel Xeon CPU cores at 2.80 GHz and 96 GB of memory.
Chapter 6
Increased Vulnerability of the Human Ventricle to Re-entrant Excitation in hERG-linked SQT1

6.1 Introduction

The SQTS was first reported as a clinical entity in 2000 [223]. It is characterised by a markedly abbreviated QT interval, poor rate adaptation of the QT interval, shortened atrial and ventricular refractory periods, tall and peaked T-waves on the ECG, by atrial and ventricular arrhythmias and an increased incidence of sudden death [117,119,346]. Genetic analysis of affected patients revealed three distinct mutations to three distinct potassium channels: KCNH2 (hERG), KCNQ1 (KvLQT1) and KCNJ2 encoded-potassium channel subunits [121,122,124,125,239].

As introduced in Chapter 3, the first variant of the SQTS (SQT1) is caused by an amino acid substitution of asparagine to lysine in position 588 (N588K) in the external S5-pore linker of the hERG potassium channel [121,122]. SQT1 causes a marked rightward shift in voltage-dependent inactivation of this channel [79,244]. In AP and voltage clamp experiments, this is reflected in the marked increase of \( I_{\text{hERG}} \) during ventricular and atrial AP repolarisation phases [79,121,244,245]. Given that hERG encodes channels responsible for the rapid delayed rectifier potassium channel current (\( I_{\text{Kr}} \)) [143], the N588K gain-of-function mutation would be expected to amplify the contribution of \( I_{\text{Kr}} \) to cardiac repolarisation, and consequently shorten the QT interval [79,121,244,245]. The ventricular effective refractory period (ERP) would in turn be anticipated to shorten, leading to increased susceptibility to re-entrant arrhythmia.

At present, no phenotypically accurate experimental model of the SQT1 exists to allow exploration of its functional consequences. In order to produce abbreviated
repolarisation experimentally, investigators have performed experiments on a perfused canine left ventricular wedge treated with the adenosine triphosphate (ATP) potassium channel (K\textsubscript{ATP}) opener pinacidil or the I\textsubscript{Kr} activator PD-118057 [347,348]. In these experiments, heterogeneous abbreviation of APs across the ventricular wall has been observed (and, thereby, amplified dispersion of repolarization), ERP abbreviation, QT interval shortening and ventricular tachycardia [347,348]. Whilst the data from these studies are valuable in understanding a link between accelerated repolarization and arrhythmogenesis, the pharmacological interventions used did not mimic precisely the changes induced by the N588K SQT1 mutation. In silico reconstruction offers an alternative approach to determining the arrhythmogenic substrates in the SQT1 [125,334,336,349,350]. Existing SQT1 simulation data though are either incomplete or based on data obtained at ambient rather than physiological temperature [125,334,336,349]. Moreover, no viable tissue substrate for ventricular arrhythmia in the SQT1 has hitherto been demonstrated in any simulation study.

Accordingly, the aims of the present study were:

i. To reproduce the kinetic changes to I\textsubscript{Kr} caused by SQT1 based on available experimental data obtained at physiological temperature, in simulations constructed with both Hodgkin-Huxley (HH) and Markov chain (MC) formulations.

ii. To incorporate control and SQT1 I\textsubscript{Kr} in human ventricular cell-based models in order to determine the functional consequences of the SQT1 mutation on AP repolarisation and the QT interval, and to compare the functional differences between the HH and MC variant models;

iii. To explore the arrhythmogenic substrate in SQT1 involving the N588K hERG mutation through the use of multicellular tissue and organ simulations.

As will be shown, the results obtained through addressing these aims provide a clear link between the kinetic changes to I\textsubscript{hERG}/I\textsubscript{Kr} in SQT1 and the altered ventricular tissue electrophysiology, favouring re-entrant arrhythmia in the SQT1.
Figure 6.1: State transition diagrams of the Markov models.

(Ai) Full Markov Chain (f-MC) state transition diagram.
(Aii) Reduced Markov Chain (r-MC) state transition diagram.
6.2 Simulation of Single Cell $I_{Kr}$ under Control and SQT1 Conditions

Two SQT1 Markov models were developed: a full Markov model (f-MC) and a reduced Markov model (r-MC). A detailed discussion of the development of the SQT1 Markov models and the Hodgkin-Huxley models is given in Section 4.2. For the reader’s convenience, the state transition diagrams for the f-MC and r-MC models are reproduced in Figure 6.1. In the f-MC model, inactivation can occur from both the closed (C3) and the open (O) state whereas it occurs only from the (O) state in the r-MC model. This is the only structural difference between the two Markov models.

As a first step in model validation, the ability of the Markov models to reproduce published experimental data [79,245] on the voltage-dependence of activation of WT and the N588K mutant hERG current at physiological temperature was tested. The same voltage clamp protocol used experimentally [265] was employed (Figure 6.2Aii and 6.2Bii). Figure 6.2A shows representative $I_{Kr hERG}$ current traces for WT (Figure 6.2Ai) and N588K (Figure 6.2Bi) elicited by the voltage clamp protocol. Current-voltage (I-V) relationships were constructed from these.

The full Markov model (f-MC) and the reduced Markov model (r-MC) were found to reproduce the experimental data quite closely in both the WT and N588K mutation conditions (Figure 6.2Aiii and 6.2Biii). The experimentally observed mutation-induced defect in inactivation of the channel, which leads to the excessive generation of $I_{Kr}$ was replicated by both Markov models. In comparative simulations, two Hodgkin Huxley models – Luo Rudy (LRd) and ten Tusscher-Noble-Noble-Panfilov (TNNP) models - failed to capture the kinetics of either the WT condition or N588K mutation. In the WT condition, all the models captured the rectification of the channel but it was rightward-shifted in the LRd model compared to the data (Figure 6.2Aiii). For the N588K mutation, the marked augmentation in $I_{Kr}$ current in the mutation was seen in all the models (Figure 6.2Bi). However, only the f-MC and r-MC models accurately reproduce the data whereas there is no rectification of the I-V relation in the LRd model (Figure 6.2Bi).
Figure 6.2: Simulated Current-Voltage Relationships for $I_{\text{hERG}}$.

(Ai, Bi) Current traces for WT (A) and N588K $I_{\text{hERG}}/I_{\text{Kr}}$ (B) elicited by the voltage protocol shown in (Aii, Bii).

(Aiii, Biii) I-V relations for end pulse currents for WT (A) and N588K $I_{\text{hERG}}/I_{\text{Kr}}$ (B). End pulse currents were normalised to the current observed at 0 mV and then plotted against membrane potential.
The ability of the models to reproduce the dynamic properties of WT and N588K $I_{Kr}$ under AP voltage-clamp (“AP clamp”) and with paired AP commands (to mimic premature electrical stimulation) [245] was then examined (Figure 6.3). Figure 6.3Ai and 6.3Aii show the time course of experimentally measured WT and N588K $I_{Kr/hERG}$ during the overlaid AP clamp [245], while Figure 6.3Aiii shows the paired AP command protocol. The simulated normalised ‘instantaneous’ I-V relationships for the WT and N588K mutant during the time course of the AP clamp are shown in Figure 6.3Bi-Ei and Figure 6.3Bii-Eiii respectively for the Markov and Hodgkin-Huxley models. The equivalent experimental data [245] are superimposed on each plot for comparison with the model results.

The middle panels in Figure 6.4 and Figure 6.5 show representative WT and N588K $I_{Kr/hERG}$ obtained during ventricular AP voltage command (Figure 6.4Ai, Aii and Figure 6.5Ai, Aii). WT $I_{Kr/hERG}$ shows a small and gradual increase in current, which peaked during the repolarisation phase of the AP before declining in amplitude. In contrast, N588K $I_{Kr/hERG}$ shows a pronounced rise in outward current earlier than WT (due to the attenuation of inactivation by the mutation), leading to a dome-shaped current. Figure 6.4Ci-Cii and 6.5Ci-Cii show the simulation results from the f-MC and TNNP models respectively. The f-MC and r-MC models reproduced quite closely the experimental instantaneous I-V data for WT (Figure 6.3Bi and 6.3Ci; Figure 6.4Ai-Ci) and N588K mutation (Figure 6.3Bii and 6.3Cii; Figure 6.4Aii-Cii), including the positive shift in the peak repolarising current [245] caused by the N588K mutation. The TNNP and LRd models failed to reproduce accurately these experimental data for either the WT (Figure 6.3Di and 6.3Ei; Figure 6.5Ai-Ci) or mutant (Figure 6.3Dii and 6.3Eii; Figure 6.5Aii-Cii) conditions including the positive shift in peak repolarising current.

Figure 6.3Bi-Eiii shows the responses of hERG/$I_{Kr}$ to the protocol comprised of paired-AP stimuli shown in Figure 6.3Aiii (see also [245]). The time-course profile of currents elicited by this protocol reflects the interaction between recovery from inactivation and deactivation of $I_{hERG}/I_{Kr}$ channels [245,288]. The f-MC, r-MC and TNNP models reproduced closely the experimental response of the hERG/$I_{Kr}$ channel to a premature stimulus [245] while the LRd model failed to do so. Under the WT condition, the $I_{Kr}$ amplitude increased with increasing inter-pulse interval reaching a peak at 30 ms after which it decreased with increasing inter-pulse intervals. Under the N588K condition, $I_{Kr}$ amplitude began at significantly higher amplitude than WT and
increased with increasing inter-pulse intervals. It peaked 10 ms earlier than for WT before it declined at greater intervals. During its decline, it was smaller than WT in amplitude between 30-70 ms of the inter-pulse interval. However, the response of the LRd H-H model to paired-AP stimuli (Figure 6.3Eiii) failed to reproduce published experimental data [245]. Considered collectively, the AP clamp simulation data suggested that the f-MC and the r-MC models recapitulated better the dynamic properties of WT and N588K I_{hERG}/I_{Kr} at 37°C than did the TNNP and Luo-Rudy H-H formulations.

In order to characterise the functional effects of the N588K mutation on ventricular APs, the Markov models (f-MC and r-MC) and the HH models (Luo-Rudy and TNNP) for WT and N588K I_{Kr} were incorporated into the TNNP human ventricular single cell AP model. Figure 6.6A shows for the f-MC model in an EPI cell: simulated APs (6.6Ai), I_{Kr} profile (6.6Aii) and instantaneous I_{Kr} I-V relationship (6.6Aiii). The MCELL and ENDO equivalents are shown in Figures 6.6B and 6.6C respectively. In all three cell types, the N588K mutation abbreviated the action potential. Under the WT condition, following the upstroke of the AP, I_{Kr} increased in amplitude gradually, reaching a peak during the plateau phase just before terminal repolarisation. It then declined during the final repolarization phase of the AP [245,247,334,348,349]. With the N588K mutation, I_{Kr} activated earlier following the upstroke of the AP, increased considerably more rapidly and achieved significantly higher maximal amplitude earlier during the plateau phase than in the WT condition. Collectively, these changes in current profile led to a marked shortening of the APD. The APD_{90} for the WT and N588K mutant conditions plus the differences in APD_{90} are shown in Table 6.1.

Greater I_{Kr} earlier during the AP (in phase 2 rather than phase 3) caused the APD shortening in the N588K mutant condition, which resulted in accelerated repolarisation of the AP (Figure 6.6Ai and 6.6Aii). The MIDDLE and ENDO cell models similarly also showed APD reduction as illustrated in Figure 6.6B and 6.6C. Figure 6.6D-6.6F summarise the results for each of the f-MC, r-MC and the two HH I_{Kr} formulations in the EPI, MIDDLE and ENDO cell types. The marked APD shortening seen in these simulations due to augmented I_{Kr} in SQT1 agrees with results seen in previous studies, in which increased I_{Kr} due to the N588K mutation produced AP shortening in the Luo-Rudy and the Priebe-Beuckelmann AP models [248,334,336,349].
Figure 6.3: $I_{hERG}/I_{Kr}$ Current-Voltage (I-V) relations during action potential clamp and effect of premature stimuli.

(A) Experimental recordings of current profiles of WT (Ai) and N588K $I_{hERG}$ (Aii) elicited by the ventricular AP command waveform overlaid [245]. In each case, instantaneous current during AP repolarisation was normalised to maximal current elicited by the waveform. (Aiii) Paired ventricular AP command waveform protocol used to elicit the $I_{hERG}/I_{Kr}$ currents from which the normalised data in Biii, Ciii, Diii and Eiii were derived [245].

(B) Full Markov chain model: Instantaneous I-V relationships for WT (Bi) and N588K $I_{hERG}/I_{Kr}$ (Bii). Thick lines show experimental recordings while the dashed lines show simulation results. (Biii) Plots of $I_{hERG}/I_{Kr}$ current during paired AP command waveforms (Aiii) for WT (squares) and N588K condition (circles) respectively.

(C) Reduced Markov chain model: Instantaneous I-V relationships for WT (Ci) and N588K $I_{hERG}/I_{Kr}$ (Cii). Thick lines show experimental recordings while the dashed lines show simulation results. (Ciii) Plots of $I_{hERG}/I_{Kr}$ current during paired ventricular AP command waveforms (Aiii) for WT (squares) and N588K condition (circles) respectively.

(D) TNNP model: Instantaneous I-V relationships for WT (Di) and N588K $I_{hERG}/I_{Kr}$
(Dii). Thick lines show experimental recordings while the dashed lines show simulation results. (Diii) Plots of $I_{hERG}/I_{Kr}$ current during paired ventricular AP command waveforms (Aiii) for WT (squares) and N588K condition (circles) respectively.

(E) Luo-Rudy model: Instantaneous I-V relationships for WT (Ei) and N588K $I_{hERG}/I_{Kr}$ (Eii). Thick lines show experimental recordings while the dashed lines show simulation results. (Eiii) Plots of $I_{hERG}/I_{Kr}$ current during paired ventricular AP command waveforms (Aii) for WT (squares) and N588K condition (circles) respectively.
Figure 6.4. Profile of \( I_{\text{hERG}} \) during ventricular AP voltage command under WT and N588K conditions obtained with the full Markov Chain model (f-MC).

A: Ventricular AP command waveform used to elicit the WT and N588K currents in B and C.

B: Example current profiles of WT (Bi) and N588K-hERG (Bii) elicited by ventricular AP voltage clamp command.

C: Simulated current profiles of WT (Ci) and N588K-hERG (Cii) elicited by ventricular AP voltage clamp command.
Figure 6.5: Profile of $I_{\text{hERG}}$ during ventricular AP voltage command under WT and N588K conditions obtained with the original TNNP $I_{\text{Kr}}$ formulation model.

A: Ventricular AP command waveform used to elicit the WT and N588K currents in B and C.

B: Example current profiles of WT (Bi) and N588K-hERG (Bii) elicited by ventricular AP voltage clamp command.

C: Simulated current profiles of WT (Ci) and N588K-hERG (Cii) elicited by ventricular AP voltage clamp command.
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<th>N588K</th>
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Table 6.1: Computed APD<sub>90</sub> (ms) and ΔAPD<sub>90</sub> (ms) under WT and N588K condition for the ENDO, MIDDLE and EPI cell models. ΔAPD<sub>90</sub> was computed as the difference of APD<sub>90</sub> between that of control condition and that of N588K condition.
Figure 6.6: Simulation of ventricular action potential and $I_{Kr}$ time courses.

(i) Steady state (1 Hz) action potentials for EPI (Ai), MIDDLE (Bi) and ENDO (Ci) cells using the full Markov chain $I_{hERG}/I_{Kr}$ model. Thick lines represent the WT and dashed lines represent the N588K condition.

(ii) Corresponding $I_{Kr}$ current profiles for EPI (Aii), MIDDLE (Bii) and ENDO (Cii) cells. Thick lines represent the WT and dashed lines represent the N588K condition.

(iii) Corresponding I-V relationships for EPI (Aiii), MIDDLE (Biii) and ENDO (Ciii) cells. Thick lines represent the WT and dotted lines represent the N588K condition.

(D,E) $I_{Kr}$ current amplitude for EPI (black bars), MIDDLE (grey bars) and ENDO (white bars) cells for all four $I_{hERG}/I_{Kr}$ formulations for WT (D) and N588K (E) conditions

(F) Computed APD difference between EPI and MIDDLE cells.
One notable finding in respect of AP shortening is that the N588K mutation abbreviated APD$_{90}$ to different degrees in the different cell types (i.e., non-uniformly), with the greatest attenuation occurring in the MIDDLE cell. This situation has been seen in previous studies [334,336]. Consequently, the mutation decreased the transmural dispersion of APD$_{90}$ across the different cell types from the ventricular wall: EPI, MIDDLE and ENDO. Simulation results with the use of the f-MC, r-MC and H-H formulations all gave consistent results of decreased dispersion of APD$_{90}$ by the N588K mutation when the models were compared (Figure 6.6F).

Figure 6.7 shows the effect of the N588K mutation on APD restitution (APD-R). The four different I$_{Kr}$ formulations were adjusted so that all the models showed similar APD restitution curves in the WT condition for EPI, MIDDLE and ENDO cells (Figure 6.7Ai-Ci). Incorporation of the N588K mutation led to flattening of the APD restitution curves and to a reduction in APD$_{90}$ in all three cell types. The mutation also shifted the APD restitution curves leftward and decreased the maximal slopes in the f-MC, r-MC and TNNP cell models (Figure 6.7D). SQTS patients tend to exhibit poor rate-adaptation of their QT intervals [224,236–238,247,258] and my results are consistent with this phenomenon as they suggested an attenuation of rate-adaptation of ventricular APD. To investigate this further, simulations of the steady state rate-dependence of the APD were performed (Figure 6.8) using the f-MC model. Again, the N588K mutation flattened the curve and caused a leftwards shift. The f-MC and r-MC I$_{Kr}$ mutant models showed greater APD abbreviation than the HH models, with the LRd I$_{Kr}$ mutant model showing the least APD attenuation.

Figure 6.9 shows the effect of the N588K mutation on ERP restitution. As with the APD-R curves, the N588K mutation led to flattening of the ERP restitution (ERP-R) curves and to abbreviation of ventricular ERP in EPI, MIDDLE and ENDO cells (Figure 6.9A-C). The mutation also shifted the ERP restitution curves leftward and decreased the maximal slopes in all the cell models (Figure 6.9D). Similar to the APD-R results, these results also suggested a loss of rate-adaptation of ventricular ERP. In the WT condition, the four different I$_{Kr}$ formulations produced similar ERP restitution curves, and as with the APD-R, the f-MC and r-MC I$_{Kr}$ mutant models showed greater ERP reduction than the HH models, with the LRd I$_{Kr}$ mutant model showing the least ERP attenuation.
Figure 6.7: Rate-dependent APD restitution.

(Ai, Bi, Ci): WT APD restitution curves for EPI, MIDDLE and ENDO cells respectively for the full Markov chain $I_{hERG}/I_{Kr}$ model formulation.

(Aii, Bii, Cii) N588K APD restitution curves for EPI, MIDDLE and ENDO cells respectively for the full Markov chain $I_{hERG}/I_{Kr}$ model formulation.

(D, Dii) Slopes of WT and N588K APD restitution curves for full Markov chain, reduced Markov chain, Luo-Rudy and TNNP hERG/I$_{Kr}$ model formulations.
Figure 6.8: Steady state APD rate dependence for the Full Markov Chain model (f-MC) incorporated into the TNNP ventricular action potential cell model.
Figure 6.9: ERP restitution curves of four models of ventricular myocytes.

(Ai, Bi, Ci) WT ERP restitution curves for EPI, MIDDLE and ENDO cells respectively for the full Markov chain $I_{\text{hERG}}/I_{\text{Kr}}$ model formulation.

(Aii, Bii, Cii) N588K ERP restitution curves for EPI, MIDDLE and ENDO cells respectively for the full Markov chain $I_{\text{hERG}}/I_{\text{Kr}}$ model formulation.

(Di, Dii) Slopes of WT and N588K ERP restitution curves respectively for the full Markov chain, reduced Markov chain, Luo-Rudy and TNNP $I_{\text{hERG}}/I_{\text{Kr}}$ model formulations.
6.3 Simulation of the ECG with WT and N588K mutant 

$\text{I}_{\text{Kr}}$

A pseudo-ECG was computed (as described in Chapter 5, section 5.3.1) using a 1D strand of cells across the ventricular wall for the WT and N588K conditions at a stimulation rate of 1Hz (Figure 6.10). Due to the limitations of the HH formulations in recapitulating the channel properties of $\text{I}_{\text{Kr}}$ as shown in Figure 6.2-6.9, these and all subsequent simulations were carried out with the f-MC model. A propagating excitation wave was initiated at the ENDO end of the strand by delivering a series of supra-threshold stimuli (section 5.2.3). The wave propagated from the ENDO through the MIDDLE and towards the EPI end of the strand. Figure 6.10A and 6.10B show space-time plots for WT and N588K conditions respectively, with space running vertically from ENDO at the bottom to EPI at top and time running horizontally from left to right. The N588K mutant shortened the QT interval to 240 ms from 378 ms for the WT condition (Figure 6.10C (WT) and 6.10D (N588K)). Although the simulation of the N588K mutation reproduced the QT interval shortening seen in SQT1 patients, it failed to reproduce another key feature of SQT1 ECGs: a significant increase in the T-wave amplitude.

To rectify this deficiency, it was necessary to consider a heterogeneous distribution of $\text{I}_{\text{Kr}}$ density in the 1D strand model. Available experimental data show that that hERG protein expression is approximately 1.5 times greater in the EPI region than in the MIDDLE region of human ventricle [75], providing a rational basis for the incorporation of heterogeneous $\text{I}_{\text{Kr}}$ distribution in the strand. The $\text{I}_{\text{Kr}}$ density was adjusted to be between 1.5-1.7 times greater in the EPI region than in the MIDDLE region. Following this adjustment, the model reproduced both key features of the SQT1 ECG under the mutation condition: QT interval abbreviation and increased T-wave amplitude. The results are shown in Figure 6.10E-H.
Figure 6.10: Space-time plot of AP propagation along a 1D transmural ventricular tissue strand and computed pseudo-ECGs.

(A, B) Colour mapping of membrane potential of cells along the 1D strand from blue (-86 mV) to red (-42 mV) (see colour key). Space runs vertically from the ENDO end to the EPI end at the top. Time runs horizontally. (A) Control (WT) condition. (B) SQT1 (N588K) condition.

(C, D) Pseudo-ECGs corresponding to the WT and SQT1 (N588K) conditions respectively.

(E, F, G and H) WT and N588K pseudo-ECGs for the different EPI: MIDDLE:ENDO I_{Kr} density ratios of 1.0:1:1, 1.5:1:1, 1.6:1:1 and 1.7:1:1 respectively.
Figure 6.11: Membrane potential heterogeneity ($\delta V$).

(A, B, C, D) Plots of $\delta V$ against time for WT (continuous lines) and N588K (dotted lines) conditions for different EPI:MIDDLE:ENDO $I_K$ density ratios; (A) 1.0:1:1 (B) 1.5:1:1 (C) 1.6:1:1 (D) 1.7:1:1.

(E) Maximum $\delta V$ during repolarization between ENDO-EPI cells in WT and N588K.
6.4 Simulation of the Spatial Gradient of the Membrane Potential

Gima and Rudy [335] previously suggested that an increased spatial gradient of the membrane potential (δV) could be responsible for the tall T-wave height seen in hyperkalemia. It was also found to be relevant to the SQTS in a prior simulation study of SQT2 from our laboratories [248]. Therefore, to find out if the tall T-wave seen in the SQT1 ECGs shown in Figure 6.10 could also be a consequence of this increased spatial gradient of δV, the effects were investigated of the N588K mutation on membrane potential heterogeneity (δV) during ventricular APs between the three cell types. The results are shown in Figure 6.11, incorporating differing ratios of EPI $I_{K_r}$ to MIDDLE and ENDO $I_{K_r}$ in the WT (solid lines) and the N588K (dotted lines) mutation conditions. It shows the time course of the pair-wise differences of δV between the cell types for both the WT and N588K conditions.

With a ratio of 1:1:1 (Figure 6.11A) of EPI $I_{K_r}$ to MIDDLE and ENDO $I_{K_r}$, the N588K mutation decreased the (δV) in each pair-wise comparison. However, with ratios of 1.5:1:1 (Figure 6.11B), 1.6:1:1 (Figure 6.11C) and 1.7:1:1 (Figure 6.11D) of the EPI $I_{K_r}$ to MIDDLE and ENDO $I_{K_r}$, the N588K mutation increased the δV between ENDO and EPI cells (Fig 6.11E), which is likely to have contributed to the increased T-wave amplitude in these simulation conditions [248,335].

Figure 6.12A shows the spatial dispersion of APD$_{90}$ across the intact transmural strand for both WT and N588K conditions with differing ratios of $I_{K_r}$ density in the EPI, MIDDLE and ENDO regions. In the intact 1D strand, the electrotonic interactions between cells smoothed out the APD distribution (Figure 6.12A) for both the WT and N588K mutation conditions. With a 1:1:1 ratio of EPI $I_{K_r}$ to MIDDLE and ENDO $I_{K_r}$, the SQT1 mutant attenuated the spatial dispersion of APD$_{90}$ relative to the WT condition. This is also illustrated by the plot of the spatial gradient of APD in Figure 6.12B and its absolute value (Figure 6.12C). However, with a ratio of 1.5:1:1 or above for the EPI $I_{K_r}$ to MIDDLE and ENDO $I_{K_r}$, the N588K mutation augmented APD dispersion at localised regions of the MIDDLE region and at the junction region between the MIDDLE and EPI regions, which also contributed to an increased T-wave amplitude. A very sharp transition in APD$_{90}$ can be seen between the MCELL and EPI
Figure 6.12: Transmural APD90 distribution and its spatial gradient along a 1D tissue strand

(A) Spatial distribution of APD90 in the 1D transmural strand for WT (blue) and N588K (red) for different EPI:MIDDLE:ENDO I_Kr density ratios. Continuous lines (1.0:1:1), dash-dot lines (1.5:1:1), dash-dash lines (1.6:1:1), dotted lines (1.7:1:1).

(B, C) Spatial gradient (B) and absolute spatial gradient (C) of APD90 in the 1D transmural strand for WT (blue) and N588K (red) for different EPI:MIDDLE:ENDO I_Kr density ratios. Continuous lines (1.0:1:1), dash-dot lines (1.5:1:1), dash-dash lines (1.6:1:1), dotted lines (1.7:1:1).
regions in both Figures 6.12B and 6.12C. This is due to a discontinuity in the electrical coupling at this border between MIDDLE and EPI cells, and is consistent with experimental observations made by Yan et al. [333] in an arterially perfused left ventricular wedge preparation and Drouin et al. [37] in their study (see Section 5.2.3).

6.5 Investigating the Arrhythmogenic Substrate in SQT1 – 1D simulations

In order to investigate the susceptibility of the N588K mutant tissue to ventricular arrhythmias, simulations were carried out that quantified the vulnerability of the tissue to unidirectional conduction block in response to a premature stimulus: the susceptibility of the tissue to a premature stimulus provides a means of quantifying the risk of generating re-entrant excitation (that could lead to fibrillatory activity [351–354]). The premature stimulus was applied at the refractory tail of a previous excitation wave. Section 5.2.3.2 provides a detailed explanation of the procedure used to determine this vulnerable period of the tissue.

The results are shown in Figure 6.13. The mutation decreased the vulnerable window throughout most of the strand except in the MIDDLE region marked by the superimposed vertical lines where the width of the vulnerable window was increased by the mutation. These results show that under the N588K mutation, the vulnerability of the tissue to arrhythmia is augmented in localised regions.

6.6 Investigating the Arrhythmogenic Substrate in SQT1 – Idealised 2D geometry simulations

An idealised 2D tissue model was used to measure the minimal spatial size of a premature test stimulus necessary to produce re-entry under both WT and N588K conditions with a 1.6:1:1 ratio of the EPI $I_{K_{r}}$ to MIDDLE $I_{K_{r}}$ to ENDO $I_{K_{r}}$. The tissue had three distinct regions (ENDO, MCELL and EPI) similar to the transmural 1D
Figure 6.13: Measured width of the vulnerable window along the 1D tissue strand. 
(A, B, C, D) Vulnerable window for WT and N588K along the 1D strand for different EPI:MIDDLE:ENDO $I_Kr$ density ratios; (A) 1.0:1:1 (B) 1.5:1:1 (C) 1.6:1:1 (D) 1.7:1:1. 
(E) Comparison of the width of the vulnerable window between WT and N588K in the MIDDLE region of the 1D strand marked by double lines.
strand; it was essentially the 1D strand swept out along the y-axis assuming the 1D strand is along the x-axis (Figure 6.14A).

A planar excitation wave propagating from the ENDO towards the EPI region of the 2D sheet was evoked at the ENDO end via a conditioning stimulus for WT (Figure 6.14Ci) and N588K (Figure 6.14Di) conditions. After a time delay, a premature stimulus was applied to a local region in the EPI region during its VW (WT; Figure 6.14Cii and N588K; Figure 6.14Dii), thereby producing unidirectional conduction, towards the EPI end (as the MCELL region was still refractory). This resulted in the formation of spiral re-entrant excitation waves in both WT (Figure 6.14Ciii) and N588K (Figure 6.14Diii) conditions, which self-terminated under the WT condition (Figure (6.14Civ) but were sustained for the N588K condition (Figure 6.14Div).

As the formation of the re-entrant excitation waves is dependent on the size of the premature test stimulus, the minimal tissue substrate size that could evoke re-entry was measured. This is proportional to the wavelength of excitation (the product of conduction velocity and APD90) and gives the minimal size of the substrate length necessary to sustain reentry in ventricular tissue. The measured size was 51 mm in the WT condition and 23 mm in the N588K condition (Figure 6.14B), showing that with the N588K mutation, it was easier to induce re-entry and hence, ventricular arrhythmias. Once initiated, reentry terminated within 284 ms in WT but was sustained in the N588K mutant condition. Thus these simulations suggest that in the N588K mutation SQT1 condition there is a greater susceptibility to ventricular arrhythmia.

6.7 Investigating the Arrhythmogenic Substrate in SQT1 – 2D and 3D Simulations with Realistic Geometry

Realistic ventricular geometry is considerably more complex structurally than an idealised 2D sheet. It also has anisotropic conduction due to the presence of fibre orientations. Therefore, it may be erroneous to assume that the results in the idealised 2D geometry necessarily translate into similar activity with realistic tissue geometry. Consequently, simulations were performed in a 2D cross-section of human ventricle tissue (Figure 5.3) and in human 3D anatomical ventricle geometry (Figure 5.5). The
Figure 6.14: Snapshots of initiation and conduction of re-entry in a 2D idealised model of transmural ventricle.

(A) Schematic representation of the 2D model.

(B) Minimal spatial length of a premature S2 stimulus that provides a sufficient substrate for the re-entrant circuit formation in WT and N588K for different EPI: MIDDLE: ENDO I_{Kr} density ratios; 1.0:1:1, 1.5:1:1, 1.6:1:1 and 1.7:1:1.

(C, D) Ci and Di: A planar conditioning wave generated by S1 stimulus at the ENDO end. Snapshots at 10 ms. Cii and Dii: S2 stimulus applied to the EPI part during the vulnerable window of the local tissue Ciii and Diii: Developed spiral wave from the S2 stimulus. Snapshots at 500 ms. Civ and Div: Snapshot of spiral wave at 1000 ms. Spiral wave self-terminated under the control condition before this recording point, but persisted under the SQT1 condition. Cv and Dv: Evolution of the action potential of a cell in the epicardial region for WT and N588K conditions.
regions in the 2D and 3D ventricular geometries were divided into the same proportions of EPI, MCELL and ENDO as in the 1D strand and the idealised 2D geometry.

6.7.1 Simulations in Realistic 2D Geometry

Figure 6.15 shows the simulation results in the 2D human ventricle slice with a 1.6:1:1 ratio of the EPI $I_{Kr}$ to MIDDLE $I_{Kr}$ to ENDO $I_{Kr}$. Figures 6.15A and 6.15B show the application of a premature stimulus (WT: 400 ms and N588K: 241 ms) during the vulnerable window of a local region in the left ventricle in both WT and N588K conditions. This led to the development of re-entrant excitation waves in both conditions (Figures 6.15B and 6.15G). These re-entrant spiral waves self-terminated within 755 ms in WT (Figure 6.15D and 6.15K) but persisted in the N588K mutant (Figure 6.15I and 6.15K). The spiral waves persisted for the entire duration (5s) of the simulation in the N588K condition (Figure 6.15K).

Figures 6.15E and 6.15J show the evolution of the AP in a local cell in the left ventricle under both WT and N588K conditions. Power spectrum analysis carried out on the APs showed a higher dominant frequency under the N588K condition compared to the WT condition (Figure 6.15L). These results together are consistent with those from the idealised 2D tissue.

6.7.2 Simulations in Realistic 3D Geometry

Figure 6.16 shows the results of the simulations performed using the anatomical human 3D ventricle geometry, with a 1.6:1:1 ratio of the EPI $I_{Kr}$ to MIDDLE $I_{Kr}$ to ENDO $I_{Kr}$. The premature stimulus (with an amplitude of -104 pA/pF) was applied from the base of the ventricle up to halfway towards its apex, covering a region of approximately 90x63 mm in both WT (380 ms) and N588K (245 ms) conditions (Figure 6.16A and 6.16B). This region included the left ventricle and the surrounding area of the right ventricular outflow tract. The premature stimulus was applied during the vulnerable window following the refractory tail of a previous excitation wave. It evoked an excitation wave that propagated uni-directionally in the retrograde direction of the control excitation.
Figure 6.15: Snapshots of initiation and conduction of re-entry in realistic 2D model cross-section of ventricles.

(A, F) Application of a premature S2 stimulus into the refractory and partially recovered region of an excitation wave after a delay of 400 ms for WT and 241 ms for N588K condition from the initial wave stimulus.
(B, G) Developed spiral wave from the S2 stimulus. Snapshot at time = 500 ms.
(C, H) Snapshot of spiral wave at time= 1000 ms. The induced spiral wave transited from transmural re-entry with tip rotating within the ventricle wall to anatomical re-entry with tip rotating around the ventricle boundary in WT. However, transmural re-entry persisted in N588K condition and broke-up forming regenerative multiple re-entrant wavelets.
(D and I) Snapshot of spiral wave at time =2000 ms. Spiral wave self-terminated in WT before this recording point, but persisted in N588K condition.
(E and J) Evolution of the action potential of a cell in the left ventricle for WT and N588K conditions.
(K) Measured lifespan of the re-entry circuits in WT and N588K condition.
(L) Computed dominant frequency of electrical activity recorded from the tissue in WT and N588K conditions (about 2.7 Hz for WT and 3.4 Hz for N588K condition).
Figure 6.16 Snapshots of initiation and conduction of re-entry in a 3D anatomical model of human ventricles.

(A, F) Application of a S2 premature stimulus (red) in a local region during the refractory period of a previous conditioning excitation wave after a time delay of 380 ms for WT and 245 ms for N588K condition from the initial conditioning wave.
stimulus.

(B, G) Developed scroll wave from the S2 stimulus. Snapshot at time = 500 ms.

(C, H) Snapshot of scroll wave at time = 750 ms. The scroll wave self-terminated in the WT condition, but persisted and broke up forming regenerative wavelets in the N588K condition,

(D and I) Snapshot of scroll wave at time = 1000 ms. The scroll wave self-terminated in WT before this recording point, but still persisted in N588K condition.

(E and J) Evolution of the action potential of a cell in the left ventricle for WT and N588K conditions.

(K) Measured lifespan of re-entry scroll wave in WT and in N588K condition.

(L) Computed dominant frequency of electrical activity recorded from ventricle in WT and N588K conditions (2.7 Hz for WT and 6.3 Hz for N588K condition).
wave, leading to the generation of re-entrant scroll waves within the ventricular wall (Figure 6.16B and 6.16G).

The re-entrant scroll waves terminated within 600 ms in the WT condition (Figure 6.16D) but broke up into sustained multiple wavelets under the N588K condition (Figure 6.16I). Figure 6.16K shows the lifespan of re-entry under both conditions while Figure 6.16E and 6.16J show the evolution of the AP in a local cell in the left ventricle under both WT and N588K conditions respectively. Power spectrum analysis on the APs showed a higher dominant frequency under the N588K condition (5.3 Hz) compared to the WT condition (2.7 Hz) (Figure 6.16L). This further supports the pro-arrhythmic nature of the N588K mutation, which was shown in the 1D and 2D simulations.

6.8 Discussion and Conclusions

6.8.1 Summary of major findings

A phenotypically accurate mammalian experimental model of SQT1 does not yet exist. Therefore, the use of in silico models and methods provides an alternative and valuable means of investigating the functional consequences of SQT mutations on genesis and maintenance of ventricular arrhythmias. The major findings of the present study are:

(i) The Markov chain $I_{kr}$ formulations tested reproduced better the dynamic properties of hERG/$I_{kr}$ under both WT and N588K hERG SQT1 conditions than did the Luo-Rudy and TNNP H-H formulations;

(ii) The N588K hERG mutation is causally linked to QT interval shortening, whether or not $I_{kr}$ is presumed to be homogeneously distributed across the ventricular wall; however, a heterogeneous $I_{kr}$ density across the ventricular strand model was found to be necessary to reproduce a taller T-wave amplitude as has been seen clinically in SQT1;

(iii) With a heterogeneous $I_{kr}$ density across the strand, the N588K mutation led to augmented membrane potential differences ($\delta V$) between ENDO and EPI cells compared pair-wise and transmural APD dispersion in localised regions of the transmural strand that contributed to the increased T-wave amplitude;
(iv) The N588K mutation increased at some localised regions the tissue’s temporal vulnerability to the genesis of uni-directional conduction by a premature excitation;

(v) The N588K mutation decreased the minimal tissue substrate size that facilitates the maintenance of re-entry as shown in both idealised and realistic tissue models of the human ventricle. These findings substantiate the causal link between the N588K mutation and QT interval shortening and, moreover, provide a comprehensive explanation for increased susceptibility to re-entry and perpetuation of re-entrant arrhythmia in the setting of SQT1.

6.8.2 Significance of the study

In silico characterisation of the functional consequences of the N588K hERG, KvLQT1 V307L (SQT2) and KCNJ2 (SQT3) mutations on ventricular cell AP shortening and characteristics of simulated ECGs have been the subject of some prior studies [125,248,334]. However, whilst for the V307L-KCNQ1 SQT2 mutation, investigation (using 1D and 2D idealised geometries) of its pro-arrhythmic effects has been conducted [248], simulations addressing the effects of the N588K mutation on perpetuating and facilitating re-entrant excitation waves in ventricular tissue have not been performed until now. Furthermore, the present study is the first to determine the arrhythmogenic consequences of the N588K mutation using multi-scale models of the human ventricles.

In an earlier study, Kogan and colleagues [355] used a simple model to demonstrate similar re-entrant propensity when the AP is shortened. They found that slowing the deactivation rate of delayed outward K\(^+\) current had a profound effect on wave-front propagation. Although the Kogan et al. study is important in that it provides a causal link between augmented K\(^+\) conductance (via deactivation alteration of deactivation characteristics) and arrhythmogenesis, the SQT1 N588K-hERG mutation is characterised by impaired inactivation not deactivation, and therefore requires targeted simulations that specifically reproduce kinetic changes to I\(_{Kr}\) by the SQT1 N588K-hERG mutation.
Prior studies [334,336] have reported inhomogeneous shortening of ventricular APD with loss of $I_{Kr}$ inactivation, which appears paradoxical in light of increased arrhythmia susceptibility in the syndrome. The present study resolves this apparent contradiction: whilst it has been demonstrated that the AP shortening as a result of N588K mutation is inhomogeneous, (resulting in a decreased $APD_{90}$ and ERP dispersion, as the greatest shortening of $APD_{90}$ occurred in MIDDLE cell APs), with heterogeneous $I_{Kr}$ in the ventricle the mutation augmented both membrane potential difference between paired ENDO and EPI cells and the APD dispersion in some localised regions of the transmural strand. The present study is the first to incorporate heterogeneous $I_{Kr}$ density in the ventricle [75] for the study of SQT1. These changes lead to an increased T-wave amplitude, which is different to previous simulation results but is consistent with clinical observations [121,235,238,356]. Another consequence of these changes is greater susceptibility of the tissue to uni-directional conduction block in response to a premature excitation stimulus.

The present study also shows that the N588K mutation reduces the minimal tissue size of the substrate required to facilitate and sustain re-entry in both idealised 2D and realistic 2D and 3D geometries. This occurs with either a homogeneous or heterogeneous distribution of $I_{Kr}$ across the ventricular wall. In all tissue models, a single reentrant excitation wave can break up into multiple re-entrant circuits, leading to a transition from tachycardia-like to fibrillation-like electrical excitation waves.

### 6.8.3 Relevance to previous studies

Extramiana and Antzelevitch [348] used the $I_{K_{ATP}}$ channel activator pinacidil on a perfused canine ventricular wedge to study arrhythmogenesis under an abbreviated repolarisation setting. This intervention resulted in preferential abbreviation of the mid-myocardial AP, increased transmural dispersion of repolarisation and easily induced ventricular tachycardia via programmed electrical stimulation [348]. The $I_{Kr}$ agonist, PD-118057, was also subsequently found to produce similar results [346], although the mechanism through which this compound increases $I_{Kr}$ is mechanistically distinct from that of the hERG N588K mutation. Unlike N588K-hERG, PD-118057 does not affect the gating or kinetic properties of the hERG/$I_{Kr}$ channel [357]; it has been postulated that this compound is able to bind directly to the channel and increase its open
probability [357]. The simulations in this chapter show for the first time that with biophysically accurate $I_{Kr}$ models of N588K-hERG, a similar pro-arrhythmic substrate does actually occur for SQT1.

A prior simulation study by Zhang et al. [248] has shown that the effects of the adult SQT2 variant also involve an augmentation of transmural APD heterogeneity, refractory dispersion and increased tissue vulnerability to arrhythmogenesis in the ventricle [248], although geometrically accurate 2D and 3D simulations were not investigated. Results of the present simulations suggest that there is however a difference in the extent and nature of transmural APD dispersion produced by SQT2 and SQT1 variants. Whilst SQT2 augments the APD dispersion across the whole transmural strand [248], SQT1 increases the APD dispersion in some localised regions (Figure 6.12). The likely underlying mechanism is probably due to the difference in the augmentation of the spatial gradient of the membrane potential ($\delta V$). Compared to the WT condition, SQT1 augments $\delta V$ pairwise only between ENDO and EPI cell types (Figure 6.11) whereas the SQT2 variant augments $\delta V$ pairwise between all cell types (Figure 7.7A).

Thus, in the present study, it was discovered that, with a heterogeneous distribution of $I_{Kr}$ density across the ventricular wall, the N588K hERG mutation may lead to augmented APD dispersion in localised regions of tissue and augmented membrane potential difference. These changes account for an increased T-wave amplitude on the ECG as observed clinically. In addition, they can also enhance the vulnerability of tissue to unidirectional conduction block in localised regions of the ventricular wall, thus facilitating reentry. Nevertheless, a similarity between SQT1 and SQT2 [248] from the simulations is that both the N588K mutation (this study) and the V307L-KCNQ1 mutation [248] reduce the minimal size of ventricular tissue to sustain reentry.

Since commencing this study, a new mutation to the KCNH2 gene, which encodes the hERG channel has been discovered in a Chinese family [123]. It involves a single base pair C $\rightarrow$ T substitution at nucleotide 1853 (C1853T) of the KCNH2 gene, which resulted in an amino-acid substitution (threonine to isoleucine exchange) at position 618 (T618I) in the hERG channel protein [123]. The T618I mutation alters hERG gating kinetics by attenuating inactivation and increasing the rate of recovery from inactivation [123]. The major benefit of the in silico approach adopted in the present study is that the models that have been developed can be used to investigate the pro-arrhythmic effects
of the T168I mutation (and indeed any further new hERG mutations identified in the future in patients with variant 1 SQTS). The same approach also has utility in investigating the efficacy of and consequently, the design of drugs to counter the pro-arrhythmic effects of the hERG mutations.

The study that constitutes the basis for this thesis chapter has been published in full paper form [358].
Chapter 7

Mathematically Modelling the Functional Consequences of the SQT2 Mutation

7.1 Introduction

Variant 2 of the SQTS (SQT2) is associated with gain-of-function mutations to the KCNQ1 protein [124,239], which when co-expressed with KCNE1 (mink; β-subunit) recapitulates the $I_{Ks}$ channel [169,170] that is partly responsible for repolarisation of the action potential. As introduced in Chapter 3, adult SQT2 was the second variant to be identified in an SQT patient [124]. A valine to leucine substitution at position 307 (V307L) on the P-loop of the KCNQ1 protein was found in a 70-year-old man successfully resuscitated from ventricular fibrillation. He was observed to have a shortened rate-corrected QT (QTc) interval of ~300 ms [124]. In vitro electrophysiological studies have revealed that the V307L mutation shifts the voltage-dependence of activation towards more negative voltages and accelerates the time-course of current activation, resulting in increased repolarising $I_{Ks}$ which has been assumed to shorten the action potential duration leading to an abbreviated QT interval [124]. However, due to lack of experimental animal models, the causal link between the V307L mutation and a shortened QT interval, especially the exact mechanism(s) by which it increases the risk of arrhythmogenesis has not been elucidated.

As discussed in previous chapters, in silico studies constitute an alternative approach that can be used to identify arrhythmogenic substrates and mechanisms in the SQTS. In reporting the SQT2 variant, Bellocq et al. [124] also conducted limited in silico investigation; while they observed the characteristic action potential shortening, they did not investigate the mutation’s impact on the QT interval or its pro-arrhythmic effects. Subsequently, using Hodgkin-Huxley style formulations [32,305] for $I_{Ks}$, Zhang et al. [248] conducted the first detailed in silico study of adult SQT2:
where $g_{ks}$ is the maximal conductance ($\mu$S/pF), $x_s$ is the activation variable, $x_{s,\infty}$ is the steady state activation variable, $E_{ks}$ is the equilibrium potential of the channel, $\tau_{xs}$ is the voltage-dependent time constant of activation, $s$ is the slope factor, $V_m$ is the membrane potential and $V_{0.5}$ is the half-activation voltage. This study [248] modified parameters in Equations (92)-(94) to reproduce the data of Bellocq et al. [124]. However, a subsequent in vitro study reported an additional kinetic change to KCNQ1+KCNE1 channel current: slowed current deactivation in the SQT2 [265]. This finding was not available at the time of the study of Zhang et al. [248], nor does the study by Zhang et al. [248] consider the functional consequences of the mutation in anatomically realistic geometries in two and three dimensions.

The present study was conducted to address these issues, and has gone further by developing a Markov chain model for the effects on $I_{ks}$ of the SQT2 V307L KCNQ1 mutation (section 4.3). Markov chain models have several advantages over HH-style models including the ability to reproduce more accurately the kinetics of ion channels (see Section 4.1 for a detailed discussion of their advantages over HH models).

The specific aims of the present study were:

i. to reproduce the kinetic changes to $I_{ks}$ in SQT2 based on available experimental data at physiological temperature by using a novel Markov chain model formulation.

ii. to use human ventricular cell-based models to determine the functional consequences of incorporating the SQT2 mutation on AP repolarisation and the QT interval.

iii. to explore the arrhythmogenic substrate in SQT2 by using “realistic” 2D tissue and 3D organ simulations.
As will be shown, the results provide a clear link between the kinetic changes to $I_{\text{KCNQ1}}$-$I_{\text{KCNE1}}$ in SQT2 and the altered ventricular tissue electrophysiology, which produces re-entrant arrhythmia in the SQT2.

7.2 Homozygote and Heterozygote Formulations

Section 4.3 gives a detailed discussion of the development of the SQT2 (homozygote – i.e. ‘pure’ SQT2 mutation). The Markov chain model developed for V307L KCNQ1 containing $I_{\text{Ks}}$ was validated against experimental data [265] and was then used to investigate the functional consequences of SQT2 in single cell and realistic multicellular (1D, 2D and 3D) tissue. Figure 4.4 shows the state transition diagram for the SQT2 Markov chain model. The developed Markov chain models in section 4.3 represent the WT and pure homozygote (V307L) conditions only. However, the proband in whom the SQT2 mutation was discovered was heterozygotic for the mutation [124]. Therefore, to mimic the heterozygous state of the proband, a heterozygous formulation (WT-V307L) consisting of 50% WT and 50% V307L was constructed and used to investigate the effects of the KCNQ1 V307L mutation in this heterozygous condition.

7.3 Simulation of Single Cell $I_{\text{Ks}}$ under Control and SQT2 Conditions

As the first step, the ability of the Markov chain model to reproduce published experimental data [265] on the voltage-dependence of activation of WT and the V307L mutant at physiological temperature was tested. The same voltage clamp protocol used experimentally [265] was employed. Figure 7.1A shows representative experimental current traces for WT (Figure 7.1A) and V307L (Figure 7.1B) $I_{\text{KCNQ1-KCNE1}}$ elicited by the voltage clamp protocol in Figures 7.2Aii and 7.2Bii. The simulated current traces are shown in Figure 7.2Ai and 7.2Bi for WT and V307L respectively. The I-V relationships for WT (Figure 7.2Aiii) and V307L (Figure 7.2Biii) were reconstructed from the current traces. Of significance is that the simulated current traces (Figure 7.2Ai and Bi) match experimental recordings (Figure 7.1A and 7.1B) and showed the slower
Figure 7.1: WT and V307L $I_{\text{KCNQ1-KCNE1}}$ experimental current traces under voltage clamp. (A) WT experimental traces and (B) V307L experimental current traces elicited by voltage clamp commands shown in Figure 7.2Aii and Bi. Figure modified from [265].

deactivation rate of the $I_{K_s}$ channel under the V307L condition [124,265], compared to the WT, which is reproduced almost faultlessly (Figure 7.2Ai and 7.2Bi).

The developed Markov chain model of $I_{K_s}$ was validated by its ability to reproduce the dynamic properties of WT and V307L $I_{K_s}$ under AP clamp. Figure 7.2Aiv and 7.2Biv show the results of the simulated $I_{K_s}$ time traces during AP clamps, which are compared to those obtained experimentally [265]. As shown in the figure, the Markov chain model reproduced the experimental data of $I_{K_s}$ during the time course of AP, including the augmented $I_{K_s}$ current in the KCNQ1 V307L mutation condition.

In order to characterise the functional effects of the V307L mutation on ventricular APs, the Markov chain model formulation was then incorporated into the 2006 TNNP ventricular single cell model [38] for WT, V307L mutation and heterozygous mutation (WT-V307L) conditions (section 7.2). Figure 7.3 shows the simulated APs (Ai), the $I_{K_s}$ profile (Aii) and $I_{K_s}$ instantaneous I-V relationship for an EPI cell model. The MIDDLE and ENDO counterparts are shown in Figure 7.3B and 7.3C respectively. In the WT condition, during the time course of the AP, $I_{K_s}$ increased progressively following the upstroke of the AP and reached maximal amplitude very late during the plateau phase before declining rapidly during terminal repolarisation. WT-V307L $I_{K_s}$ activated earlier than WT and increased in amplitude more rapidly. Unlike in the WT condition, it
Figure 7.2: Simulated Current-Voltage Relationships for $I_{K_s}$.

Current traces for WT (Ai) and V307L $I_{K_s}$ (Bi) elicited by the voltage protocol shown in (Aii, Bii). I-V relation for $I_{K_s}$ for WT (Aiii) and V307L $I_{K_s}$ (Biii). Profile of $I_{K_s}$ during ventricular AP voltage command under WT (Aiv) and V307L (Biv) conditions.
Figure 7.3: Simulation of action potential and $I_{Ks}$ time courses.

(i) Steady state (1 Hz) action potentials for EPI (Ai), MIDDLE (Bi) and ENDO (Ci) cells. Blue lines represent WT, green lines represent WT-V307L and red lines represent the V307L condition.

(ii) Corresponding $I_{Ks}$ current profiles for EPI (Aii), MIDDLE (Bii) and ENDO (Cii) cells. Blue lines represent WT, green lines represent WT-V307L and red lines represent the V307L condition.

(iii) Corresponding I-V relationships for EPI (Aiii), MIDDLE (Biii) and ENDO (Ciii) cells. Blue lines represent WT, green lines represent WT-V307L and red lines represent the V307L condition.
reached significantly higher maximal amplitude early during the plateau leading to the abbreviation of the APD. V307L \( I_{Ks} \) activated the earliest of the three conditions, increased the most rapidly and attained the greatest maximal amplitude. Consequently, it abbreviated the APD to the greatest extent.

Under the WT condition, the computed \( \text{APD}_{90} \) was 326 ms, 454 ms and 327 ms for EPI, MIDDLE and ENDO cells respectively. These were shortened respectively to 233 ms, 355 ms and 234 ms under the WT-V307L condition and to 194 ms, 306 ms and 194 ms under the V307L mutation condition. The \( \text{APD}_{90} \) values for all the conditions are summarised in Table 7.1. The APD shortening resulted from the augmented \( I_{Ks} \) early during the plateau phase of the AP as shown by time course of \( I_{Ks} \) (Figure 7.3Aii-Cii) and the I-V phase plots in Figures 7.3Aiii-Ciii. These results agree with the findings of Bellocq et al. [124] who observed an increase in \( I_{Ks} \) and APD shortening under V307L condition using the Priebe-Beuckelmann AP model [307].

The APD abbreviation was rate-dependent as shown by the APD-Restitution (APD-R) curves in Figure 7.4A-C for the EPI, MIDDLE and ENDO cell types respectively. Over the range of diastolic intervals (DI) studied, the APD was smaller in the WT-V307L and V307L mutants than in the WT condition. The mutations also steepened the APD-R curves in each cell type as shown by the computed maximal slopes for each APD-R curve in Figure 7.4D. In the EPI cell, the maximal slopes of the WT-V307L and V307L mutations were similar, while there was a progressive increase in steepness of the slopes in the MIDDLE cell type. In the ENDO cell, while the slopes under the WT-V307L and V307L conditions were steeper than WT, the slope of the WT-V307L mutant was steeper than that of the V307L mutant.

<table>
<thead>
<tr>
<th></th>
<th>WT (ms)</th>
<th>WT-V307L (ms)</th>
<th>V307L (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPI</td>
<td>325.56</td>
<td>232.98</td>
<td>193.60</td>
</tr>
<tr>
<td>MCELL</td>
<td>453.62</td>
<td>355.38</td>
<td>305.94</td>
</tr>
<tr>
<td>ENDO</td>
<td>327.36</td>
<td>233.76</td>
<td>194.04</td>
</tr>
</tbody>
</table>

Table 7.1: Computed \( \text{APD}_{90} \) (ms) for EPI, MCELL and ENDO cell cell types under WT, WT-V307L and V307L conditions.
Figure 7.4: APD and ERP restitution curves.

(A, B, C): APD restitution curves for EPI (A), MIDDLE (B) and ENDO (C) cells respectively for the WT, WT-V307L and V307L conditions.

(D) Measured slopes of APD restitution curves for EPI, MIDDLE and ENDO cells in WT, WT-V307L and V307L conditions.

(E, F, G) ERP restitution curves for EPI (E), MIDDLE (F) and ENDO (G) cells respectively for the WT, WT-V307L and V307L conditions.

(H) Measured slopes of ERP restitution curves for EPI, MIDDLE and ENDO cells for WT, WT-V307L and V307L conditions.
The ERP reduction was also rate-dependent. It was reduced under the WT-V307L and V307L mutation conditions compared to the WT condition across the range of basic stimulus cycle lengths (BCL) as shown in ERP-R curve in Figure 7.4E-G for the EPI, MIDDLE and ENDO cell types respectively. In the EPI and ENDO cells, there was little difference in the slopes of the ERP-R curves between the WT, WT-V307L and V307L conditions (Figure 7.4H). The slope was steeper for the mutation conditions in the MIDDLE cell compared to WT cell but the slope for the WT-V307L condition was slightly steeper than that for the V307L mutant. The mutations also shifted the ERP-R curve leftwards implying that the KCNQ1 V307L mutation enabled ventricular cells to support electrical activity at higher rates (as normally seen during VT and VF).

Figure 7.5 shows the state occupancy of the WT, WT-V307L and V307L I\textsubscript{Ks} channels during the AP in zone 1 (blue), zone 2 (green) and the open states; O\textsubscript{1} and O\textsubscript{2} (red) Figure (4.4). Zone 2 is occupied by channels where the voltage sensors still need to make the slow transition to zone 1 before transitioning to the open state while channels in zone 1 have already made this transition and only need to undertake a fast transition to the open states (see Section 4.3.1). Under the WT condition (Figure 7.5Ai,Bi), approximately all channels accumulate in zone 2 at AP initiation and thus need to make the slow transition to zone 1 before full activation, which accounts for the delay in activation seen in I\textsubscript{Ks} channels [71–73,175].

Under the WT-V307L condition (Figure 7.5Aii, Bii), at the initiation of the AP, approximately 2% of the channels occupy zone 1 (compared with 0% in WT) while the rest occupy zone 2. Therefore, the delay in activation is shorter under the WT-V307L condition and channels transition to the open states faster. Nevertheless, one would assume a similar activation rate and progression as the WT condition, but due to the slower deactivation under the WT-V307L condition, channels accumulate in the open state resulting in excess current during the plateau phase of the AP and hence AP shortening. Under the V307L condition (Figure 7.5Aiii,Biii), on AP initiation, ~3.5% of the channels occupy zone 1 with the remainder occupying zone 2. Therefore, just as with the WT-V307L condition, as the AP progresses, there is faster open state transition and more open state accumulation (due to slower channel deactivation) compared to both WT and WT-V307L conditions leading to the even greater AP shortening.

These results imply that under both the WT-V307L and V307L conditions, the primary
Figure 7.5: WT, WT-V307L and V307L state occupancy during the AP.

Ai, Aii, Aiii: Action potential and $I_{Ks}$ under the WT (Ai), WT-V307L (Aii), V307L (Aiii).

Bi, Bii, Biii: State occupancy during the APs. Green shows zone 2 occupancy, blue shows zone 1 occupancy and red shows open state occupancy. Accumulation in zone 2 leads to a slow transition to zone 1 and hence a delay in activation under WT (Bi). Accumulation in zone 1 and open-state accumulation due to slow channel deactivation accelerates channel opening and larger $I_{Ks}$ during the AP under WT-V307L condition (Bii) leading to APD abbreviation (Aii). Slow channel deactivation results in open-state accumulation under the V307L condition coupled with greater accumulation in zone 1 (Biii) leading to AP shortening (Aiii).
mechanism for excessive current that causes AP shortening is the greater channel occupancy of zone 1 with a fast transition to the open state coupled with accumulation in the open state due to slower channel deactivation. Under the V307L condition, this open state accumulation is greater than under the V307L condition.

7.4 Simulation of the ECG with WT and SQT2 Mutant $\text{I}_\text{Ks}$

Using a 1D strand model of the ventricular wall, pseudo-ECGs were computed under the WT, WT-V307L and V307L conditions (Figure 7.6D-F). These were extracted from a propagating wave from the ENDO end of the strand towards the EPI end (Figure 7.6A-C). Time runs horizontally from left to right in Figure 7.6A-C while space runs vertically from the ENDO end at the bottom to the EPI end at the top. The QT interval was shortened from 351 ms in the WT condition to 292 ms in the WT-V307L condition and to 262 ms in the V307L condition (Figure 7.6G). T-wave width (measured as the time interval between $T_{\text{peak}}$ and $T_{\text{end}}$) also changed from 49 ms (WT) to 60 ms (WT-V307L) and 64 ms (V307L). These simulations thus reproduce the key features observed in the ECGs of SQTS patients; abbreviated QT interval, tall and peaked T-waves and wider $T_{\text{peak}}$ to $T_{\text{end}}$ [116,223,236–238,247]. As the only difference between these simulations is the altered kinetics of the $\text{I}_\text{Ks}$ channel by the mutations, the observed changes in the QT interval, T-wave height and width can confidently be attributed to the parameters corresponding to the V307L mutation.

Gima and Rudy [335] suggested that an increased spatial gradient of the membrane potential ($\delta V$) could be responsible for the increase in T-wave height seen in hyperkalemia. Therefore, in order to determine if the same effect was responsible for the taller T-wave amplitudes in the WT-V307L and V307L ECGs (Figure 7.6D-G), the effects of the V307L mutation on membrane potential ($\delta V$) heterogeneity during ventricular APs between the three cell types were investigated. The results are shown in Figure 7.8. Figure 7.7A shows the pairwise differences between EPI, MIDDLE and ENDO cells during an AP. Under the KCNQ1 WT-V307L and V307L mutation conditions, the maximal $\delta V$ between EPI-MIDDLE (Figure 7.7B) and ENDO-MIDDLE cells were greater than under the WT condition, which contributed to the augmented T-wave amplitude [335,358,359].
Figure 7.6: Pseudo ECGs under the WT, WT-V307L and V307L conditions
(A, B, C) Colour mapping of membrane potential of cells along the 1D strand from blue (-86 mV) to red (-42 mV) (see colour key, in mV). Space runs vertically from the ENDO end to the EPI end at the top. Time runs horizontally. (A) WT condition. (B) WT-V307L condition. (C) V307L condition.
(D, E, F) Pseudo-ECGs corresponding to the WT, WT-V307L and V307L conditions respectively.
(G) Superimposed pseudo-ECGs for the WT, WT-V307L, V307L conditions respectively and their associated QT intervals.
Figure 7.7: Membrane potential heterogeneity ($\delta V$), transmural distribution and spatial gradient of APD$_{90}$.

(A) Plots of $\delta V$ against time for WT (continuous lines), WT-V307L (dotted lines) and V307L (dashed lines) conditions.

(B) Maximum $\delta V$ during repolarization between MIDDLE-EPI cells in WT, WT-V307L and V307L.

(C) Spatial distribution of APD$_{90}$ in the 1D transmural strand for WT (blue), WT-V307L (green) and V307L (red).

(D, E) Absolute spatial gradient (E) and actual spatial gradient (D) of APD$_{90}$ in the 1D transmural strand for WT (blue), WT-V307L (green) and V307L (red).
The dispersion of APD$_{90}$ across the intact 1D strand under the WT, WT-V307L and V307L conditions was also measured. Figure 7.7C-E show the measured spatial distribution of APD$_{90}$ (Figure 7.7C), the spatial gradient of APD$_{90}$ (Figure 7.7D) and the absolute value of the spatial gradient of APD$_{90}$ (Figure 7.7E). The spatial gradient of APD$_{90}$ was augmented across the strand and markedly so in the ENDO region. The sharp transition of APD$_{90}$ and spike in the spatial gradient between the EPI-MIDDLE border is due to the discontinuity in the electrical coupling at this border and is consistent with experimental observations made by Yan et al. [333] in an arterially perfused left ventricular wedge preparation and Drouin et al. in a normal human heart [37].

7.5 Investigating the Arrhythmogenic Substrate in SQT2 – 1D simulations

Using the 1D strand, the vulnerability of WT, WT-V307L and V307L tissue to unidirectional block in response to a premature stimulus applied during the refractory tail of a previous excitation wave was investigated. Figure 7.8 shows the width of the vulnerability window across the strand during which the tissue is predisposed to a premature stimulus that can lead to ventricular fibrillation. Under the WT-V307L condition, the tissue’s vulnerability was increased across the whole strand compared to WT except for a very small region in the middle of the strand (Figure 7.8A). For the V307L condition, the tissue’s vulnerability was greater than WT and WT-V307L in the region marked by the arrow in Figure 7.8A. Figure 7.8B shows the temporal vulnerability window width for the marked region (arrow) under WT, WT-V307L and V307L conditions. On either side of the middle of the strand, within the region marked with the arrow, the vulnerability window of the tissue increased from 15.8 ms in the WT condition to 21.7 ms and 28.8 ms under the WT-V307L and V307L conditions respectively.
Figure 7.8: Vulnerable window across the transmural 1D strand.
(A) Measured vulnerable window for WT, WT-D172N and D172N along the 1D strand.
(B) Measured vulnerable window width in the region depicted by the arrow in (A).
7.6 Investigating the Arrhythmogenic Substrate in SQT2 – 2D and 3D Simulations with Realistic Geometry

7.6.1 Simulations in 2D Realistic Geometry

In a realistic human ventricle cross-sectional slice (Figure 7.9), the response of WT, WT-V307L and V307L tissue to a local premature stimulus applied within the left ventricular wall during the tissue’s vulnerable window was investigated (WT: 370 ms after the arrival of conditional wavefront; WT-V307L: 310 ms after the arrival of conditional wavefront; V307L: 230 ms after the arrival of conditional wavefront). The results of the 2D simulations are shown in Figure 7.9.

Following the premature stimulus, a re-entrant excitation wave was initiated within the left ventricular wall as shown in Figure 7.9Ai-Di for WT, Figure 7.9Aii-Dii for WT-V307L and Figure 7.9Aiii-Diii for the V307L condition. The snap-shots shown in Figure 7.9A-D show subsequent conduction of the induced re-entrant excitation waves from the applied premature stimulus for the WT (Figure 7.9Bi-Di), WT-V307L (Figure 7.9Bii-Dii) and V307L (Figure 7.9Biii-Diii) conditions. Under the WT condition, the initiated re-entry self-terminated after 1.1 s (Figure 7.9Di, Figure 7.9F) but it persisted under the mutation conditions throughout the 5s simulation period (WT-V307L: Figure 7.9Bii-Dii; V307L: Figure 7.9Biii-Diii) and (Figure 7.9F). The time course of an AP in the left ventricle is shown for the WT, WT-V307L and V307L conditions in Figures 7.9Ei-Eiii respectively.

Power spectrum analysis of the recorded whole-field averaged electrical activity from the tissue revealed a higher dominant frequency in the mutation conditions (3.32 Hz for WT-V307L and 4.30 Hz for V307L compared to the WT condition (1.96 Hz) (Figure 7.9G). These 2D simulation results illustrate that the KCNQ1 V307L mutation increases tissue susceptibility to arrhythmogenesis and maintenance of re-entrant excitation waves.
Figure 7.9: Snapshots of initiation and conduction of re-entry in realistic 2D model cross-section of ventricles

(Ai, Aii, Aiii) Application of a premature S2 stimulus into the refractory and partially recovered region of an excitation wave after a delay of 370 ms for WT, 310 ms for WT-V307L and 230 ms for V307L condition from the initial wave stimulus.

(Bi, Bii, Biii) Developed spiral wave from the S2 stimulus. Snapshot at time = 800 ms.

(Ci, Cii, Ciii) Snapshot of spiral wave at time = 1000 ms. The induced spiral wave
transited from transmural re-entry with tip rotating within the ventricle wall to anatomical re-entry with tip rotating around the ventricle boundary in WT and WT-V307L conditions. However, transmural re-entry persisted in the V307L condition and broke-up forming regenerative multiple re-entrant wavelets.

(Di, Dii, Diii) Snapshot of spiral wave at time = 1500 ms. Spiral wave self-terminated in WT before this recording point, but persisted in WT- V307L and V307L conditions.

(Ei, Eii, Eiii) Recorded time series of the action potential of a cell in the left ventricle for the WT, WT- V307L and V307L conditions.

(F) Measured lifespan of re-entry scroll wave in WT, WT- V307L and V307L conditions.

(G) Computed dominant frequency of electrical activity recorded from ventricle in WT, WT- V307L and V307L conditions. (1.96 Hz for WT, 3.32 Hz for WT- V307L and 4.30 Hz for V307L condition).
Figure 7.10: Snapshots of initiation and conduction of re-entry in a 3D anatomical model of human ventricles.

(Ai, Aii, Aiii) Application of a S2 premature stimulus in a local region at refractory period of a previous conditioning excitation wave after a time delay of 355 ms for WT, 315 ms for WT-V307L and 260 ms for V307L conditions from the initial conditioning wave stimulus.

(Bi, Bii, Biii) Developed scroll wave from the S2 stimulus for the WT, WT-V307L and V307L conditions. Snapshot at time = 500 ms.

(Ci, Cii, Ciii) Snapshot of scroll wave at time = 750 ms for the WT, WT-V307L and
V307L conditions.

(Di, Dii, Diii) Snapshot of scroll wave at time =1000 ms. The scroll wave self-terminated in WT, but persisted and broke up forming regenerative wavelets in WT-V307L and V307L conditions.

(Ei, Eii, Eiii) Recorded time series of the action potential of a cell in the left ventricle for WT, WT-V307L and V307L conditions.

(F) Measured lifespan of re-entry scroll wave in WT, WT-V307L and V307L conditions.

(G) Computed dominant frequency of electrical activity recorded from ventricle in WT, WT-V307L and V307L conditions (2.34 Hz for WT, 3.13 Hz for WT-V307L and 7.42 Hz for V307L condition).
7.6.2 Simulations in 3D Realistic Geometry

Realistically, the ventricles are three-dimensional and have a much more complex anisotropic geometry compared to the 2D ventricular slice. Therefore, it would be erroneous to assume that sustained reentry in the 2D tissue model necessarily translates to similar activity in 3D tissue. Consequently, further simulations were performed using a 3D anatomical human ventricle geometry. The results are shown in Figure 7.10, which shows snapshots of the evolution of re-entrant scroll waves (WT: Figure 7.10Ai-Di; WT-V307L: Figure 7.10Aii-Dii; V307L: Figure 7.10Aiii-Diii) developing as a response to a premature stimulus. For the WT condition, the scroll wave self-terminated with a lifespan of 0.5s (Figure 7.10F). However, under WT-V307L and V307L mutation conditions, the scroll wave broke up forming multiple re-entrant wavelets that self-terminated within 2.5s in WT-V307L tissue but were sustained throughout the 5s simulation period in V307L tissue (Figure 7.10F).

Power spectrum analysis of the recorded whole-field averaged electrical activity shows the dominant frequency of ventricle excitation to be 2.34 Hz for the WT condition, 3.13 Hz for the WT-V307L mutation condition and 7.42 Hz for the V307L mutation condition (Figure 7.10G). Figures 7.10Ei-Eiii show a recording of the evolution of the AP of a cell in the left ventricle for the WT, WT-V307L and V307L conditions. Although, in the WT-V307L mutation condition, the re-entrant wavelets self-terminated as opposed to being sustained as in the 2D ventricular slice (Figure 7.9F), these 3D results further illustrate the pro-arrhythmic effects of the KCNQ1 V307L mutation.

7.7 Investigating Blockade of I_{Ks} as a Potential Therapeutic Target in the SQT2

As a theoretical “pseudo-pharmacological” approach to treating patients with the adult SQT2 variant, blockade of the I_{Ks} channel by drugs was mimicked in order to determine the extent of blockade required to normalise the QT interval. First, I_{Ks} was blocked in the EPI, MIDDLE and ENDO single cell types. Figure 7.11A-E show the results for an EPI cell under the WT-V07L (Figure 7.11A-B) and V307L (Figure 7.11D-E) conditions (results for MIDDLE and ENDO cells are similar). In all three cell types, approximately
Figure 7.11 Blockade of $I_{Ks}$ in the single cell under the WT-V307L and V307L conditions

(A,D) EPI action potentials of WT-V307L (A) and V307L (D) resulting from varying percentage block of $I_{Ks}$. Dashed line represents WT and boxed percentage represents the $I_{Ks}$ blockade required to normalise the AP under the mutation conditions.

(B,E) Corresponding $I_{Ks}$ current profile to APs in (A) and (D) for the WT-V307L (B) and V307L (E) conditions.

(C,F) Resulting ECGs from blockade of $I_{Ks}$ in the 1D transmural strand under the WT(C) and (WT-V307L (F) conditions. Blue line represents WT and boxed percentage represents the $I_{Ks}$ blockade required to normalise the QT interval under the mutation conditions.
60% $I_{Ks}$ blockade was required to make the APD comparable to that of WT under the WT-V307L mutation condition while approximately 76% $I_{Ks}$ blockade was necessary under the V307L mutation condition.

Using the intact 1D tissue strand, a similar investigation on the normalisation of the QT interval was carried out. In tissue, due to the electrical coupling between cells via gap junctions, the APD between the different cell types are smoothed out. Thus, for the WT-V307L mutation condition (Figure 7.11C), 59% $I_{Ks}$ blockade was needed to normalise the QT interval to that of WT while under the V307L mutation condition, approximately 75% $I_{Ks}$ blockade was necessary (Figure 7.11F). These results are similar to the single cell simulations.

Finally, simulations to determine if $I_{Ks}$ blockade could terminate re-entrant activity under the mutation conditions in the 3D anatomical human ventricle (Figure 7.12) were performed. Figure 7.12A shows the WT condition with a premature stimulus applied during the tissue’s vulnerable window at 355 ms. If $I_{Ks}$ blockade under the mutation conditions normalises the QT interval, then the application of a premature stimulus at this same time (355 ms) should produce somewhat similar activity to WT. Figures 7.11Bi and 7.10Ci show WT-V307L and V307L mutated tissue with no $I_{Ks}$ blockade, i.e., the pure heterozygote and homozygote mutants respectively with a premature stimulus applied at 315 ms for WT-V307L and 260 ms for V307L leading to re-entrant activity that persists beyond the WT reentry lifespan. It transpired that 58% $I_{Ks}$ blockade was sufficient to make the WT-V307L reentry lifespan (Figure 7.12Bii) comparable to that of WT while 65% $I_{Ks}$ blockade was adequate under the V307L mutation condition (Figure 7.12Cii). These simulations illustrate the possibility of $I_{Ks}$ as a relevant drug target to treat tachyarrhythmia in the SQT2 setting.

### 7.8 Discussion and Conclusions

#### 7.8.1 Summary of major findings

The proband in whom the KCNQ1 V307L mutation was discovered was heterozygotic for the SQT2 mutation (WT-V307L) [124]. He was successfully resuscitated following
Figure 7.12: Termination of reentry by $I_{Ks}$ blockade

(A) Application of a premature stimulus during the vulnerable window of a local region of WT tissue (355 ms) leads to the development of a spiral wave (500 ms) that terminates before 1000 ms.

(Bi) WT-V307L mutated tissue with no blockade of $I_{Ks}$. Application of a premature
stimulus during the vulnerable window of a local region of the tissue (315 ms) leads to the development of a spiral wave (500 ms) that is sustained beyond 1000 ms.

(Bii) WT-V307L mutated tissue with 58% blockade of $I_{Ks}$. Application of a premature stimulus during the vulnerable window of a local region of the tissue (355 ms) leads to the development of a spiral wave (500 ms) that terminates before 1000 ms.

(Bi) V307L mutated tissue with no blockade of $I_{Ks}$. Application of a premature stimulus during the vulnerable window of a local region of the tissue (260 ms) leads to the development of a spiral wave (500 ms) that is sustained beyond 1000 ms.

(Bii) V307L mutated tissue with 65% blockade of $I_{Ks}$. Application of a premature stimulus during the vulnerable window of a local region of the tissue (355 ms) leads to the development of a spiral wave (500 ms) that terminates before 750 ms.
a VF episode. It is of significance that with the WT-V307L expression model, mimicking the heterozygous state of the proband, the 3D simulations (Figure 7.10) showed that re-entrant activity in the heterozygote condition (WT-V307L) (Figure 7.10Aii-Dii) self-terminated within 2.5s (Figure 7.10F) with a dominant frequency of 3.13 Hz (Figure 7.10G). However, in the homozygote condition (V307L) (Figure 7.10Aiii-Diii), re-entrant activity was sustained (Figure 7.10F) with a dominant frequency of 7.42 Hz (Figure 7.10G).

The major findings are:

1. the development of a novel Markov chain model of the SQT2 mutation which reproduces better the kinetic changes of I_Ks under both the WT and V307L conditions compared to previous studies [124,248]. It also reproduces the slowed deactivation of the I_Ks channel due to the V307L mutation.
2. the KCNQ1 V307L mutation abbreviates the AP duration and steepens the APD-R curve.
3. the KCNQ1 V307L mutation shortens the QT interval, increases T wave amplitude and T_peak – T_end duration, all of which are concordant with clinical observations regarding the SQTS.
4. the KCNQ1 V307L mutation leads to augmented membrane potential differences (δV) between paired cells and transmural APD dispersion in localised regions of the transmural strand that contributes to the increased T-wave amplitude.
5. the KCNQ1 V307L mutation increases the tissue’s temporal vulnerability to the genesis of uni-directional conduction by a premature excitation at some localised regions;
6. the KCNQ1 V307L mutation reduces the minimal tissue substrate size that facilitates the maintenance of re-entry and accelerates reentrant excitation waves.
7. I_Ks blockade is a potential therapeutic target in normalizing the QT interval and terminating re-entrant activity in the SQT2 setting.

These findings provide a causal link between the KCNQ1 V307L mutation, QT interval shortening and tachyarrhythmias. Moreover, they provide a comprehensive explanation
for the increased susceptibility to re-entry and perpetuation of re-entrant arrhythmia in the SQT2 setting.

**7.8.2 Significance of the Study and Relevance to Previous Studies**

In first reporting the existence of the KCNQ1 V307L mutation in a patient with the SQTS, Bellocq et al. [124] used a Priebe-Beuckelmann ventricular cell AP model [307] to demonstrate AP shortening. In the initial report of the KCNJ2-linked SQT3 [125], a simulated pseudo-ECG for the KCNQ1 V307L mutation showing QT interval shortening was presented in comparison to that of the KCNH2-linked SQT1 and the KCNJ2-linked SQT3 mutants. Zhang et al. [248], in a previous study, used the 2004 TNNP human ventricular AP cell model [305] with modified Hodgkin-Huxley I\textsubscript{Ks} formulations reproducing the KCNQ1 V307L kinetics to also demonstrate AP abbreviation, QT interval shortening, T wave morphology changes, reduced minimal substrate size for re-entry and reentrant activity in idealised 2D geometry. However, the current study is the first to develop a Markov Model of the KCNQ1 V307L mutation, which reproduces the kinetics of the mutation including the faster deactivation of the I\textsubscript{Ks} channel under the KCNQ1 V307L mutation and to examine the mutation’s functional consequences in realistic 2D and 3D anatomical geometries. This current study is also the first to investigate the blockade of I\textsubscript{Ks} as a potential pharmacological intervention for treating SQT2 patients.

The advantage of and the greater insight into arrhythmogenic mechanisms provided by the Markov chain model can be seen in Figure 7.5, which shows the channel state occupancy under WT, WT-V307L and V307L conditions. Under the WT condition (Figure 7.5 Ai, Bi), at AP initiation, the channels reside in zone 2 (Figure 7.5Bi), where the voltage sensors need to complete a slow transition to zone 1 and then a fast transition to the open state. This leads to a delay in activation (Figure 7.5Ai). This delay is a kinetic property of the WT I\textsubscript{Ks} channel [71–73,175]. However, under the WT-V307L condition (Figure 7.5Aii, Bii), 2% of the channels reside in zone 1 on AP initiation (Figure 7.5Bii), which facilitates channel opening and larger I\textsubscript{Ks} during the AP resulting in AP abbreviation (Figure 7.5Aii). Open-state accumulation is also greater than in WT due to slower deactivation. Thus, the primary mechanism for larger I\textsubscript{Ks} in the WT-V307L mutation is greater zone 1 occupancy coupled with slower deactivation.
In the V307L condition, compared to WT, 3.5% of channels occupy zone 1 and because the V307L mutation slows channel deactivation considerably, open-state accumulation is greater than under the WT or WT-V307L conditions. Consequently, this leads to larger $I_{Ks}$ during the AP. This kind of insight is not possible with simple Hodgkin-Huxley models, as channel state occupancy is not accounted for in such formulations in the way that is possible with a Markov-based approach.

7.8.3 Arrhythmogenic mechanisms of the KCNQ1 V307L mutation

The SQTS is associated with malignant tachycardias [117,119,124,360] and some patients present with ventricular fibrillation episodes [124,356] including the SQT2 proband [124] who was successfully resuscitated. Bellocq et al. [124] hypothesized that heterogeneous APD abbreviation would be anticipated to provide a substrate for increased risk of re-entrant arrhythmia.

The simulations in the present study support this hypothesis that the transmural dispersion of APD$_{90}$ due to differential APD abbreviation among EPI, MIDDLE and ENDO cells is pro-arrhythmic. It resulted in an augmented APD dispersion in some regions of the transmural strand, which increased the tissue’s vulnerable time window in which uni-directional conduction block could occur as shown in Figure 7.8. The KCNQ1 V307L mutation also increased the membrane potential heterogeneity ($\delta V$) between paired cell type APs: MIDDLE-ENDO and MIDDLE-EPI (Figure 7.7A-B). This augmented the APD of cells in the intact tissue model via electrical gap junction coupling between cells and contributed to the heterogeneous transmural APD$_{90}$ dispersion. The augmented $\delta V$ also accounted for the increased T-wave amplitude in the pseudo-ECGs (Figure 7.6). Maintenance of re-entrant activity is also aided by the ERP reduction under the KCNQ1 V307L mutation as it decreases the wavelength of ventricular excitation waves, allowing higher activation frequencies of re-entrant excitation waves (Figure 7.10). Under the heterozygote (WT-V307L) mutation condition, re-entrant activity self-terminated in the 3D simulation (Figure 7.10Aii-Dii and Figure 7.10F). This is the relevant clinical condition with which the proband is associated [124]). Given that there’s only one known case of SQT2 with this mutation [124] and that the patient reached the age of 70 without any complaints including VF [124], this can be considered a strength of the present study. In addition, during
electrophysiological study, no arrhythmias could be induced [124]. The 3D data are also suggestive of perhaps other factors being required to make ventricular tachycardia (VT) persist into VF in the heterozygote condition.

7.8.4 SQT2 treatment

The use of Implantable Cardioverter Defibrillators (ICD) is the current treatment for the SQTS [8,117,119,254,255]. However, as the SQTS is characterised by tall and peaked T-waves, there is the risk of inappropriate shocks to the patient due to T-wave oversensing [8,9,117,119,254]. Additionally, ICDs do not restore the QT interval to its normal duration and are not suitable for all patients, e.g., infants. Therefore, pharmacological alternatives that can restore the normal duration of the QT interval and offer protection from arrhythmias are being actively pursued [117,119,256,257]. Pharmacologically, there is very little information available on SQT2 (likely due to a complete lack of clinically used pure I\textsubscript{Ks} blockers). Although I\textsubscript{Ks} is selectively blocked by chromanol compounds such as chromanol 293B [8,264,361], its blocking potency was reduced by the KCNQ1 V307L mutation [8,264]. However, a recent study by El Harchi et al. [265] found that recombinant I\textsubscript{Ks} channels incorporating the KCNQ1 V307L mutation were effectively inhibited by mefloquine.

The simulations in the present study show in silico simulation of the effects of a drug that selectively blocks the I\textsubscript{Ks} channel. In the SQT2 setting, under the WT-V307L heterozygote condition, a blockade of I\textsubscript{Ks} by ~58% was sufficient to restore the QT interval to its original WT duration and make the tissue behave like WT tissue while under the V307L homozygote condition, a blockade of I\textsubscript{Ks} by ~65% was sufficient to achieve the same result. These QT interval restorations terminate re-entrant activity in the tissue. Of course, as there is a potential risk with I\textsubscript{Ks} blockade of torsade de pointes [361–363], caution must be exercised in designing and using a drug that blocks I\textsubscript{Ks}; however, in the setting of accelerated repolarisation such a drug might have utility. It should also be noted that the precise quantitative information on amount of I\textsubscript{Ks} block required to normalise the QT interval may be model dependent. Therefore, for this reason, it would be informative in the future to conduct similar pseudo-pharmacological simulations with other human ventricular myocyte models.
Chapter 8

Proarrhythmia in KCNJ2-linked Short QT Syndrome: Insights from Modelling

8.1 Introduction

As discussed in Chapters 1 and 2, a number of K\(^+\) channels contribute to ventricular AP repolarisation. Due to its voltage dependence, \(I_{K1}\) plays little or no role during phases 0 or 1 of the AP, it contributes slightly during phase 2 (the plateau phase) but its major effect is seen during phase 3, particularly during terminal repolarisation [364–366] (also see Section 2.4 and Figure 2.10). At this stage of the AP, \(I_{Kr}\) declines and \(I_{K1}\) increases. \(I_{K1}\) also plays a significant role in maintaining a stable resting potential in atrial and ventricular cells [204,367].

Mutations to channels responsible for \(I_{K1}\) can be arrhythmogenic [204]. For example, loss-of-function mutations to the Kir 2.1 protein result in the Andersen-Tawil syndrome. This syndrome delays ventricular repolarisation and facilitates ventricular arrhythmia [368,369]. On the other hand, Kir 2.1 over-expressing mice exhibit up-regulation of \(I_{K1}\), which enhances the risk of ventricular arrhythmia via the production of a substrate that stabilises and facilitates high frequency rotor development [370]. Similarly, a gain-in-function (V93I) Kir2.1 mutation has been implicated in a familial form of atrial fibrillation [371].

The SQT3 variant of the SQTS is due to a gain-of-function mutation in the KCNJ2 gene [125] that encodes Kir2.1. SQT3 was first identified in a 5-year-old child and her father, both of whom had abbreviated QTc of 315 ms and 320 ms respectively. Their ECGs showed abnormally narrow and peaked T-waves [125]. Genetic analysis showed an amino acid substitution of aspartate to asparagine (D\(\rightarrow\)N) substitution at position 172 in the Kir 2.1 protein. At both ambient and physiological temperatures, whole cell patch clamp recordings showed that the D172N mutation preferentially increased the outward over the inward current through recombinant Kir 2.1 channels [125,251].
In vitro ventricular AP clamp experiments have provided a direct demonstration of the D172N mutation on Kir2.1 current during physiological waveforms [251]. The results of these experiments have shown increased outward Kir2.1 current during terminal repolarisation [251].

As for the SQT1 and SQT2 variants, at present there is no phenotypically accurate experimental model of the SQT3 mutation for the investigation of the arrhythmia substrate in this variant of the SQTS. There are limited simulation data available; an example being one for which the kinetic changes to Kir2.1 current induced by the D172N Kir2.1 mutation were incorporated into a Priebe-Beucklemann model [307]. Simulations incorporating D172N Kir2.1 exhibited a shortened AP duration and steepened the AP restitution [125]. Simulations using a 1D tissue model reproduced the T-wave morphology seen clinically [125]. However detailed simulations to investigate the arrhythmogenic substrate in SQT3 were not performed.

The aim of this study was to investigate the arrhythmogenic substrate in the SQT3 syndrome. This aim was pursued by using physiologically accurate ventricular cell and tissue models (1-3D) with transmural and structural heterogeneity, incorporating kinetic changes to Kir2.1 current seen during voltage and AP clamp.

### 8.2 Simulation of Single Cell $I_{K1}$ under Control and SQT3 Conditions

A detailed discussion of the development of the SQT3 model formulations for the WT condition, the WT-D172N (heterozygote) and D172N (homozygote) conditions is given in Section 4.4. Figure 8.1Ai shows the simulated I-V relationship for the WT, WT-D172N and D172N mutations compared to the experimental data [251]. The WT conductance was adjusted to show the same peak current density as the original ten Tusscher et al. [38,305,306] $I_{K1}$ formulation current density during the I-V relation (Figure 8.1Aii), thus maintaining the overall current densities, APD$_{90}$ and dynamic properties of the ten Tusscher et al. human ventricular model [38,305,306]. Relative current proportions for WT, WT-D172N and D172N conditions were then scaled using relative proportions of peak $I_{K1}$ obtained previously from AP clamp.
Figure 8.1: SQT3 model fit to experimental data

(Ai, Aii) Model fit to normalised experimental current-voltage (I-V) relations (Ai) for WT (blue), WT-D172N (green) and D172N (red) conditions and model-derived $I_{K1}$ current densities (Aii) based on the original TNNP $I_{K1}$ channel current density.

(Bi, Bii, Biii) Simulated profile of $I_{K1}$ for WT (Bi), WT-D172N (Bii) and D172N (Biii) during an epicardial ventricular AP command.

(Ci, Cii, Ciii) Experimental profile of $I_{K1}$ for WT (Ci), WT-D172N (Cii) and D172N (Ciii) during an epicardial ventricular AP command. Modified from [251].
experiments [251] ($V_{\text{hold}}$ of -80 mV and $E_{\text{rev}}$ of -88 to -89 mV). The SQT3 model reproduced quite closely the experimental data (Figure 8.1Ci-Ciii) in all the three conditions (Figure 8.1Bi-Biii).

In order to characterise the functional effects of the WT-D172N and D172N mutation on ventricular APs, the SQT3 model formulations for the WT, WT-D172N and D172N mutant conditions were incorporated into the 2006 TNNP single cell model for human ventricular cell action potentials [38]. Figure 8.2 shows simulated APs (8.2Ai), $I_{K1}$ profile (8.2Aii) and instantaneous $I_{K1}$ I-V relationship (8.2Aiii) of an EPI ventricular cell. The MCELL and ENDO counterparts are shown in Figures 8.2B and 8.2C respectively. In all three cell types, the WT-D172N and D172N mutations abbreviated the AP. Under the WT condition, $I_{K1}$ was inactive during practically all of the plateau phase but its outward component began to increase in amplitude gradually during phase 3 (repolarisation phase) of the AP and reached a peak during terminal repolarisation after which it declined. The same pattern was observed for the WT-D172N mutation except that the rise of the outward component of $I_{K1}$ began slightly earlier during phase 3 of the AP and it reached a higher amplitude than in the WT condition. With the D172N mutation, $I_{K1}$ was activated earlier than the WT-D172N condition, increased more rapidly and achieved significantly higher maximal amplitude during terminal repolarisation than WT and WT-D172N. This led to the marked shortening of the APD in both the WT-D172N and D172N conditions.

The measured WT APD$_{90}$ was 312 ms, 431 and 312 ms, respectively, for the EPI, MIDDLE and ENDO cells. These values were decreased respectively to 283 ms, 382 ms and 284 ms for the WT-D172N condition and to 265 ms, 354 ms and 265 ms for the D172N condition (Table 8.1). The shortening in APD was due to the increased $I_{K1}$ during the AP repolarisation phase as shown by the time-course of $I_{K1}$ (Figure 8.2Aii-Cii) and the instantaneous I-V plot during AP repolarisation (Figure 8.2Aiii-Ciii). The observed resting potential values were -86.2, -86.4 and -86.5 mV for the WT, WT-D172N and D172N conditions respectively.

The abbreviation of APD$_{90}$ by the WT-D172N and D172N conditions was rate-dependent. The APD restitution (APD-R) curve is shown in Figure 8.3A-C. The APD$_{90}$ was smaller in the WT-D172N and D172N conditions than in the WT condition across the range of diastolic intervals (DIs) investigated. The mutant conditions also steepened
Figure 8.2: Simulations of ventricular action potentials for WT, WT-D172N and D172N conditions.

(Ai, Aii, Aiii) Epicardial simulation of action potentials (Ai), time course and amplitude of $I_{K1}$ (Aii) and instantaneous I-V relations (Aiii) under WT, WT-D172N and D172N conditions.

(Bi, Bi, Biii) Mid-myocardial simulation of action potentials (Bi), time course and amplitude of $I_{K1}$ (Bii) and instantaneous I-V relations (Biii) under WT, WT-D172N and D172N conditions.

(Ci, Cii, Ciii) Endocardial simulation of action potentials (Ci), time course and amplitude of $I_{K1}$ (Cii) and instantaneous I-V relations (Ciii) under WT, WT-D172N and D172N conditions.
the APD-R curve as shown by the computed maximal slopes in Figure 8.3D. WT-D172N and D172N conditions increased the slope of the APD-R curves in the EPI and ENDO cells. However, in the MCELL, there was a reduction in the slope in the WT-D172N condition, but a significant increase in slope by the D172N condition.

The WT-D172N and D172N mutations also shortened the effective refractory period (ERP) for all three cell types and resulted in a leftward shift of the ERP restitution (ERP-R) curve (Figure 8.3E-G). This shortening was also rate-dependent and was smaller in the mutation conditions than in WT across the range of basic stimulus cycle lengths (BCL) studied. As with the APD-R curves, the mutation conditions also steepened the ERP-R curves as shown by the computed maximal ERP slopes (Figure 8.3H). Steepening of the APD-R and ERP-R curves is associated with increased instability of re-entrant excitation waves [372], which predisposes towards their breakup and consequently leads to the formulation of multiple re-entrant excitation wavelets [372–374]. The leftward shift of the ERP-R curves by the mutation conditions also implies an ability of the mutation conditions to sustain electrical activity at higher rates (as seen under VT and VF conditions). These results therefore provide evidence for the pro-arrhythmic nature of the KCNJ2 Kir 2.1 WT-D172N and D172N mutations.

### 8.3 Simulation of the ECG with WT, WT-D172N and D172N Mutant I<sub>K1</sub>

A pseudo-ECG was computed using a 1D strand of cells across the ventricular wall for the WT, WT-D172N and D172N conditions at a stimulation rate of 1Hz (Figure 8.4D-F). Details of the method used to simulate the pseudo-ECG can be found in

<table>
<thead>
<tr>
<th></th>
<th>EPI</th>
<th>MCELL</th>
<th>ENDO</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (ms)</td>
<td>311.84</td>
<td>430.76</td>
<td>312.00</td>
</tr>
<tr>
<td>WT-D172N (ms)</td>
<td>283.20</td>
<td>382.40</td>
<td>283.50</td>
</tr>
<tr>
<td>D172N (ms)</td>
<td>264.86</td>
<td>353.98</td>
<td>265.20</td>
</tr>
</tbody>
</table>

Table 8.1: Computed APD<sub>90</sub> (ms) for EPI, MCELL and ENDO cell types under WT, WT-D172N and D172N conditions.
Figure 8.3: Rate-dependent APD and ERP restitution curves.

(A, B, C): APD restitution curves for EPI (A), MIDDLE (B) and ENDO (C) cells respectively for the WT, WT-D172N and D172N conditions.

(D) Measured slopes of APD restitution curves for EPI, MIDDLE and ENDO cells in WT, WT-D172N and D172N conditions.

(E, F, G) ERP restitution curves for EPI (E), MIDDLE (F) and ENDO (G) cells respectively for the WT, WT-D172N and D172N conditions.

(H) Measured slopes of ERP restitution curves for EPI, MIDDLE and ENDO cells for WT, WT-D172N and D172N conditions.

DI- Diastolic interval; PCL – Pacing cycle length.
Figure 8.4: Space-time plot of AP propagation along a 1D transmural ventricular strand, computed pseudo-ECGs and conduction velocity.

(A, B, C) Colour mapping of membrane potential of cells along the 1D strand from blue (-86 mV) to red (-42 mV) (see colour key). Space runs vertically from the ENDO end to the EPI end at the top. Time runs horizontally. (A) WT condition. (B) WT-D172N condition. (C) D172N condition.

(D, E, F) Pseudo-ECGs corresponding to the WT, WT-D172N and D172N conditions respectively.

(G) Superimposed pseudo-ECGs for the WT, WT-D172N, D172N conditions respectively and their associated QT intervals.

(H) Conduction velocity (CV) restitution under WT (blue), WT-D172N (green) and D172N (red) conditions. PCL – pacing cycle length.
A propagating excitation wave was initiated at the ENDO end of the strand by delivering a series of supra-threshold stimuli (section 5.2.3). The wave propagated from the ENDO through the MIDDLE and towards the EPI direction.

Figures 8.4A-C show a space-time plot with space running vertically from ENDO at the bottom to EPI at top and time running horizontally from left to right. The WT-D172N and D172N mutants shortened the QT interval from 363 ms for WT to 319 ms and 295 ms respectively (Figure 8.4G). The T-waves for the mutant conditions were also taller and peaked, and the T-wave width (measured as the difference between $T_{\text{peak}} - T_{\text{end}}$) decreased from 51 ms (WT) to 39 ms (WT-D172N) and 33 ms (D172N). Given that only the channel kinetics of $I_{K1}$ were different in the three simulation conditions, the observed shortening of the QT interval, the changes in T-wave height and width can be attributed with confidence to the effect on the simulations of the altered WT-D172N and D172N parameters.

### 8.4 Simulation of Transmural APD dispersion and Temporal Vulnerability

Figure 8.5A shows the spatial dispersion of APD$_{90}$ across the transmural strand for WT, WT-D172N and D172N conditions. The mutants reduced the APD$_{90}$ across the strand but did not significantly alter the spatial dispersion of APD$_{90}$ relative to WT. Figure 8.5B shows the spatial gradient of the transmural dispersion of the APD$_{90}$ across the strand and its absolute value is shown in Figure 8.5C. The spatial gradient of APD$_{90}$ dispersion was increased in the mutant conditions in the EPI region and in localised parts of the MCELL and ENDO regions, which also contributed to the increased T-wave amplitude. A very sharp transition in APD$_{90}$ can be seen between the MCELL and EPI region in Figures 8.5A-C. This is due to the discontinuity in the electrical coupling at this border and is consistent with experimental observations made by Yan et al. [333] in an arterially perfused left ventricular wedge preparation and by Drouin et al. [37] in a normal heart.
Figure 8.5: Transmural APD$_{90}$ distribution and its spatial gradient along a 1D transmural strand

(A) Spatial distribution of APD$_{90}$ in the 1D transmural strand for WT (blue), WT-D172N (green) and D172N (red).

(B) Actual spatial gradient of APD$_{90}$ in the 1D transmural strand for WT (blue), WT-D172N (green) and D172N (red).

(C) Absolute spatial gradient of APD$_{90}$ in the 1D transmural strand for WT (blue), WT-D172N (green) and D172N (red).
8.5 Investigating the Conduction Velocity in SQT3

The conduction velocity (CV) across the ventricular transmural strand was computed under the WT, WT-D172N and D172N conditions (Figure 8.4H). The mutants decreased CV at low rates (PCL > 560 ms; rate < 107 beats/minute) but enhanced it at higher rates. The decreased CV at low rates was due to reduced tissue excitability (Figure 8.6) as no change in the inter-cellular electrical coupling was considered. At a rate of 60 beats/minute (BCL=1000 ms), the measured CV was 66 cm s^{-1} for the WT, 64 cm s^{-1} for the WT-D172N and 62 cm s^{-1} for the D172N conditions. However, at higher rates between 158 and 196 beats/minute (305 ms < PCL < 380 ms), the measured CV was much higher in the WT-D172N and D172N conditions than in the WT condition.

The increased CV at high rates under the mutant conditions is due to shorter ERP under these conditions compared to the WT condition. At a stimulus rate above 167 beats/minute (SI < 360 ms), conduction failed in the WT condition, as a large part of the tissue was still refractory but conduction was sustained in the WT-D172N and D172N conditions. The highest rate for ventricular tissue to support conduction was 183 beats/minute (SI = 327 ms) under the WT-D172N condition and 201 beats/minute (SI = 298 ms) for the D172N condition. Thus the increased I_{K1} facilitated ventricular conduction at high rates close to those observed in clinical ventricular tachycardia [375].

8.6 Investigating the Arrhythmogenic Substrate in SQT3 – 1D simulations

The vulnerable window of the mutant tissue to a premature stimulus, i.e., its temporal vulnerability to unidirectional conduction in response to premature stimuli was then investigated. The period of vulnerability occurs during the refractory period of a previous excitation wave and represents the period during which a premature stimulus can elicit unidirectional conduction, which can act as a substrate for arrhythmogenesis [351–354]. Figure 8.7A, 8.7B and 8.7C show the width of the vulnerable window across the tissue in the WT, WT-D172N and D172N conditions respectively. The window at any point in the strand – from the ENDO (0 mm on the x- axis) to the EPI (15 mm on
Figure 8.6: Excitation threshold plotted against stimulus intervals (SIs) for the WT, WT-D172N and D172N mutation conditions.
the x-axis) end –is the difference between the upper envelope T1 on the y-axis and the lower envelope T2. It can be seen that in the D172N mutation condition, the tissue’s temporal vulnerability was increased across the whole strand, but only at most of the MIDDLE region in the WT-D172N mutation condition.

8.7 Investigating the Arrhythmogenic Substrate in SQT3 – Idealised 2D geometry simulations

An idealised 2D tissue model was used to measure the minimal spatial size of a premature test stimulus necessary to produce re-entry under WT, WT-D172N and D172N conditions. Similar to the 1D transmural strand, the 2D tissue had three distinct regions (ENDO, MCELL and EPI) and can be considered to be equivalent to the 1D strand, but swept out in the y-direction (assuming the 1D strand is in the x-direction). A planar excitation wave propagating from the ENDO towards the EPI region of the 2D sheet was evoked at the ENDO via a conditioning stimulus. After a time delay, a premature stimulus was applied to a localized portion of the EPI region and during its VW, thereby producing unidirectional conduction (towards the EPI end, as the MCELL region was still refractory). This resulted in the formation of spiral re-entrant excitation waves, which self-terminated for the WT condition but were sustained for the WT-D172N and D172N conditions.

As the formation of the re-entrant excitation waves is dependent on the size of the premature test stimulus, the minimal stimulus size that could evoke re-entry was measured. The measured size was 49 mm in the WT condition, 21 mm in the WT-D172N condition and 18 mm in the D172N condition, showing that under conditions with the D172N mutation present, it was easier to induce reentry and hence, ventricular fibrillation. The reduction in the critical size of the substrate required to support the formation and maintenance of re-entrant spiral waves was significant; it was reduced by 57% in the WT-D172N condition and 63% in the D172N condition. Thus, under the WT-D172N and D172N mutation conditions, there was a greater susceptibility of idealised 2D tissue to ventricular arrhythmia.
Figure 8.7: The measured width of the temporal vulnerable window along the 1D strand for WT (A), WT-D172N (B) and D172N (C) conditions.
8.8 Investigating the Arrhythmogenic Substrate in SQT3 – 2D and 3D Simulations with Realistic Geometry

8.8.1 Simulations with Realistic 2D Geometry

Realistic ventricular geometry is considerably more complex than an idealised 2D sheet and in addition, involves anisotropic conduction due to the presence of differing fibre orientations. Consequently, similar to SQT1 (Chapter 6) and SQT2 (Chapter 7), simulations were performed in a 2D cross-section of human ventricle tissue and in an anatomical, human 3D ventricle geometry (see Section 5.2.4.2). Transmural heterogeneity (EPI, MCELL and ENDO regions) in the ventricle was also considered.

Figure 8.8 shows simulation results obtained for the 2D human ventricle slice. Figures 8.8A and 8.8B show the application of a premature stimulus (WT: 335 ms, WT-D172N: 301 ms and D172N: 285 ms) during the vulnerable window of a local region in the left ventricle in WT (Figure 8.8Ai-Di), WT-D172N (Figure 8.8Aii-Dii) and D172N (Figure 8.8Aiii-Diii) conditions. This led to the generation of re-entrant excitation waves in WT (Figure 8.8Bi-Di), WT-D172N (Figure 8.8Bii-Dii) and D172N (Figure 8.8Biii-Diii) conditions. The re-entrant spiral waves self-terminated within 0.7 s in WT (Figure 8.8Di and Figure 8.9A) but not in the WT-D172N (Figure 8.8Dii) or D172N (Figure 8.8Diii) conditions, where they persisted for the entire duration (5s) of the simulation conditions (Figure 8.9A).

Figures 8.8Ei-Eiii show the evolution of the AP in a local cell in the ventricle under the WT (Figure 8.8Ei), WT-D172N (Figure 8.8Eii) and D172N (Figure 8.8Eiii) conditions. Power spectrum analysis of the recorded whole-field averaged electrical activity from the tissue carried out on the APs showed a higher dominant frequency (Figure 8.9B) under the mutant conditions (WT-D172N: 3.1 Hz and D172N: 3.6 Hz) compared to the WT (2.8 Hz) condition (Figure 8.9B). Taken together, these results show that the D172N mutation is able to increase the susceptibility of the ventricular tissue to genesis and maintenance of re-entrant excitation waves leading to ventricular arrhythmia.
Figure 8.8: Snapshots of initiation and conduction of re-entry in realistic 2D model cross-section of ventricles.

(Ai, Aii, Aiii) Application of a premature S2 stimulus into the refractory and partially recovered region of an excitation wave after a delay of 335 ms for WT, 301 ms for WT-D172N and 285 ms for D172N condition from the initial wave stimulus.

(Bi, Bii, Biii) Developed spiral wave from the S2 stimulus. Snapshot at time = 500 ms.

(Ci, Cii, Ciii) Snapshot of spiral wave at time = 750 ms. The induced spiral wave transited from transmural re-entry with tip rotating within the ventricle wall to anatomical re-entry with tip rotating around the ventricle boundary in WT and WT-D172N conditions. However, transmural re-entry persisted in the D172N condition and broke-up forming regenerative multiple re-entrant wavelets.

(Di, Dii, Diii) Snapshot of spiral wave at time = 1000 ms. Spiral wave self-terminated in WT before this recording point, but persisted in WT-D172N and D172N conditions.

(Ei, Eii, Eiii) Recorded time series of the action potential of a cell in the left ventricle for the WT, WT-D172N and D172N conditions.
Figure 8.9: Reentry lifespan and dominant frequency in realistic 2D model cross-section of ventricles.

(A) Measured lifespan of re-entry spiral waves in WT, WT-D172N and D172N conditions.

(B) Computed dominant frequency of electrical activity recorded from ventricle in WT, WT-D172N and D172N conditions (WT: 2.8 Hz; WT-D172N: 3.1 Hz; D172N: 3.6 Hz)
8.8.2 Simulations with 3D Realistic Geometry

Due to the complex geometry and anisotropic properties of ventricular tissue, it cannot be assumed that sustained re-entry in a 2D tissue model under the mutation conditions studied above necessarily translates into similar activity in a 3D tissue model. Therefore, similar to SQT1 (Chapter 6) and SQT2 (Chapter 7), further simulations were performed using three-dimensional anatomical human ventricle geometry (Figure 8.10).

Figure 8.10 shows the results of the simulations obtained using anatomical human 3D ventricle geometry. The premature stimulus was applied from the base of the ventricle up to half-way towards its apex, covering a region of approximately 90x63 mm in the WT, WT-D172N and D172N conditions (Figure 8.10Ai, 8.10Aii and 8.10Aiii). This region included the left ventricle and the surrounding area of the right ventricular outflow tract. The premature stimulus was applied during the vulnerable window of the aforementioned region following the refractory tail of a previous excitation wave.

This generated re-entrant scroll waves in all conditions, which terminated under the WT condition within 0.7s but were sustained in the WT-D172N and D172N conditions (Figure 8.10 and 8.11A) throughout the entire simulation period of 10 s. Figures 8.10Ei-Eiii show the evolution of the AP in a local cell in the ventricles and Figure 8.11A shows the lifespan of re-entry under all conditions. Power spectrum analysis on the APs showed a higher dominant frequency under the WT-D172N and D172N conditions compared to the WT condition (Figure 8.11B). This further supports the pro-arrhythmic nature of the D172N mutation, as shown in the 1D and 2D simulations.

8.9 Discussion

The results from these simulations constitute novel evidence regarding the pro-arrhythmic effects of the augmented outward current component of the KCNJ2 D172N mutations. The WT-D172N and D172N expression conditions increased the susceptibility of the tissue to the initiation, stabilization and acceleration of re-entry. Priori et al. [125] in their first report of the mutation used the Priebe-Beuckelmann ventricular AP model [307] to demonstrate AP shortening, steeper APD and ERP.
Figure 8.10: Snapshots of initiation and conduction of re-entry in a 3D anatomical model of human ventricles.

(Ai, Aii, Aiii) Application of an S2 premature stimulus in a local region at refractory period of a previous conditioning excitation wave after a time delay of 380 ms for WT, 350 ms for WT-D172N and 343 ms for D172N conditions from the initial conditioning wave stimulus.

(Bi, Bi, Bi) Developed scroll wave from the S2 stimulus for the WT, WT-D172N and D172N conditions. Snapshot at time = 500 ms.

(Ci, Ci, Ci) Snapshot of scroll wave at time = 750 ms for the WT, WT-D172N and D172N conditions.

(Di, Di, Di) Snapshot of scroll wave at time = 1000 ms. The scroll wave self-terminated in WT, but persisted and broke up forming regenerative wavelets in WT-D172N and D172N conditions.

(Ei, Eii, Eiii) Recorded time series of the action potential of a cell in the left ventricle for WT, WT-D172N and D172N conditions.
Figure 8.11: Reentry lifespan and dominant frequency in realistic 3D anatomical model of human ventricles.

(A) Measured lifespan of re-entry scroll wave in WT, WT-D172N and D172N conditions.

(B) Computed dominant frequency of electrical activity recorded from ventricle in WT, WT-D172N and D172N conditions (2.3 Hz for WT, 4.8 Hz for WT-D172N and 6.0 Hz for D172N condition).
restitution curves, QT interval shortening and alterations to T-wave morphology. The present study not only reproduces those findings but has also for the first time examined the functional consequences of the mutation at the 2D tissue and 3D organ levels. In addition to QT interval shortening, my simulations show increased susceptibility to the initiation and stabilisation of re-entry under both WT-D172N and D172N expression conditions. It is significant that under the WT-D172N condition (which mimics the heterozygote state of the proband), the QT interval shortened by ~12% and was practically equivalent to that of the proband. Tissue vulnerability and re-entry lifespan, which are factors of augmented arrhythmogenic susceptibility, were also increased.

8.9.1 Pro-fibrillatory mechanisms of the Kir2.1 D172N mutation

Clinically, SQTS patients present with ventricular tachycardias [119,124,360] and some have been known to have ventricular fibrillation [356]. Ventricular fibrillation was elicited via programmed electrical stimulation in the SQT3 setting. The proband in whom the SQT3 mutation was discovered had no prior history of cardiac arrhythmias but her father had prior incidents of presyncopal events and palpitations [125]. Priori et al. conjectured that the steeper APD and ERP restitution curves in the D172N mutation condition would provide a substrate for increased risk of stable reentry in tissue [125]. The simulation results in this chapter provide evidence that these steepening of the APD and ERP restitution curves plus the APD and ERP shortening by the D172N mutation are pro-arrhythmic. They reduce the minimal size of the substrate required to initiate and maintain reentry. In the WT-D172N and D172N conditions, the measured minimal substrate size was reduced by 57% and 63% respectively compared to the WT condition. Reentry self-terminated in the WT condition but was perpetuated under the WT-D172N and D172N conditions. It self-terminated in the WT condition because the ERP and consequently the wavelength of the reentrant circuit(s) was too large to be sustained in such a limited mass of tissue. However, in the mutation conditions, the reduced ERP and consequently the reduced wavelength of the reentrant circuit(s) allowed its accommodation within the tissue mass. The SQT2 mutation also shortened APD and ERP, steepened the APD-R curve but did so less differentially across the ventricular wall for the ERP-R curve and was also able to support and facilitate re-entry (Chapter 7). Similarly, the SQT1 mutation also shortened APD and
ERP but reduced the slopes of the APD-R and ERP-R curves. It was also able to support and facilitate re-entry via mechanisms described in Chapter 6.

The KCNJ2 D172N mutation differentially abbreviated the APD and ERP in the EPI, MIDDLE and ENDO cells (i.e., it augmented transmural heterogeneity). This led to augmentation of the transmural dispersion of APD/ERP in different regions of the transmural strand (Figure 8.5 and 8.7). The effect of this was increased susceptibility of the tissue to arrhythmogenic stimuli, as the tissue’s vulnerable window to premature stimuli was increased (Figure 8.7).

The D172N mutation also stabilised and accelerated reentry in 2D and 3D tissue further reflecting its pro-arrhythmic nature. This is consistent with findings in previous studies of the role of $I_{K1}$ in arrhythmogenesis [204,370,376]. There are however, some differences between those studies and that of the present chapter. In the previous studies, both the outward and inward components of $I_{K1}$ were increased or scaled proportionally, which had the effect of steepening the slope of the I-V curve. Consequently, any membrane potential change close to the potassium equilibrium potential ($E_K$) would act in such a way as to shift the resting potential towards $E_K$. Therefore, during high excitation rates, sodium current ($I_{Na}$) recovery from inactivation is increased, thereby helping to stabilise reentry. In my study, only the outward component of the $I_{K1}$ current is enhanced (the inward component is unaffected). There is thus less effect on the slope of the I-V curve and the resting potential was found to be little altered by the D172N mutation. Consequently, the stabilisation of reentry under these conditions was not through increased $I_{Na}$ recovery but via increased tissue excitability at high excitation rates (Figure 8.6) and the shorter ERP of the WT-D172N and D172N mutations (Figure 8.3). The reduced ERP of the mutations also reduced the wavelength of the reentrant excitation waves thus allowing their activation at higher frequencies (Figure 8.8 and 8.10) and once formed, the waves were stable and persistent.

8.9.2 Relevance to Previous $I_{K1}$ Studies

Channelopathies related to $I_{K1}$ are known to lead to cardiac arrhythmias, e.g., Anderson’s syndrome [377,378], long QT syndrome [379–381] and short QT syndrome
In experimental animal models, a few studies have also implicated $I_{K1}$ in the genesis and maintenance of ventricular fibrillation [382–384]. In transgenic mouse heart with overexpression of Kir2.1, the initiation and stabilisation of ventricular fibrillation was the consequence of increased $I_{K1}$ [370]. In a guinea pig heart model, it was found that $I_{K1}$ played a significant part in rotor dynamics and its blockade terminated ventricular fibrillation [382,383]. These studies have resulted in greater awareness of the role played by $I_{K1}$ in cardiac arrhythmias.

In summary, the results of this chapter support this accumulating evidence of the prominent role played by $I_{K1}$ in the initiation and maintenance of cardiac arrhythmias [204,382,383,385]. The Kir 2.1 D172N mutation stabilised and accelerated reentry, which is consistent with the experimental study of mice with Kir 2.1 overexpression [370]. The D172N Kir2.1 mutation also increased human ventricular tissue susceptibility to the initiation of reentry. Thus, in some settings $I_{K1}$ may offer a potential therapeutic target for the treatment of cardiac arrhythmias.

The study that constitutes the basis for this thesis chapter has been published in full paper form [359].
Chapter 9

Relationship between Electrical and Mechanical Systole in the Short QT Syndrome: Insights from Modelling

9.1 Electromechanical Cardiac Myocyte Model

In Chapters 6 to 8, a pure electrophysiology model – the ten Tusscher et al. human ventricular cell model (TNNP) [38] – representing the electrical activity of the human ventricle was used to investigate the functional consequences of the SQTS on cardiac electrical excitation wave conduction. It is feasible that changes to repolarisation time might influence electromechanical coupling in the SQTS setting. In order to investigate the effects of the SQTS on cardiac mechanical dynamics, a contracting or force-generating human ventricular myocyte, an electromechanically coupled model of cardiac cell and tissue is required.

9.2 Myofilament Model

To describe the mechanics of the cardiac myocyte, the Rice et al. [386] myocyte contraction model (RMM) was adopted. It is based on the cross-bridge cycling model of mechanical contraction as explained in section 1.9.2.3. It is able to replicate a wide range of experimental data [386] including:

- steady state force-sarcomere length relations (F-SL relations).
- steady state force-calcium relations (F-Ca relations) including SL effects.
- steady state sarcomere length-calcium relations (SL-Ca relations) for unloaded cells.
- steady state force-velocity relations (F-V relations).
The biophysical processes of cell contraction are represented by systems of ordinary differential equations (ODE) [386].

9.3 Coupling the Electrophysiology model with the Myofilament Mechanics Model

The essential link between the electrophysiology model and the myofilament mechanics model (MM) is the intracellular calcium concentration \([Ca^{2+}]_i\) and associated \(Ca^{2+}\)-handling dynamics. \([Ca^{2+}]_i\) is produced as dynamic output from the electrophysiology model and used as an input to the mechanics model from where the amount that is buffered to troponin is calculated. In the TNNP electrophysiology model, the myoplasmic calcium concentration is calculated via

\[
\frac{dCa_i}{dt} = Ca_{ibufc} \left( \frac{V_v}{V_c} (I_{leak} - I_{up}) + I_{sfer} \right) - C_m \frac{I_{bCa} + I_{pCa} - 2I_{NaCa}}{2V_c F} \tag{95}
\]

where \(Ca_{ibufc}\) is the total cytoplasmic buffer concentration, \(V_{sr}\) is the volume of the sarcoplasmic reticulum (SR), \(V_c\) is the cytoplasmic volume, \(I_{leak}\) is the sarcoplasmic reticulum (SR) \(Ca^{2+}\) leak current, \(I_{up}\) is the SR \(Ca^{2+}\) pump current, \(I_{sfer}\) is diffusive \(Ca^{2+}\) current between diadic \(Ca^{2+}\) subspace and bulk cytoplasm, \(C_m\) is the membrane cell capacitance per unit surface area, \(I_{bCa}\) is the background \(Ca^{2+}\) current, \(I_{pCa}\) is the plateau \(Ca^{2+}\) current, \(I_{NaCa}\) is the \(Na^+\)/\(Ca^{2+}\) exchanger current and \(F\) is the Faraday constant.

The flux of the binding of calcium to troponin from the myofilament mechanics model is added to the calcium concentration calculation in Equation (95), which then becomes:

\[
\frac{dCa_i}{dt} = Ca_{ibufc} \left( \frac{V_v}{V_c} (I_{leak} - I_{up}) + I_{sfer} \right) - C_m \frac{I_{bCa} + I_{pCa} - 2I_{NaCa}}{2V_c F} - \frac{dTrop_{Ca}}{1000} \tag{96}
\]

where \(dTrop_{Ca}\) represents the concentration of calcium bound to troponin. With all the state variables describing the myofilament mechanics model included in the TNNP electromechanical model, and with Equation (96) substituted for Equation (95), the two models become effectively coupled, yielding an electromechanical cell model of a
human ventricular myocyte. Consequently, the electromechanical model is formulated as a system of differential-algebraic equations (DAE) of the form [38,386]:

\[
\frac{dy}{dt} = f(y,z) \quad (97)
\]
\[
g(y,z) = 0 \quad (98)
\]

Figure 9.1 shows the APs from the EPI, MIDDLE and ENDO cells for the TNNP model and the newly coupled electromechanical model at 1Hz under the WT, SQT1, SQT2 and SQT3 conditions. The changing transmembrane potential of the electromechanics model during each AP waveform is identical to and visually indistinguishable from that of the TNNP model.

9.3.1 Stretch-activated Channel

In cardiac tissue, a number of ionic channels that are activated by cell stretch have been identified [387–391]. These are known as stretch-activated channels (SACs) and can have significant effects on cardiac electrophysiological properties [387–391] (see Section 1.8). They respond to mechanical stimuli by an increase in open probability rather than conductance [88,392].

A stretch-activated current ($I_{sac}$) was incorporated into the electromechanics model following the work of Kelderman and Panfilov [393], Lunze et al. [394], Kuijpers [395], Youm et al. [86] and Kohl and Sachs [88]. $I_{sac}$ is formulated as:

\[
I_{sac} = G_{sac} \cdot P_m \cdot (V_m - E_{sac}) \quad (99)
\]

where $V_m$ is the membrane potential, $G_{sac}$ and $E_{sac}$ are the maximum membrane conductance and reversal potential of the stretch-activated channels respectively. $P_m$ is the open channel probability and is modelled as:

\[
P_m = \frac{1.0}{1 + e^{-\frac{V_m - E_{1/2}}{k_v}}} \quad (100)
\]
Figure 9.1: Action potentials from the epicardial, mid-myocardial and endocardial cell types of the original ten Tusscher et al. [38] model (the pure electrophysiology model) and the newly coupled electromechanics model.
where $\varepsilon_{1/2}$ is the half-activation strain, $\varepsilon$ is the strain, which has an explicit dependence on the sarcomere length and $k_e = 0.02$ [86,394,396] is the activation slope. $E_{\text{sac}}$ in the electromechanics model was typically 1 and describes the experimentally observed depolarising effect of the channel [397,398].

Additionally, in the electromechanics model, the channel is permeable to Na$^+$, K$^+$ and Ca$^{2+}$ with $I_{\text{sac}}$ defined by:

$$I_{\text{sac}} = I_{\text{sac,Na}} + I_{\text{sac,K}} + I_{\text{sac,Ca}}$$ (101)

where $I_{\text{sac,Na}}$, $I_{\text{sac,K}}$ and $I_{\text{sac,Ca}}$ represent the contributions of Na$^+$, K$^+$ and Ca$^{2+}$ to $I_{\text{sac}}$ respectively. In order to investigate the effects of the channel’s permeability to Na$^+$, K$^+$ and Ca$^{2+}$, two cases were considered in the single cell simulations: $P_{\text{Na}} : P_{\text{K}} : P_{\text{Ca}} = 1:1:1$ and $P_{\text{Na}} : P_{\text{K}} : P_{\text{Ca}} = 1:1:0$ with $P_{\text{Na}}$, $P_{\text{K}}$ and $P_{\text{Ca}}$ being the relative permeabilities to Na$^+$, K$^+$ and Ca$^{2+}$ respectively.

### 9.4 Tissue Mechanics Model

The cardiac tissue is modelled within the theoretical framework of nonlinear finite elasticity. Within this framework, any boundary value problem is comprised of three basic relations: kinematics relations, equations of motion and constitutive relations [399–401].

The kinematics relations govern the motion and deformation of the body under investigation; they define the relationship between the displacement of the body and the strain field. The equations of motion or the equilibrium relations are balance principles that describe the fundamental laws of physics governing the motion of a continuum. The constitutive relations describe the behaviour of a particular material, i.e., its response to an applied load [401–407].

Similar to other studies, cardiac tissue is modelled as an inhomogeneous, anisotropic, incompressible, nonlinear material [408–416]. The undeformed tissue occupies the region $\Omega_0$ with coordinates $\mathbf{X} = (X_1, X_2, X_3)$ while the deformed tissue occupies the
region \( \Omega \) with coordinates \( \mathbf{x} = (x_1, x_2, x_3) \). The deformation gradient \( \mathbf{F} \), an important quantity in nonlinear continuum mechanics, is a tensor that maps or transforms elements from the undeformed configuration to the deformed configuration [401–407]. It is given by:

\[
\mathbf{F} = \frac{\partial \mathbf{x}}{\partial \mathbf{X}}
\]  

(102)

where:

\[
F_{ij} = \frac{\partial x_i}{\partial X_j}
\]  

(103)

which provides a means of computing the components of the tensor \( \mathbf{F} \) given a referential description of the motion relative to a Cartesian coordinate system [401–407].

The Right Cauchy-Green stress tensor, \( \mathbf{C} \) is defined as:

\[
\mathbf{C} = \mathbf{F}^T \mathbf{F}
\]  

(104)

It quantifies the squared length of infinitesimal fibres in the deformed configuration.

The Green-Lagrange strain tensor, \( \mathbf{E} \), quantifies the length changes in a material fibre and angles between pairs of fibres in a deformed solid. It is defined as:

\[
\mathbf{E} = \frac{1}{2} (\mathbf{C} - \mathbf{I})
\]  

(105)

where \( \mathbf{I} \) is the second-order identity tensor. In the absence of body forces, and assuming that the body is always in instantaneous equilibrium and no inertial effects, the coordinates of the deformed body then satisfy the steady-state equilibrium equation with incompressibility enforced, i.e., the volume of the material is preserved:
\[
\frac{\partial}{\partial X_M} (T_{MN} F_{IN}) = 0 \tag{106}
\]

\[
\det(F) = 1 \tag{107}
\]

where \(i = 1, 2, 3\). \(T = (T_{MN})\) is the second Piola-Kirchhoff stress tensor; it relates a stress measure (in this case, \(C\)) to a strain measure (in this case, \(E\)) and refers to the deformed body. The incompressibility constraint is enforced via Equation (107), which simply states that the volume of the body is preserved with deformation.

\(T\) is composed of two parts; an elastic force component (the passive stress due to deformation) and a biochemically-generated force component [408]. Therefore, it can be written as:

\[
T = T_{\text{elastic}} + T_{\text{biochem}} \tag{108}
\]

To calculate \(T\), a strain energy function \((W)\) is required, which defines the constitutive behaviour of the material. For cardiac tissue, many strain energy functions have been proposed [409,412,414,417–419]. In this work, the Pole-Zero strain energy function was used (see Section 9.5). Written in terms of the strain energy function, \(W\), the entries of \(T_{\text{elastic}}\) are:

\[
T_{MN}^{\text{elastic}} = \frac{1}{2} \left( \frac{\partial W}{\partial E_{MN}} + \frac{\partial W}{\partial E_{NM}} \right) \tag{109}
\]

In this work, it assumed that the fibre direction is parallel to the X1-axis and similar to previous studies [408,412,414–416], it is assumed that the direction of the biochemically-generated force/tension acts only in the fibre direction. For example, for a 2D rectangular sheet with width (base) parallel to the x-axis, the fibre direction is chosen as the x-axis and this would also be the direction of the biochemically-generated force/tension. Following the work of Pathmanathan and Whiteley [408], \(T_{\text{biochem}}\) is calculated as:

\[
T_{\text{biochem}} = \frac{T_d}{C_{11}} \delta_{M1} \delta_{N1} \tag{110}
\]
where $Ta$ is the active force/tension obtained from the electromechanics cell model, $C_{11}$ is the indexed component of the Right Cauchy-Green stress tensor, $C$, and $\delta_{M1}\delta_{N1}$ ensure that $Ta$ acts only in the fibre direction. Equation (108) can then be written as:

$$T_{MN} = \frac{1}{2} \left( \frac{\partial W}{\partial E_{MN}} + \frac{\partial W}{\partial E_{NM}} \right) + \frac{Ta}{C_{11}} \delta_{M1}\delta_{N1}$$  \hspace{1cm} (111)

### 9.5 The Pole-Zero Strain Energy Function

For cardiac tissue, Nash and Hunter [412] used the pole-zero strain energy function, which is also employed in the current study. It encapsulates microstructural observations and bi-axial test results for myocardium [412]. It is given as:

$$W = \sum_{M,N=1}^{3} k_{MN} \frac{E_{MN}^2}{(a_{MN} - E_{MN})^b_{MN}}$$  \hspace{1cm} (112)

where $a_{MN}$ are physical properties of the tissue that are measured directly from microstructural observations. They are referred to as limiting strains or poles. $b_{MN}$ are related to the curvature of the uniaxial stress-strain relationships and were estimated by biaxial tension test results [420,421]. $k_{MN}$ are weighting factors that determine the contribution of the deformation to the strain energy of the material. $E$ is the Green Lagrange strain tensor (see section 9.4). Greater detail on the estimation of these parameter and coefficients can be found in [421].

### 9.6 Mechanical Feedback in the Electrophysiology Tissue Model

As with the electrical simulations in Chapters 6-8, the monodomain representation of cardiac tissue is employed [320–322]. However, the equation is modified to take into account the effect of the deforming tissue by incorporating a feedback in the diffusion term:
\[
C_m \frac{dV}{dt} = -(I_{\text{ion}} + I_{\text{stim}}) + \nabla \cdot (DC^{-1}\nabla V)
\] (113)

Here, the only difference from Equation (81) is the \(C^{-1}\) term, which is the inverse of the Right Cauchy-Green deformation tensor. This is in common with previous studies [408,416,422]. Physiologically, this term reflects the fact that the gap junctions between the cells distort with a deforming body. Hence, the electrical propagation across these gap junctions and throughout the tissue in deformed tissue is different to that in undeformed tissue.

9.7 Numerical Methods

9.7.1 Meshes

The electromechanical investigations were carried out on idealised, anisotropic, 2D tissue measuring 15 mm by 50 mm (see Section 5.2.4.1). The mesh used had a spatial resolution of 0.15 mm in both the x and y directions and 100 by 333 P1 finite elements [329,330,423]. The spatial and temporal resolution for cardiac electrophysiological simulations is well understood (in terms of the granularity necessary to sufficiently capture the essential features of electrical wave propagation in cardiac tissue) [305,323,325] and the mesh resolution is similar to the lengths of ventricular myocytes (80 – 150 \(\mu m\)) as suggested by Feigenbaum [332]. The mesh also consisted of three transmural regions; EPI, MCELL and ENDO as described in section 5.2.3, 5.2.4.1 and Figure 5.2.

9.7.2 Electrophysiology Problem

Obtaining a solution to the electromechanics problem in tissue involves solving two distinct sub-problems: an electrophysiology problem and a mechanics problem. The electrophysiological problem is solved with a Strang splitting method [324]. It is discretised in time using the Crank-Nicholson method [328] and in space using the Finite Element Method [328–331] (see Section 5.2.2.2). \(I_{\text{ion}}\) in Equation (113)
represents the coupled electromechanics single cells discussed in section 9.3. It is solved as described in section 5.2.1 using the forward Euler method with a time step of 0.02 ms. In addition to obtaining the membrane potential as a solution from $I_{ion}$, it also produces the active tension/force, which is passed as input to the Tissue Mechanics model to calculate tissue deformation.

### 9.7.3 The Mechanics Problem

The active tension/force ($T_a$) output from the electrophysiology solution is projected onto the mechanics mesh via an optimisation calculation that involves the solution of the Laplace equation. The active tension produces a state of stress in the tissue that causes it to deform. $T_a$ is incorporated into the mechanics problem as discussed in Equations (108)-(111).

The mechanics problem consists of only an elliptic component, which is also discretised using the Finite Element Method [328–331]. In the finite element analysis of systems which are subject to constraint conditions such as incompressibility (in this particular case, the incompressibility constraint in Equation 107), numerical difficulties, often termed locking phenomena arise [331,424–426]. This is an excessive stiffness of the system where the finite element is unable to distort while simultaneously satisfying the incompressibility constraint at every point in the body [331,401,405,424–426].

In order to avoid locking phenomena, a mixed formulation based on a three-field Hu-Washizu variational principle [401,405,427–430] was employed to solve Equation (106). It incorporated three independent variables: the deformation ($u$), Green-Lagrange strain tensor ($E$ from Equation 105) and the second Piola-Kirchhoff stress tensor ($T$ from Equation 108). The functional on the three-field Hu-Washizu principle in the absence of body forces (see Equation 106) is defined as:

$$
\Pi(u,E,T) = \int_{\Omega} \left[ W(E) + T : (E(u) - E) \right] d\Omega
$$

(114)
where $W(E)$ is the stored strain energy function given in Equation (112). The stationary condition on this functional with respect to $u$, $E$ and $T$ yields three independent equations:

$$D\Pi(u,E,T)[\delta u] = \int_{\Omega} T \cdot E(\delta u) d\Omega$$

(115)

$$D\Pi(u,E,T)[\delta E] = \int_{\Omega} \delta E \left( \frac{\partial W}{\partial E} - T \right) d\Omega$$

(116)

$$D\Pi(u,E,T)[\delta T] = \int_{\Omega} \delta T \cdot (E(u) - E) d\Omega$$

(117)

where $\delta u$, $\delta E$ and $\delta T$ are variations in the displacement, strain and stresses respectively.

Equations (115)-(117) represent the weak forms of the momentum balance equation (Equation 106), the displacement-strain equation and a hyperelastic constitutive equation for $T$ respectively. Equations (116)-(117) represent constraint terms enforcing incompressibility of the cardiac tissue (Equation 107), which have to be fulfilled together with Equation (115). Thus, $\delta E$ and $\delta T$ can be interpreted as Lagrange multipliers.

Over a typical finite element domain, $P1$ elements [329,330,423] are used to discretize the displacement variable, $u$, while the Green-Lagrange strain, $E$ and second Piola-Kirchhoff, $T$ variables are discretized with discontinuous (constant) functions. The nonlinear system in Equation (115)-(117) is linearized and solved iteratively using the Newton-Raphson method [400,405,406,429] to determine the system’s equilibrium configuration. At mechanical equilibrium, a state of deformation is attained, which is represented by the Right Cauchy-Green stress tensor $C$. This is used to update the conductivity tensor of the electrophysiology problem in Equation (113).

### 9.7.4 Combining the Electrophysiology and Mechanics Problems

In common with similar studies [408,422,431–433], the electrophysiology problem was solved with time step $\Delta t$ for $N$ steps. Then, the mechanics problem was solved with time step $N\Delta t$ to update the deformation of the tissue as it is more computationally intensive. The implementation and solution of both the electrophysiology and mechanics problems were developed using FEniCS, the automated finite element computing software suite [434,435].
9.8 Single Cell Electromechanical Simulations without $I_{sac}$

9.8.1 SQT1

Figure 9.2 shows the effects of the SQT1 mutation on the intracellular Ca$^{2+}$ concentration, the sarcomere length and the active force development in an electromechanically coupled single cell without the stretch-activated current ($I_{sac}$). The abbreviation of the AP by SQT1 is shown for the different cell types: EPI (Figure 9.2Ai), MCELL (Figure 9.2Bi) and ENDO (Figure 9.2Ci). The difference in shortening between the pure electrophysiology model and the coupled electromechanics model was insignificant in all the cell types (less than 0.01%, Figure 9.1).

Compared to WT, the SQT1 mutation reduced the intracellular calcium concentration by ~36% in EPI (Figure 9.2Aii) and ENDO (Figure 9.2Cii) and by ~47% in MCELL (Figure 9.2Bii). The initial sarcomere length for all cell types in both WT and SQT1 was 2.17 µm (Figure 9.2Aiii-Ciii). The minimum contracted sarcomere length (SL) in the WT condition was ~1.92 µm (EPI), ~1.80 µm (MCELL) and ~1.89 µm (ENDO). This was reduced with the SQT1 mutation to ~2.10 µm (EPI), ~2.01 µm (MCELL) and ~2.06 µm (ENDO). This is shown in Figure 9.2Aiii-Ciii for EPI, MCELL and ENDO respectively. The reduction in the intracellular calcium concentration was accompanied by a consequent reduction in the contractile force compared to WT by ~70% in EPI (Figure 9.2Aiv), ~56% in MCELL (Figure 9.2Biv) and ~59% in ENDO (Figure 9.2Civ). A summary of these results is shown in Table 9.1.

9.8.2 SQT2

Figure 9.3 shows the effects of the SQT2 mutation on the intracellular Ca$^{2+}$ concentration, the sarcomere length and the active force development in an electromechanically coupled single cell without the stretch-activated current ($I_{sac}$). The abbreviation of the AP by SQT2 is shown for the different cell types: EPI (Figure 9.3Ai), MCELL (Figure 9.3Bi) and ENDO (Figure 9.3Ci). The difference in shortening between the pure electrophysiology model and the coupled electromechanics model was insignificant in all the cell types (less than 0.01%, Figure 9.1).
Figure 9.2 Single cell electromechanical effects of the SQT1 mutation without $I_{\text{ sac}}$.

(Ai,Bi,Ci) WT (black) and SQT1 (red) action potentials in the EPI (Ai), MCELL (Bi) and ENDO (Ci) cells.

(Aii,Bii,Cii) WT (black) and SQT1 (red) calcium concentration in the EPI (Aii), MCELL (Bii) and ENDO (Cii) cells.

(Aiii,Biii,Ciii) WT (black) and SQT1 (red) sarcomere length in the EPI (Aiii), MCELL (Biii) and ENDO (Ciii) cells.

(Aiv,Biv,Civ) WT (black) and SQT1 (red) normalised active force in the EPI (Aiv), MCELL (Biv) and ENDO (Civ) cells.
Compared to WT, the SQT2 mutation reduced the calcium concentration by ~47% in EPI (Figure 9.3Aii) and ENDO (Figure 9.3Cii) and by ~26% in MCELL (Figure 9.3Bii). The initial sarcomere length for all cell types in both WT and SQT2 was 2.17 µm (Figure 9.3Aiii-Ciii). The minimum contracted sarcomere length (SL) in the WT condition was ~1.92 µm (EPI), ~1.80 µm (MCELL) and ~1.89 µm (ENDO). This was reduced with the SQT2 mutation to ~2.14 µm (EPI), ~1.89 µm (MCELL) and ~2.12 µm (ENDO) and is shown in Figure 9.3Aiii-Ciii for EPI, MCELL and ENDO respectively. The reduction in the intracellular calcium concentration was accompanied by a consequent reduction in the contractile force compared to WT by ~87% in EPI (Figure 9.3Aiv) and ~79% in ENDO (Figure 9.3Civ) but was only reduced by ~26% in MCELL (Figure 9.3Biv). A summary of these results is shown in Table 9.2.

9.8.3 SQT3

Figure 9.4 shows the effects of the SQT3 mutation on the intracellular Ca\(^{2+}\) concentration, the sarcomere length, and the active force development in an electromechanically coupled single cell without the stretch-activated current (\(I_{\text{sac}}\)). The abbreviation of the AP by SQT3 is shown for the different cell types: EPI (Figure 9.4Ai), MCELL (Figure 9.4Bi) and ENDO (Figure 9.4Ci). The difference in shortening between the pure electrophysiology model and the coupled electromechanics model was insignificant in all the cell types (less than 0.01%, Figure 9.1).

Compared to WT, the SQT3 mutation reduced the intracellular calcium concentration by ~17% in EPI (Figure 9.4Aii) and ENDO (Figure 9.4Cii) and by ~18% in MCELL (Figure 9.4Bii). The initial sarcomere length for all cell types in both WT and SQT3 was 2.17 µm (Figure 9.4Aiii-Ciii). The minimum contracted sarcomere length (SL) in the WT condition was ~1.92 µm (EPI), ~1.80 µm (MCELL) and ~1.89 µm (ENDO). This was reduced with SQT3 mutation to ~1.99 µm (EPI), ~1.86 µm (MCELL) and ~1.96 µm (ENDO) and is shown in Figure 9.4Aiii-Ciii for EPI, MCELL and ENDO respectively. The reduction in the intracellular calcium concentration was accompanied by a consequent reduction in the contractile force compared to WT by ~25% in EPI (Figure 9.4Aiv), and ~22% in ENDO (Figure 9.4Civ) but was only reduced by ~17% in MCELL (Figure 9.4Biv). A summary of these results is shown in Table 9.3.
Figure 9.3 Single cell electromechanical effects of the SQT2 mutation without $I_{\text{sac}}$.

(Ai,Bi,Ci) WT (black) and SQT2 (red) action potentials in the EPI (Ai), MCELL (Bi) and ENDO (Ci) cells.

(Aii,Bii,Cii) WT (black) and SQT2 (red) calcium concentration in the EPI (Aii), MCELL (Bii) and ENDO (Cii) cells.

(Aiii,Biii,Ciii) WT (black) and SQT2 (red) sarcomere length in the EPI (Aiii), MCELL (Biii) and ENDO (Ciii) cells.

(Aiv,Biv,Civ) WT (black) and SQT2 (red) normalised active force in the EPI (Aiv), MCELL (Biv) and ENDO (Civ) cells.
Figure 9.4 Single cell electromechanical effects of the SQT3 mutation without $I_{\text{sar}}$.

(Ai,Bi,Ci) WT (black) and SQT3 (red) action potentials in the EPI (Ai), MCELL (Bi) and ENDO (Ci) cells.

(Aii,Bii,Cii) WT (black) and SQT3 (red) calcium concentration in the EPI (Aii), MCELL (Bii) and ENDO (Cii) cells.

(Aiii,Biii,Ciii) WT (black) and SQT3 (red) sarcomere length in the EPI (Aiii), MCELL (Biii) and ENDO (Ciii) cells.

(Aiv,Biv,Civ) WT (black) and SQT3 (red) normalised active force in the EPI (Aiv), MCELL (Biv) and ENDO (Civ) cells.
<table>
<thead>
<tr>
<th>Sarcomere Length (µM)</th>
<th>Force of Contraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPI</td>
<td>MCELL</td>
</tr>
<tr>
<td>WT</td>
<td>1.92</td>
</tr>
<tr>
<td>SQT1</td>
<td>2.10</td>
</tr>
</tbody>
</table>

Table 9.1: Without $I_{sac}$ - Minimal length of contracted sarcomere with the SQT1 mutation and the consequent active force of contraction relative to WT. Initial sarcomere length is 2.17 µM. The smaller the sarcomere length, the greater is the force of contraction.

<table>
<thead>
<tr>
<th>Sarcomere Length (µM)</th>
<th>Force of Contraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPI</td>
<td>MCELL</td>
</tr>
<tr>
<td>WT</td>
<td>1.92</td>
</tr>
<tr>
<td>SQT2</td>
<td>2.14</td>
</tr>
</tbody>
</table>

Table 9.2: Without $I_{sac}$ - Minimal length of contracted sarcomere with the SQT2 mutation and the consequent active force of contraction relative to WT. Initial sarcomere length is 2.17 µM. The smaller the sarcomere length, the greater is the force of contraction.

<table>
<thead>
<tr>
<th>Sarcomere Length (µM)</th>
<th>Force of Contraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPI</td>
<td>MCELL</td>
</tr>
<tr>
<td>WT</td>
<td>1.92</td>
</tr>
<tr>
<td>SQT3</td>
<td>1.99</td>
</tr>
</tbody>
</table>

Table 9.3: Without $I_{sac}$ - Minimal length of contracted sarcomere with the SQT3 mutation and the consequent active force of contraction relative to WT. Initial sarcomere length is 2.17 µM. The smaller the sarcomere length, the greater is the force of contraction.
9.8.4 Simulated AP Clamp

The effects of the mutations on contractile force (particularly SQT1 and SQT2) are quite profound (Figure 9.2-9.3). A patient with such a severe reduction in contractility would be expected to suffer heart failure and die. In order to gain a mechanistic insight into these large effects on the contractile force, a simulated AP clamp was performed on the WT electromechanics model only (Figure 9.5). This model (with no changes) was subjected to two different AP waveforms of differing durations – a normal waveform and a shortened waveform (Figure 9.5A) – in order to observe how these waveforms influence the amplitude of the intracellular Ca\(^{2+}\) concentration, the sarcomere length and force of contraction in the WT electromechanics model. The reasoning was that if on application of the AP waveforms with different durations to the same model under AP clamp, the same profound differences seen in the SQT models are observed, this would imply that the key factor is the effect of AP shortening on Ca\(^{2+}\) handling (and on SR content in particular).

Figure 9.5 shows the results of the simulated AP clamp, which shows the same pronounced effects as the SQT models on the intracellular calcium concentration amplitude (Figure 9.5C), the sarcomere length shortening (Figure 9.5D) and contractility (Figure 9.5E). The peak I\(_{\text{CaL}}\) is the same with both normal and shortened waveforms (Figure 9.5B) but with a slight increase in amplitude during terminal repolarisation in the shortened waveform (Figure 9.5B).

9.9 Single Cell Electromechanical Simulations with \(I_{\text{sac}}\)

Figure 9.6 shows the effects of incorporating a stretch-activated current into the electromechanics model. It shows three cases; the effects on the WT electromechanics model:

1. without \(I_{\text{sac}}\).
2. with \(I_{\text{sac}}\) and the SACs having relative permeabilities to Na\(^{+}\), K\(^{+}\) and Ca\(^{2+}\) in the ratio 1:1:1 (\(P_{\text{Na}} : P_{\text{K}} : P_{\text{Ca}} = 1:1:1\)) and
3. with \(I_{\text{sac}}\) and the SACs having relative permeabilities to Na\(^{+}\), K\(^{+}\) and Ca\(^{2+}\) in the ratio 1:1:0 (\(P_{\text{Na}} : P_{\text{K}} : P_{\text{Ca}} = 1:1:0\)).
Figure 9.5: Simulated AP clamp using the WT electromechanics model without $I_{sat}$.

A. The normal (black) and shortened (red) AP waveforms applied to the WT electromechanics model.

B. $I_{CaL}$ elicited by the two AP waveforms in A.

C. $[Ca]_i$ elicited by the two AP waveforms in A.

D. SL shortening elicited by the two AP waveforms in A.

E. Contractile force elicited by the two AP waveforms in A.

All units are normalised to the maximum value.
Figure 9.6: Effects of stretch-activated current ($I_{sac}$) on the WT electromechanics model (Ai,Bi,Ci) Action potentials in the EPI (Ai), MCELL (Bi) and ENDO (Ci) cells of WT with no $I_{sac}$ (black), WT with $I_{sac}$ ($P_{Na} : P_{K} : P_{Ca} = 1:1:0$, green) and WT with $I_{sac}$ ($P_{Na} : P_{K} : P_{Ca} = 1:1:1$, blue).

(Aii,Bii,Cii) Calcium concentration in the EPI (Aii), MCELL (Bii) and ENDO (Cii) cells of WT with no $I_{sac}$ (black), WT with $I_{sac}$ ($P_{Na} : P_{K} : P_{Ca} = 1:1:0$, green) and WT with $I_{sac}$ ($P_{Na} : P_{K} : P_{Ca} = 1:1:1$, blue).

(Aiii,Biii,Ciii) Sarcomere length in the EPI (Aiii), MCELL (Biii) and ENDO (Ciii) cells of WT with no $I_{sac}$ (black), WT with $I_{sac}$ ($P_{Na} : P_{K} : P_{Ca} = 1:1:0$, green) and WT with $I_{sac}$ ($P_{Na} : P_{K} : P_{Ca} = 1:1:1$, blue).

(Aiv,Biv,Civ) Normalised active force in the EPI (Aiv), MCELL (Biv) and ENDO (Civ) cells of WT with no $I_{sac}$ (black), WT with $I_{sac}$ ($P_{Na} : P_{K} : P_{Ca} = 1:1:0$, green) and WT with $I_{sac}$ ($P_{Na} : P_{K} : P_{Ca} = 1:1:1$, blue).
Across the ventricular wall, the resting potential of EPI (Figure 9.6Ai), MCELL (Figure 9.6Bi) and ENDO (Figure 9.6Ci) increased from -86 mV (WT without $I_{sac}$) to -76 mV (WT with $I_{sac} = P_{Na} : P_{K} : P_{Ca} = 1:1:0$) and to -79 mV (WT with $I_{sac} = P_{Na} : P_{K} : P_{Ca} = 1:1:1$). This is an experimentally observed effect of SACs [97,98].

$I_{sac}$ with both permeability ratios shortened the action potential in EPI (Figure 9.6Ai), MCELL (Figure 9.6Bi) and ENDO (Figure 9.6Ci) with the greater shortening occurring with $P_{Na} : P_{K} : P_{Ca} = 1:1:1$. AP shortening is one of the effects that has been observed experimentally with SACs [93–96] (also see Section 1.8). AP lengthening has also been observed [90–92]. These changes are thought to be related to the inward Ca$^{2+}$ current and to changes in the Na$^+$/Ca$^{2+}$ exchanger [86,436]. $I_{sac}$ with both permeability ratios also increased the amplitude of the intracellular Ca$^{2+}$ concentration (Figure 9.6Aii-Cii), the Sarcomere length shortening (Figure 9.6Aiii-Cii) and the active force of contraction (Figure 9.6Aiv-Civ) [91,101–106,436] with the greater lengthening occurring with $P_{Na} : P_{K} : P_{Ca} = 1:1:1$. Tables 9.4-9.7 give quantitative comparison of the degree of AP shortening, intracellular calcium concentration amplitude augmentation, greater SL shortening and increased active force respectively. Table 9.8 shows the effects of $I_{sac}$ on the APDs of the SQT1-SQT3 variants with $P_{Na} : P_{K} : P_{Ca} = 1:1:0$ and $P_{Na} : P_{K} : P_{Ca} = 1:1:1$.

### 9.9.1 SQT1

#### 9.9.1.1 $P_{Na}:P_{K}:P_{Ca} = 1:1:0$

Figure 9.7 shows the effects of the SQT1 mutation on the intracellular Ca$^{2+}$ concentration, the sarcomere length and the active force development in an electromechanically coupled single cell with the stretch-activated current ($I_{sac}$) at a relative permeability ratio to Na$^+$, K$^+$ and Ca$^{2+}$ of 1:1:0. Both the WT and SQT1 electromechanics models used this permeability ratio. The abbreviation of the AP by SQT1 is shown for the different cell types: EPI (Figure 9.7Ai), MCELL (Figure 9.7Bi) and ENDO (Figure 9.7Ci).
<table>
<thead>
<tr>
<th>APD (ms)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>EPI</td>
<td>MCELL</td>
<td>ENDO</td>
</tr>
<tr>
<td>WT</td>
<td>310</td>
<td>433</td>
<td>314</td>
</tr>
<tr>
<td>WT ((I_{\text{sac}} P_{Na} : P_{K} : P_{Ca} = 1:1:0))</td>
<td>306</td>
<td>420</td>
<td>310</td>
</tr>
<tr>
<td>WT ((I_{\text{sac}} P_{Na} : P_{K} : P_{Ca} = 1:1:1))</td>
<td></td>
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</tr>
</tbody>
</table>

Table 9.4: WT Changes in APD due to \(I_{\text{sac}}\) at \(P_{Na} : P_{K} : P_{Ca} = 1:1:0\) and \(P_{Na} : P_{K} : P_{Ca} = 1:1:1\).

<table>
<thead>
<tr>
<th>(Ca_{i} (% \text{ WT}))</th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPI</td>
<td>MCELL</td>
<td>ENDO</td>
</tr>
<tr>
<td>WT</td>
<td>118</td>
<td>108</td>
<td>122</td>
</tr>
<tr>
<td>WT ((I_{\text{sac}} P_{Na} : P_{K} : P_{Ca} = 1:1:0))</td>
<td>154</td>
<td>136</td>
<td>164</td>
</tr>
<tr>
<td>WT ((I_{\text{sac}} P_{Na} : P_{K} : P_{Ca} = 1:1:1))</td>
<td></td>
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</table>

Table 9.5: WT Changes in \(Ca_{i}\) due to \(I_{\text{sac}}\) at \(P_{Na} : P_{K} : P_{Ca} = 1:1:0\) and \(P_{Na} : P_{K} : P_{Ca} = 1:1:1\).

<table>
<thead>
<tr>
<th>SL ((\mu\text{m}))</th>
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<tbody>
<tr>
<td></td>
<td>EPI</td>
<td>MCELL</td>
<td>ENDO</td>
</tr>
<tr>
<td>WT</td>
<td>1.85</td>
<td>1.77</td>
<td>1.84</td>
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<tr>
<td>WT ((I_{\text{sac}} P_{Na} : P_{K} : P_{Ca} = 1:1:0))</td>
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<td>1.71</td>
<td>1.75</td>
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<tr>
<td>WT ((I_{\text{sac}} P_{Na} : P_{K} : P_{Ca} = 1:1:1))</td>
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</tr>
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</table>

Table 9.6: WT changes in minimal length of contracted sarcomere due to \(I_{\text{sac}}\) at \(P_{Na} : P_{K} : P_{Ca} = 1:1:0\) and \(P_{Na} : P_{K} : P_{Ca} = 1:1:1\). Initial sarcomere length is 2.17 \(\mu\text{M}\). The smaller the sarcomere length, the greater is the force of contraction.

<table>
<thead>
<tr>
<th>Force of Contraction (%WT)</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>EPI</td>
<td>MCELL</td>
<td>ENDO</td>
</tr>
<tr>
<td>WT</td>
<td>126</td>
<td>107</td>
<td>120</td>
</tr>
<tr>
<td>WT ((I_{\text{sac}} P_{Na} : P_{K} : P_{Ca} = 1:1:0))</td>
<td>167</td>
<td>123</td>
<td>153</td>
</tr>
<tr>
<td>WT ((I_{\text{sac}} P_{Na} : P_{K} : P_{Ca} = 1:1:1))</td>
<td></td>
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</tr>
</tbody>
</table>

Table 9.7: WT changes in contractile force due to \(I_{\text{sac}}\) at \(P_{Na} : P_{K} : P_{Ca} = 1:1:0\) and \(P_{Na} : P_{K} : P_{Ca} = 1:1:1\).
<table>
<thead>
<tr>
<th></th>
<th>APD (ms)</th>
<th>Without $I_{sac}$</th>
<th>$I_{sac}$ $P_{Na}:P_{K}:P_{Ca} = 1:1:0$</th>
<th>$I_{sac}$ $P_{Na}:P_{K}:P_{Ca} = 1:1:1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPI</td>
<td>212</td>
<td>214</td>
<td>218</td>
<td></td>
</tr>
<tr>
<td>SQT1 MCELL</td>
<td>232</td>
<td>230</td>
<td>237</td>
<td></td>
</tr>
<tr>
<td>ENDO</td>
<td>211</td>
<td>218</td>
<td>218</td>
<td></td>
</tr>
<tr>
<td>EPI</td>
<td>233</td>
<td>232</td>
<td>230</td>
<td></td>
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<tr>
<td>SQT2 MCELL</td>
<td>355</td>
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<td></td>
</tr>
<tr>
<td>ENDO</td>
<td>234</td>
<td>228</td>
<td>226</td>
<td></td>
</tr>
<tr>
<td>EPI</td>
<td>283</td>
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<tr>
<td>SQT3 MCELL</td>
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<tr>
<td>ENDO</td>
<td>284</td>
<td>270</td>
<td>257</td>
<td></td>
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</tbody>
</table>

Table 9.8: SQT1, SQT2 and SQT3 changes in APD due to $I_{sac}$ at $P_{Na}:P_{K}:P_{Ca} = 1:1:0$ and $P_{Na}:P_{K}:P_{Ca} = 1:1:1$. 
Figure 9.7 Single cell electromechanical effects of the SQT1 mutation with $I_{sac}$ at $P_{Na} : P_{K} : P_{Ca} = 1:1:0$.

(Ai,Bi,Ci) WT (black) and SQT1 (red) action potentials in the EPI (Ai), MCELL (Bi) and ENDO (Ci) cells.

(Aii,Bii,Cii) WT (black) and SQT1 (red) calcium concentration in the EPI (Aii), MCELL (Bii) and ENDO (Cii) cells.

(Aiii,Biii,Ciii) WT (black) and SQT1 (red) sarcomere length in the EPI (Aiii), MCELL (Biii) and ENDO (Ciii) cells.

(Aiv,Biv,Civ) WT (black) and SQT1 (red) normalised active force in the EPI (Aiv), MCELL (Biv) and ENDO (Civ) cells.

<table>
<thead>
<tr>
<th>SL ($\mu$m)</th>
<th>EPI</th>
<th>MCELL</th>
<th>ENDO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WT ($I_{sac} P_{Na} : P_{K} : P_{Ca} = 1:1:0$)</strong></td>
<td>1.85</td>
<td>1.77</td>
<td>1.84</td>
</tr>
<tr>
<td><strong>SQT1 ($I_{sac} P_{Na} : P_{K} : P_{Ca} = 1:1:0$)</strong></td>
<td>1.97</td>
<td>1.93</td>
<td>1.97</td>
</tr>
</tbody>
</table>

**Force of Contraction (%WT)**

| **WT ($I_{sac} P_{Na} : P_{K} : P_{Ca} = 1:1:0$)** | 100 | 100 | 100 |
| **SQT1 ($I_{sac} P_{Na} : P_{K} : P_{Ca} = 1:1:0$)** | 60  | 61  | 64  |

Table 9.9: SQT1 ($I_{sac}$ at $P_{Na} : P_{K} : P_{Ca} = 1:1:0$) - minimal length of contracted sarcomere with the SQT1 mutation and the consequent active force of contraction relative to WT. Initial sarcomere length is 2.17 $\mu$m. The smaller the sarcomere length, the greater is the force of contraction.
Compared to WT, the SQT1 mutation reduced the intracellular calcium concentration by ~31% in EPI (Figure 9.7Aii) and ENDO (Figure 9.7Cii) and by ~39% in MCELL (Figure 9.7Bii). The initial sarcomere length for all cell types in both WT and SQT1 was 2.17 µm (Figure 9.7Aiii-Ciii). The minimum contracted sarcomere length (SL) in the WT condition was ~1.85 µm (EPI), ~1.77 µm (MCELL) and ~1.84 µm (ENDO). This was reduced by the SQT1 mutation to ~1.97 µm (EPI), ~1.93 µm (MCELL) and ~1.97 µm (ENDO). This is shown in Figure 9.7Aiii-Ciii for EPI, MCELL and ENDO respectively. The reduction in the intracellular calcium concentration was accompanied by a consequent reduction in the contractile force compared to WT by ~40% in EPI (Figure 9.7Aiv), ~39% in MCELL (Figure 9.7Biv) and ~36% in ENDO (Figure 9.7Civ). A summary of these results is shown in Table 9.9.

9.9.1.2 $P_{Na}:P_{K}:P_{Ca} = 1:1:1$

Figure 9.8 shows the effects of the SQT1 mutation on the intracellular $Ca^{2+}$ concentration, the sarcomere length and the active force development in an electromechanically coupled single cell with the stretch-activated current ($I_{so}$) and relative permeability ratio to $Na^+$, $K^+$ and $Ca^{2+}$ of 1:1:1. Both the WT and SQT1 electromechanics models used this permeability ratio. The abbreviation of the AP by SQT1 is shown for the different cell types: EPI (Figure 9.8Ai), MCELL (Figure 9.8Bi) and ENDO (Figure 9.8Ci).

Compared to WT, the SQT1 mutation reduced the intracellular calcium concentration by ~16% in EPI (Figure 9.8Aii) and ENDO (Figure 9.8Cii) and by ~25% in MCELL (Figure 9.8Bii). The initial sarcomere length for all cell types in both WT and SQT1 was 2.17 µm (Figure 9.8Aiii-Ciii). The minimum contracted sarcomere length (SL) in the WT condition was ~1.75 µm (EPI), ~1.71 µm (MCELL) and ~1.75 µm (ENDO). This was reduced by the SQT1 mutation to ~1.81 µm (EPI), ~1.78 µm (MCELL) and ~1.80 µm (ENDO). This is shown in Figure 9.8Aiii-Ciii for EPI, MCELL and ENDO respectively. The reduction in the intracellular calcium concentration was accompanied by a consequent reduction in the contractile force compared to WT by ~13% in EPI (Figure 9.8Aiv), ~15% in MCELL (Figure 9.8Biv) and ~11% in ENDO (Figure 9.8Civ). A summary of these results is shown in Table 9.10.
Figure 9.8 Single cell electromechanical effects of the SQT1 mutation with $I_{sac}$ at $P_{Na} : P_{K} : P_{Ca} = 1:1:1$.

(Ai,Bi,Ci) WT (black) and SQT1 (red) action potentials in the EPI (Ai), MCELL (Bi) and ENDO (Ci) cells.

(Aii,Bii,Cii) WT (black) and SQT1 (red) calcium concentration in the EPI (Aii), MCELL (Bii) and ENDO (Cii) cells.

(Aiii,Biii,Ciii) WT (black) and SQT1 (red) sarcomere length in the EPI (Aiii), MCELL (Biii) and ENDO (Ciii) cells.

(Aiv,Biv,Civ) WT (black) and SQT1 (red) normalised active force in the EPI (Aiv), MCELL (Biv) and ENDO (Civ) cells.

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<th>MCELL</th>
<th>ENDO</th>
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<td>WT ($I_{sac} P_{Na} : P_{K} : P_{Ca} = 1:1:1$)</td>
<td>1.75</td>
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<table>
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<th>Force of Contraction (%WT)</th>
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<tr>
<td>WT ($I_{sac} P_{Na} : P_{K} : P_{Ca} = 1:1:1$)</td>
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<tr>
<td>SQT1 ($I_{sac} P_{Na} : P_{K} : P_{Ca} = 1:1:1$)</td>
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Table 9.10: SQT1 ($I_{sac}$ at $P_{Na} : P_{K} : P_{Ca} = 1:1:1$) - minimal length of contracted sarcomere with the SQT1 mutation and the consequent active force of contraction relative to WT. Initial sarcomere length is 2.17 µM. The smaller the sarcomere length, the greater is the force of contraction.
9.9.2 SQT2

9.9.2.1 $P_{Na}:P_{K}:P_{Ca} = 1:1:0$

Figure 9.9 shows the effects of the SQT2 mutation on the intracellular Ca\(^{2+}\) concentration, the sarcomere length and the active force development in an electromechanically coupled single cell with the stretch-activated current ($I_{sac}$) and relative permeability ratio to Na\(^{+}\), K\(^{+}\) and Ca\(^{2+}\) of 1:1:0. Both the WT and SQT2 electromechanics models used this permeability ratio. The abbreviation of the AP by SQT2 is shown for the different cell types: EPI (Figure 9.9Ai), MCELL (Figure 9.9Bi) and ENDO (Figure 9.9Ci).

Compared to WT, the SQT2 mutation reduced the intracellular calcium concentration by ~42% in EPI (Figure 9.9Aii) and ENDO (Figure 9.9Cii) and by ~21% in MCELL (Figure 9.9Bii). The initial sarcomere length for all cell types in both WT and SQT2 was 2.17 µm (Figure 9.9Aiii-Ciii). The minimum contracted sarcomere length (SL) in the WT condition was ~1.85 µm (EPI), ~1.77 µm (MCELL) and ~1.84 µm (ENDO). This was reduced by the SQT2 mutation to ~2.04 µm (EPI), ~1.85 µm (MCELL) and ~2.05 µm (ENDO). This is shown in Figure 9.9Aiii-Ciii for EPI, MCELL and ENDO respectively. The reduction in the intracellular calcium concentration was accompanied by a consequent reduction in the contractile force compared to WT by ~57% in EPI (Figure 9.9Aiv), ~18% in MCELL (Figure 9.9Biv) and ~60% in ENDO (Figure 9.9Civ). A summary of these results is shown in Table 9.11.

9.9.2.2 $P_{Na}:P_{K}:P_{Ca} = 1:1:1$

Figure 9.10 shows the effects of the SQT2 mutation on the intracellular Ca\(^{2+}\) concentration, the sarcomere length and the active force development in an electromechanically coupled single cell with the stretch-activated current ($I_{sac}$) and relative permeability ratio to Na\(^{+}\), K\(^{+}\) and Ca\(^{2+}\) of 1:1:1. Both the WT and SQT2 electromechanics models used this permeability ratio. The abbreviation of the AP by SQT2 is shown for the different cell types: EPI (Figure 9.10Ai), MCELL (Figure 9.10Bi) and ENDO (Figure 9.10Ci).
Figure 9.9 Single cell electromechanical effects of the SQT2 mutation with $I_{\text{sac}}$ at $P_{\text{Na}} : P_{\text{K}} : P_{\text{Ca}} = 1:1:0$.

(Ai,Bi,Ci) WT (black) and SQT2 (red) action potentials in the EPI (Ai), MCELL (Bi) and ENDO (Ci) cells.

(Aii,Bii,Cii) WT (black) and SQT2 (red) calcium concentration in the EPI (Aii), MCELL (Bii) and ENDO (Cii) cells.

(Aiii,Biii,Ciii) WT (black) and SQT2 (red) sarcomere length in the EPI (Aiii), MCELL (Biii) and ENDO (Ciii) cells.

(Aiv,Biv,Civ) WT (black) and SQT2 (red) normalised active force in the EPI (Aiv), MCELL (Biv) and ENDO (Civ) cells.

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<td>1.77</td>
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<td>2.05</td>
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<td><strong>SQT2 ($I_{\text{sac}} P_{\text{Na}} : P_{\text{K}} : P_{\text{Ca}} = 1:1:0$)</strong></td>
<td>43</td>
<td>82</td>
<td>40</td>
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Table 9.11: SQT2 ($I_{\text{sac}}$ at $P_{\text{Na}} : P_{\text{K}} : P_{\text{Ca}} = 1:1:0$) - minimal length of contracted sarcomere with the SQT2 mutation and the consequent active force of contraction relative to WT. Initial sarcomere length is 2.17 $\mu$m. The smaller the sarcomere length, the greater is the force of contraction.
Figure 9.10 Single cell electromechanical effects of the SQT2 mutation with $I_{\text{sac}}$ at $P_{\text{Na}}:P_{K}:P_{\text{Ca}} = 1:1:1$.

(Ai,Bi,Ci) WT (black) and SQT2 (red) action potentials in the EPI (Ai), MCELL (Bi) and ENDO (Ci) cells.

(Aii,Bii,Cii) WT (black) and SQT2 (red) calcium concentration in the EPI (Aii), MCELL (Bii) and ENDO (Cii) cells.

(Aiii,Biii,Ciii) WT (black) and SQT2 (red) sarcomere length in the EPI (Aiii), MCELL (Biii) and ENDO (Ciii) cells.

(Aiv,Biv,Civ) WT (black) and SQT2 (red) normalised active force in the EPI (Aiv), MCELL (Biv) and ENDO (Civ) cells.

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<tr>
<td>1.75</td>
<td>1.71</td>
<td>1.75</td>
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<td><strong>SQT2 ($I_{\text{sac}}P_{\text{Na}}:P_{K}:P_{\text{Ca}} = 1:1:1$)</strong></td>
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<td>1.84</td>
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<td><strong>Force of Contraction (%WT)</strong></td>
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<td>100</td>
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<tr>
<td><strong>SQT2 ($I_{\text{sac}}P_{\text{Na}}:P_{K}:P_{\text{Ca}} = 1:1:1$)</strong></td>
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<td>79</td>
<td>91</td>
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Table 9.12: SQT2 ($I_{\text{sac}}$ at $P_{\text{Na}}:P_{K}:P_{\text{Ca}} = 1:1:1$) - minimal length of contracted sarcomere with the SQT2 mutation and the consequent active force of contraction relative to WT. Initial sarcomere length is 2.17 µM. The smaller the sarcomere length, the greater is the force of contraction.
Compared to WT, the SQT2 mutation reduced the intracellular calcium concentration by ~25% in EPI (Figure 9.10Aii) and ENDO (Figure 9.10Cii) and by ~17% in MCELL (Figure 9.10Bii). The initial sarcomere length for all cell types in both WT and SQT2 was 2.17 µm (Figure 9.10Aiii-Ciii). The minimum contracted sarcomere length (SL) in the WT condition was ~1.75 µm (EPI), ~1.71 µm (MCELL) and ~1.75 µm (ENDO). This was reduced by the SQT2 mutation to ~1.84 µm (EPI), ~1.75 µm (MCELL) and ~1.84 µm (ENDO). This is shown in Figure 9.10Aiii-Ciii for EPI, MCELL and ENDO respectively. The reduction in the intracellular calcium concentration was accompanied by a consequent reduction in the contractile force compared to WT by ~21% in EPI (Figure 9.10Aiv), ~9% in MCELL (Figure 9.10Biv) and ~20% in ENDO (Figure 9.10Civ). A summary of these results is shown in Table 9.12.

9.9.3 SQT3

9.9.3.1 $P_{Na}:P_{K}:P_{Ca} = 1:1:0$

Figure 9.11 shows the effects of the SQT3 mutation on the intracellular Ca$^{2+}$ concentration, the sarcomere length and the active force development in an electromechanically coupled single cell with the stretch-activated current ($I_{sac}$) and relative permeability ratio to Na$^+$, K$^+$ and Ca$^{2+}$ of 1:1:0. Both the WT and SQT3 electromechanics models used this permeability ratio. The abbreviation of the AP by SQT3 is shown for the different cell types: EPI (Figure 9.11Ai), MCELL (Figure 9.11Bi) and ENDO (Figure 9.11Ci).

Compared to WT, the SQT3 mutation reduced the intracellular calcium concentration by ~19% in EPI (Figure 9.11Aii), ENDO (Figure 9.11Cii) and MCELL (Figure 9.11Bii). The initial sarcomere length for all cell types in both WT and SQT3 was 2.17 µm (Figure 9.11Aiii-Ciii). The minimum contracted sarcomere length (SL) in the WT condition was ~1.85 µm (EPI), ~1.77 µm (MCELL) and ~1.84 µm (ENDO). This was reduced by the SQT3 mutation to ~1.91 µm (EPI), ~1.83 µm (MCELL) and ~1.92 µm (ENDO). This is shown in Figure 9.11Aiii-Ciii for EPI, MCELL and ENDO respectively. The reduction in the intracellular calcium concentration was accompanied by a consequent reduction in the contractile force compared to WT by ~20% in EPI.
Figure 9.11 Single cell electromechanical effects of the SQT3 mutation with $I_{\text{sac}}$ at $P_{Na}:P_{K}:P_{Ca} = 1:1:0$.

(Ai,Bi,Ci) WT (black) and SQT3 (red) action potentials in the EPI (Ai), MCELL (Bi) and ENDO (Ci) cells.

(Aii,Bii,Cii) WT (black) and SQT3 (red) calcium concentration in the EPI (Aii), MCELL (Bii) and ENDO (Cii) cells.

(Aiii,Biii,Ciii) WT (black) and SQT3 (red) sarcomere length in the EPI (Aiii), MCELL (Biii) and ENDO (Ciii) cells.

(Aiv,Biv,Civ) WT (black) and SQT3 (red) normalised active force in the EPI (Aiv), MCELL (Biv) and ENDO (Civ) cells.

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<th>ENDO</th>
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<tbody>
<tr>
<td>WT ($I_{\text{sac}} ; P_{Na}:P_{K}:P_{Ca} = 1:1:0$)</td>
<td>1.85</td>
<td>1.77</td>
<td>1.84</td>
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<td>1.83</td>
<td>1.92</td>
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**Force of Contraction (%WT)**

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<tr>
<td>WT ($I_{\text{sac}} ; P_{Na}:P_{K}:P_{Ca} = 1:1:0$)</td>
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<td>SQT3 ($I_{\text{sac}} ; P_{Na}:P_{K}:P_{Ca} = 1:1:0$)</td>
<td>80</td>
<td>86</td>
<td>79</td>
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Table 9.13: SQT3 ($I_{\text{sac}}$ at $P_{Na}:P_{K}:P_{Ca} = 1:1:0$) - minimal length of contracted sarcomere with the SQT3 mutation and the consequent active force of contraction relative to WT. Initial sarcomere length is 2.17 $\mu$M. The smaller the sarcomere length, the greater is the force of contraction.
(Figure 9.11Aiv), ~14% in MCELL (Figure 9.11Biv) and ~21% in ENDO (Figure 9.11Civ). A summary of these results is shown in Table 9.13.

9.9.3.2 $P_{Na}:P_{K}:P_{Ca} = 1:1:1$

Figure 9.12 shows the effects of the SQT3 mutation on the intracellular Ca$^{2+}$ concentration, the sarcomere length and the active force development in an electromechanically coupled single cell with the stretch-activated current ($I_{sac}$) and relative permeability ratio to Na$^+$, K$^+$ and Ca$^{2+}$ of 1:1:1. Both the WT and SQT3 electromechanics models used this permeability ratio. The abbreviation of the AP by SQT3 is shown for the different cell types: EPI (Figure 9.12Ai), MCELL (Figure 9.12Bi) and ENDO (Figure 9.12Ci).

Compared to WT, the SQT3 mutation reduced the intracellular calcium concentration by ~13% in EPI (Figure 9.12Aii) and ENDO (Figure 9.12Cii) and by ~17% in MCELL (Figure 9.12Bii). The initial sarcomere length for all cell types in both WT and SQT3 was 2.17 $\mu$m (Figure 9.12Aiii-Ciii). The minimum contracted sarcomere length (SL) in the WT condition was ~1.75 $\mu$m (EPI), ~1.71 $\mu$m (MCELL) and ~1.75 $\mu$m (ENDO). This was reduced by the SQT3 mutation to ~1.78 $\mu$m (EPI), ~1.75 $\mu$m (MCELL) and ~1.79 $\mu$m (ENDO). This is shown in Figure 9.12Aiii-Ciii for EPI, MCELL and ENDO respectively. The reduction in the intracellular calcium concentration was accompanied by a consequent reduction in the contractile force compared to WT by ~8% in EPI (Figure 9.12Aiv), MCELL (Figure 9.12Biv) and ENDO (Figure 9.12Civ). A summary of these results is shown in Table 9.14.

9.10 Tissue Simulations

Figure 9.13 shows the results of the electromechanical simulations in an idealised, transmural 2D sheet of the left ventricle for the WT, SQT1, SQT2 and SQT3 conditions without $I_{sac}$ while Figure 9.14 shows simulations with $I_{sac}$ present. The 2D mesh is fixed along the left edge ($X = 0$) in order to avoid rigid body rotation while the unrestricted edges are free to move and have no externally applied force. In a realistic setting, the fixed edge would move in tandem with the ventricular wall.
Figure 9.12 Single cell electromechanical effects of the SQT3 mutation with $I_{sac}$ at $P_{Na} : P_K : P_{Ca} = 1:1:1$.

(Ai,Bi,Ci) WT (black) and SQT3 (red) action potentials in the EPI (Ai), MCELL (Bi) and ENDO (Ci) cells.

(Aii,Bii,Cii) WT (black) and SQT3 (red) calcium concentration in the EPI (Aii), MCELL (Bii) and ENDO (Cii) cells.

(Aiii,Biii,Ciii) WT (black) and SQT3 (red) sarcomere length in the EPI (Aiii), MCELL (Biii) and ENDO (Ciii) cells.

(Aiv,Biv,Civ) WT (black) and SQT3 (red) normalised active force in the EPI (Aiv), MCELL (Biv) and ENDO (Civ) cells.

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<tbody>
<tr>
<td><strong>WT (I_{sac} P_{Na} : P_K : P_{Ca} = 1:1:1)</strong></td>
<td>1.75</td>
<td>1.71</td>
<td>1.75</td>
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<td><strong>SQT3 (I_{sac} P_{Na} : P_K : P_{Ca} = 1:1:1)</strong></td>
<td>1.78</td>
<td>1.75</td>
<td>1.79</td>
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**Force of Contraction (%WT)**

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<tr>
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<tr>
<td><strong>SQT3 (I_{sac} P_{Na} : P_K : P_{Ca} = 1:1:1)</strong></td>
<td>92</td>
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Table 9.14: SQT3 ($I_{sac}$ at $P_{Na} : P_K : P_{Ca} = 1:1:1$) - minimal length of contracted sarcomere with the SQT3 mutation and the consequent active force of contraction relative to WT. Initial sarcomere length is 2.17 µM. The smaller the sarcomere length, the greater is the force of contraction.
The 2D simulations show the electrical wave propagation in the electrophysiology mesh on which is superimposed the mechanics mesh showing deformations induced by the active tension calculated from the electrical wave propagation at 10 ms (Figure 9.13A), 100 ms (Figure 9.13B), 200 ms (Figure 9.13C), 250 ms (Figure 9.13D) and 300 ms (Figure 9.13E). The 2D simulations without $I_{\text{sac}}$ (Figure 9.13) reflect the results in the single cell simulations without $I_{\text{sac}}$ (Figure 9.2-9.4). The stimulus (applied along the left edge of the sheet, $X = 0$) initiated electrical propagation from the ENDO end towards the EPI end of the sheet (Figure 9.13A), and induced deformation of the sheet (Figure 9.13B-E). The induced deformation was strongest for the WT condition (Figure 9.13B-E, top row) but there was extremely weak deformation in SQT1 (Figure 9.13, second row) and SQT2 (Figure 9.13, third row) and some degree of deformation in SQT3 (Figure 9.13, bottom row). In addition, by 300 ms, repolarisation was complete in SQT1 and SQT2 (Figure 9.13E) but was still in progress in WT and SQT3 (Figure 9.13E) due to their longer APDs compared to SQT1 and SQT2.

As the contractile function in SQT patients does not significantly differ from that of control patients [121,437], the 2D simulations with $I_{\text{sac}}$ incorporated were performed with the relative permeability ratio $P_{\text{Na}} : P_{\text{K}} : P_{\text{Ca}} = 1:1:1$ as it had the greater contractile force in the single cell compared to $P_{\text{Na}} : P_{\text{K}} : P_{\text{Ca}} = 1:1:0$ (Figures 9.7-9.12 and Tables 9.9-9.14).

Figure 9.14 shows the effects on tissue deformation with the addition of $I_{\text{sac}}$ to the electromechanics models. There is deformation in all the conditions (Figure 9.14A-E). The vertical displacement of the node at the bottom right-hand corner of the undeformed sheet under all the conditions is indicated with an arrow. At 100 ms, under the WT condition without $I_{\text{sacs}}$, this node was displaced by 0.99 mm but was displaced by 1.20 mm with the incorporation of $I_{\text{sac}}$. In the SQT electromechanics models with the incorporation of $I_{\text{sac}}$, the node was displaced by 0.79 mm (SQT1), 0.79 mm (SQT2) and 0.83 mm (SQT3) at 100 ms. At 200 ms, under the WT condition without $I_{\text{sacs}}$, the node was displaced by 1.27 mm but was displaced by 1.59 mm with the incorporation of $I_{\text{sac}}$. In the SQT electromechanics models with the incorporation of $I_{\text{sac}}$, the node was displaced by 0.95 mm (SQT1), 0.91 mm (SQT2) and 1.15 mm (SQT3) at 200 ms. This results also reflect the single cell electromechanics simulations with $I_{\text{sac}}$ incorporated at a relative permeability ratio $P_{\text{Na}} : P_{\text{K}} : P_{\text{Ca}} = 1:1:1$ (Figures 9.8, 9.10 and 9.12).
Figure 9.13: Electromechanical coupling in 2D ventricular tissue under the SQTS mutations (Results without stretch-activated current, $I_{sat}$).
Snapshots of tissue deformation induced by the superimposed electrical wave propagation in WT, SQT1, SQT2 and SQT3 at 10 ms (A), 100 ms (B), 200 ms (C), 250 ms (D) and 300 ms (E).
Figure 9.14: Electromechanical coupling in 2D ventricular tissue under the SQTS mutations with stretch-activated current, $I_{sac}$.

Snapshots of tissue deformation induced by the superimposed electrical wave propagation at 100 ms and 200 ms in WT without $I_{sac}$ (A), WT with $I_{sac}$ (B), SQT1 with $I_{sac}$ (C), SQT2 with $I_{sac}$ (D) and SQT3 with $I_{sac}$ (E).

The vertical displacement of the node in the bottom right-hand corner of the undeformed sheet is depicted with arrows.
9.11 Discussion

9.11.1 Simulations without $I_{sac}$

Associated with the contraction of the sarcomere is the generated (active) force of contraction. In the single cell simulations without consideration of $I_{sac}$, this was severely impaired in the SQT1 and SQT2 mutations. The force generated was only 30% in EPI, 44% in MCELL and 41% in ENDO in the SQT1 condition. It was only 13% in EPI, 74% in MCELL and 21% in ENDO in the SQT2 condition. The greater force in the MCELL under both conditions is due to its longer APD compared to EPI and ENDO cells. This profound reduction in contractile force is very severe and in a real heart would probably lead to heart failure, instant collapse and death of the patient. The situation was more favourable with the SQT3 mutation. The force of contraction was 76% in EPI, 83% in MCELL and 78% in ENDO. This is because SQT3 acts during terminal repolarisation and thus has longer APDs across the ventricular wall compared to SQT1 and SQT2. A key question that arises is how these modelled changes relate to the real situation in SQTS patients?

At present, there are few experimental data on the effects of the SQTS on ventricular mechanical contraction with which to validate and compare the above findings. Gaita et al. [5] performed echocardiography, cardiac MRI and stress tests on the two families in which the SQTS was first reported [5] and found no structural abnormalities. In a 70-year male patient with the SQT2 mutation, Bellocq et al. [124] also found no structural abnormalities. Tests performed on this patient, including echocardiography, exercise testing, coronary angiography, left and right ventricular angiography, scintigraphy, and ergonovine coronary spasm test showed no abnormalities. In addition, ejection fraction from the left ventricle was 49% [124].

Of particular significance is the finding by Schimpf et al. [437], who in their study to determine whether abbreviation of cardiac repolarisation altered mechanical function in the SQTS found that both SQTS patients and control subjects had no significant differences in end systolic, end diastolic volume and ejection fraction. This particularly calls into question, the severe reduction in contractility in the SQT1 and SQT2 electromechanical simulation results without the incorporation of $I_{sac}$ (Figure 9.2Aiv-
Civ, Figure 9.3Aiv-Civ and Figure 9.13). Nevertheless, the SQTS is characterised by an increased risk of sudden death [5,9,117,118,238].

Simulated AP clamp experiments in which the WT electromechanics model (without $I_{sac}$) was subjected to AP waveforms of different durations (normal and shortened) provided a mechanistic insight into the reason for the profound reduction and effects on contractility. It showed AP shortening to be intrinsically linked to contractile force and hence calcium binding to troponin (which seems to be impaired). Therefore, a possible explanation is that under the SQTS, the severely reduced contractility is most likely due to reduced SR Ca$^{2+}$ loading. AP shortening alters currents and dynamics and provides less time for SR Ca$^{2+}$ loading and therefore SR Ca$^{2+}$ content may be compromised. There would then be reduced SR Ca$^{2+}$ for release and consequently reduced contraction. Thus, calcium binding to the troponin-tropomyosin complex becomes defective, insufficient active ADP sites are uncovered, which in turn affects cross-bridge binding by the myosin filaments to the actin filaments. Consequently, the power stroke required to generate the contraction of the sarcomere becomes weakened.

**9.11.2 Simulations with $I_{sac}$**

The coupling of electrical and mechanical activity in the heart is an active area of research and an important mechanism of electromechanical coupling is the presence of cardiac ion channels activated by mechanical stimuli such as changes in cell volume or cell stretch [387–391]. Consequently, a non-selective cationic stretch-activated current ($I_{sac}$) was incorporated into the electromechanics models. The stretch-activated channel was assumed to be permeable to Na$^{+}$, K$^{+}$ and Ca$^{2+}$. Two relative permeability cases were considered: $P_{Na}:P_{K}:P_{Ca} = 1:1:0$ and $P_{Na}:P_{K}:P_{Ca} = 1:1:1$. 

Incorporation of $I_{sac}$ with both permeability ratios resulted in increased amplitude of the intracellular Ca$^{2+}$, increased shortening of the sarcomere length and consequently greater contractile force under all the conditions; WT, SQT1, SQT2, SQT3 (Tables 9.6, 9.7, 9.9–9.14). These effects are similar to those reported experimentally [5,121,124,436,437]. Permeability ratio $P_{Na}:P_{K}:P_{Ca} = 1:1:1$ had the greater effect on these features. With $I_{sac}$ in the electromechanics models, the contractile force under the SQT variants increased considerably particularly with permeability ratio...
\[ P_{Na} : P_K : P_{Ca} = 1:1:1; \] SQT1 (87% in EPI (from 30% without \( I_{sac} \)), 85% in MCELL (from 44% without \( I_{sac} \)) and 89% in ENDO (from 41% without \( I_{sac} \)) – Table 9.10). Under the SQT2 condition, the contractile force was 79% in EPI (from 13% without \( I_{sac} \)), 91% in MCELL (from 74% without \( I_{sac} \)) and 80% in ENDO (from 21% without \( I_{sac} \)) – Table 9.12). Under the SQT3 condition, the contractile force was 92% in EPI (from 76% without \( I_{sac} \)), 92% in MCELL (from 83% without \( I_{sac} \)) and 92% in ENDO (from 78% without \( I_{sac} \)) – Table 9.14). Hence, it seems that a compensatory mechanism for the profound reduction in contractile force is the presence of stretch-activated channels in cardiac tissue.

Without \( I_{sac} \), there was very little deformation in 2D tissue under the SQT1 and SQT2 conditions (Figure 9.13). However, there was some degree of deformation under the SQT3 mutation because across the ventricular wall, its APD is greater than the SQT1 and SQT2 mutations as it acts during terminal repolarisation. With the incorporation of \( I_{sac} \) at a permeability ratio of \( P_{Na} : P_K : P_{Ca} = 1:1:1 \), there was greater deformation (contraction) in the SQT1-3 conditions (Figure 9.14). With foreknowledge that contractile function in SQT patients is not significantly altered from that of control patients [5,121,124,437], \( I_{sac} \) was incorporated in the tissue simulation at the permeability ratio of \( P_{Na} : P_K : P_{Ca} = 1:1:1 \) as it generates the greater contractile force compared to \( P_{Na} : P_K : P_{Ca} = 1:1:0 \).

The simulations in this chapter constitute a first attempt to link electrical and mechanical systems in the setting of the SQTS. The dissonance between the magnitude of effects predicted from the simulations and the information available on SQTS patients indicates that further work is required. Example areas that need to be investigated are the link between APD and SR loading and myofilament sensitivity to released Ca\(^{2+}\). It is hoped that these simulations form a basis for further investigation and dissemination of relevant experimental data by clinicians, experimentalists and mathematical modellers, out of which, over the next few years, a clearer picture will emerge.
Chapter 10

Discussion and Conclusion

The work presented in this thesis centres on the *in silico* investigation of arrhythmia substrates in the inherited cardiac condition: the short QT syndrome (SQTS). It has focused on the functional consequences of the gene mutations associated with the first three variants of the SQTS; SQT1, SQT2 and SQT3. SQT1 affects the hERG channel, which is responsible for the rapid-delayed outward rectifier potassium current ($I_{K_r}$) \([121–123]\), SQT2 affects the KCNQ1 gene, which encodes the $\alpha$ subunit of channels mediating slow-delayed outward rectifier potassium channel ($I_{K_s}$) \([124]\). SQT3 affects KCNJ2, which encodes the Kir2.1 protein that contributes to inwardly-rectifying potassium channel current ($I_{K_1}$) \([125]\).

In the absence of phenotypically accurate models of the SQTS variants (SQT1 – SQT3), *in silico* models offer the best complementary method to investigating the functional consequences of these and other gene mutations. Consequently, Markov models that reproduced the kinetic properties of the SQT1 and SQT2 mutations were developed while a Hodgkin-Huxley style model was developed for the SQT3 mutation with the same objective. These models were incorporated into the TNNP single cell human ventricular AP model, which was in turn extended into multicellular models. Investigations were carried out at the single cell, 1D, 2D and 3D organ levels with anatomically realistic geometries.

On the basis of these *in silico* investigations, insights into the arrhythmogenic mechanisms of the SQT1-SQT3 mutations were attained, which elucidated the commonalities and differences between these SQTS variants.
10.1 Summary of the Functional Consequences of the SQT1, SQT2 and SQT3 Variants

10.1.1 SQT1

1. The SQT1 mutation abbreviated AP duration. AP shortening occurred regardless of $I_{Kr}$ density distribution across the ventricular wall, i.e., homogeneous or heterogeneous $I_{Kr}$ density distribution. However, in the ventricular TNNP strand model, a heterogeneous distribution of $I_{Kr}$ density across the ventricular wall was found to be necessary to reproduce a tall and peaked T-wave as seen clinically in SQT1. Szabo et al. [75] reported a greater abundance in the expression of hERG/$I_{Kr}$ in the epicardium compared to the midmyocardium in the human left-ventricular wall.

2. With the heterogeneously distributed $I_{Kr}$ density, the SQT1 mutation augmented the membrane potential differences ($\delta V$) between paired ENDO-EPI cells across a left-ventricular strand. In addition, in localised regions of the strand, the SQT1 mutation augmented the transmural dispersion of APD. These augmentations of $\delta V$ and APD served to help increase the T-wave amplitude on the ECG.

3. With the heterogeneously distributed $I_{Kr}$ density, the SQT1 mutation increased the temporal vulnerability of the tissue to unidirectional condition by a premature excitation.

4. The SQT1 mutation substantially reduced the minimal substrate size of tissue required to initiate and facilitate the maintenance of re-entry.

5. In the absence of a stretch-activated current ($I_{sac}$) from the electromechanics model, the SQT1 mutation compromised the binding of calcium to troponin and as a consequence, the contractile force of the left ventricular myocyte was severely impaired. The contractile force was $\sim$38% of WT across the ventricular wall. With the incorporation of $I_{sac}$ at a relative permeability ratio $P_{Na} : P_{K} : P_{Ca} = 1:1:1$, the contractile force was increased to $\sim$87% of WT across the ventricular wall.

These findings substantiate the causal link between the N588K mutation and QT interval shortening and, moreover, provide a comprehensive explanation for increased susceptibility to re-entry and perpetuation of re-entrant arrhythmia in the setting of
SQT1. The present study is the first to determine the arrhythmogenic consequences of the N588K mutation using multi-scale models of the human ventricles.

In an earlier study, Kogan and colleagues [355] used a simple model to demonstrate re-entrant activity when the AP is shortened. They found that slowing the deactivation rate of delayed outward K⁺ current had a profound effect on wave-front propagation. Although the Kogan et al. study is important in that it provides a causal link between augmented K⁺ conductance (via deactivation alteration of deactivation characteristics) and arrhythmogenesis, the SQT1 N588K-hERG mutation is characterised by impaired inactivation not deactivation, and therefore requires targeted simulations that specifically reproduce kinetic changes to I_{Kr} by the SQT1 N588K-hERG mutation.

Prior studies [334,336] have reported inhomogeneous shortening of ventricular APD with loss of I_{Kr} inactivation, which appears paradoxical in light of increased arrhythmia susceptibility in the syndrome. The present study resolves this apparent contradiction: whilst the present study has demonstrated that the AP shortening as a result of N588K mutation is inhomogeneous, (resulting in a decreased APD_{90} and ERP dispersion, as the greatest shortening of APD_{90} occurred in MIDDLE cell APs), with heterogeneous I_{Kr} in the ventricle, the mutation augmented both membrane potential difference between paired ENDO-EPI cells and the APD dispersion in some localised regions of the transmural strand. The present study is the first to incorporate heterogeneous I_{Kr} density in the ventricle [75] for the study of SQT1. These changes lead to an increased T-wave amplitude, which is different to previous simulation results but is consistent with clinical observations [117], [231], [234], [346]. Another consequence of these changes is greater susceptibility of the tissue to unidirectional conduction block in response to a premature excitation stimulus.

The present study also shows that the N588K mutation reduces the minimal tissue size of the substrate required to facilitate and sustain re-entry in both idealised 2D and realistic 2D and 3D geometries. This occurs with either a homogeneous or heterogeneous distribution of I_{Kr} across the ventricular wall. In all tissue models, a single reentrant excitation wave can break up into multiple re-entrant circuits, leading to a transition from tachycardia-like to fibrillation-like electrical excitation waves.
10.1.2 SQT2

1. The SQT2 mutation abbreviated AP duration and steepened the APD-R curve. It consequently shortened the QT interval, increased T wave amplitude and T$_{peak}$ – T$_{end}$ duration, all of which are concordant with clinical observations regarding the SQTS [124,235,237].

2. The SQT2 mutation augmented the δV between paired cells across a left-ventricular strand. In addition, in localised regions of the strand, the mutation augments the transmural dispersion of APD. These augmentations of δV and APD served to help increase the T-wave amplitude on the ECG.

3. The SQT2 mutation increased the temporal vulnerability of the tissue to the genesis of unidirectional conduction by a premature excitation at some localised regions of transmural strand.

4. The SQT2 mutation reduced the minimal tissue substrate size required to initiate and facilitate the maintenance of re-entry and accelerate reentrant excitation waves.

5. In the absence of a stretch-activated current (I$_{sac}$) from the electromechanics model, the SQT2 mutation compromised the binding of calcium to troponin and as a consequence, impaired the contractile force of the left ventricular myocyte. The contractile force was ~36% of WT across the ventricle wall. With the incorporation of I$_{sac}$ at a relative permeability ratio $P_{Na}:P_{K}:P_{Ca}=1:1:1$, the contractile force was increased to ~83% of WT across the ventricular wall.

Just as with SQT1, these findings also provide a causal link between the KCNQ1 SQT2 mutation, QT interval shortening and tachyarrhythmias. They also provide a comprehensive explanation for the increased susceptibility to re-entry and the perpetuation of re-entrant arrhythmia in the SQT2 setting.

The novel SQT2 Markov model developed in this thesis provides greater utility and insight into the arrhythmogenic mechanisms of the mutation. With the Markov model, the state occupancy of the channels during the AP could be monitored. On AP initiation, 2% of the I$_{Ks}$ channels reside in zone 1, which is a fast transition zone to the open state. The remaining 98% reside in zone 2 (a slow transition zone to zone 1), i.e., the channels need to make a slow transition from zone 2 to zone 1 before transitioning to the open
state. In contrast, WT has 100% of its channels residing in zone 2, leading to a delay in WT activation.

The greater occupancy of zone 1 by the SQT2 mutant channels facilitates channel opening and larger $I_{Ks}$ during the AP resulting in AP abbreviation. Open-state accumulation is also greater than in WT due to slower deactivation of the mutant channel. Thus, the primary mechanism for larger $I_{Ks}$ in the SQT2 mutation is greater zone 1 occupancy coupled with slower channel deactivation.

The use of Implantable Cardioverter Defibrillators (ICD) is the current treatment for the SQTS [8], [74], [76], [216], [217]. However, as the SQTS is characterised by tall and peaked T-waves, there is the risk of inappropriate shocks to the patient due to T-wave over-sensing [8], [9], [74], [76], [216]. Additionally, ICDs do not restore the QT interval to its normal duration and are not suitable for all patients, e.g., infants. Therefore, pharmacological alternatives that can restore the normal duration of the QT interval and offer protection from arrhythmias are being actively pursued [74], [76], [218], [219]. Pharmacologically, there is very little available on SQT2 but a recent study by El Harchi et al. [227] found that recombinant $I_{Ks}$ channels incorporating the KCNQ1 V307L mutation were effectively inhibited by mefloquine. Although $I_{Ks}$ is selectively blocked by chromanol compounds such as chromanol 293B [8], [226], [314], its blocking potency was reduced by the KCNQ1 V307L mutation [8], [226].

The present study shows by mimicking drugs that selectively block the $I_{Ks}$ channel in the SQT2 setting that under the WT-V307L heterozygote condition, a blockade of $I_{Ks}$ by ~58% is sufficient to restore the QT interval to its original WT duration. Under the V307L homozygote condition, a blockade of $I_{Ks}$ by ~65% is sufficient to achieve the same result. These QT interval restorations terminate re-entrant activity in the tissue.

10.1.3 SQT3

1. The SQT3 mutation abbreviated AP duration and steepened the APD and ERP restitution curves. It consequently shortened the QT interval and modified the T-wave characteristics on the ECG; the T-wave became peaked, tall and
asymmetric with a slow rise and sharp decline as seen clinically in SQT3 patients [125].

2. The SQT3 mutation increased the transmural APD dispersion across the ventricular wall in different regions of the transmural strand, the effect of which was increased susceptibility of the tissue to premature stimuli.

3. The SQT3 mutation stabilised, accelerated and perpetuated reentry. It reduced the minimal substrate size of tissue required to initiate and facilitate the maintenance of re-entry and accelerated re-entrant excitation waves.

4. In the absence of a stretch-activated current ($I_{sac}$) from the electromechanics model, the SQT3 mutation compromised the binding of calcium to troponin and as a consequence, impaired the contractile force of the left ventricle. The contractile force was $\sim 79\%$ of WT across the ventricle wall. With the incorporation of $I_{sac}$ at a relative permeability ratio $P_{Na} : P_{K} : P_{Ca} = 1:1:1$, the contractile force was increased to $\sim 92\%$ of WT across the ventricular wall.

As with the SQT1 and SQT2 mutations, these characteristics of the KCNJ2 D172N mutation reveal a causal link between the mutation and QT interval shortening, and offer a novel explanation for increased vulnerability of tissue to re-entry and maintenance of re-entrant arrhythmia in the SQT3 setting.

The SQT3 mutation stabilised and accelerated reentry in tissue reflecting its pro-arrhythmic nature. This is consistent with findings in previous studies of the role of $I_{K1}$ in arrhythmogenesis [204,370,376]. There are however, some differences between those studies and mine. In the previous studies, both the outward and inward components of $I_{K1}$ were increased or scaled proportionally, which had the effect of steepening the slope of the $I$-$V$ curve. Consequently, any membrane potential change close to the potassium equilibrium potential ($E_K$) would act in such a way as to shift the resting potential towards $E_K$. Therefore, during high excitation rates, sodium current ($I_{Na}$) recovery from inactivation is increased, thereby helping to stabilise reentry.

In my study, only the outward component of the $I_{K1}$ current is enhanced (the inward component is unaffected) There is thus less effect on the slope of the $I$-$V$ curve and the resting potential was found to be little altered by the D172N mutation. Consequently, the stabilisation of reentry under these conditions was not through increased $I_{Na}$ recovery but via increased tissue excitability at high excitation rates and the shorter ERP.
of the SQT3 mutation. The reduced ERP of the mutation also reduced the wavelength of the reentrant excitation waves thus allowing their activation at higher frequencies and once formed, the waves were stable and persistent.

10.2 Common Mechanisms between the SQT1-3 variants

The three short QT variants (SQT1, SQT2 and SQT3) considered in this thesis share common elements and arrhythmia mechanisms. These are depicted in Figure 10.1. Each mutation is a gain-of-function mutation (SQT1: gain-of-function mutation of the \( I_{Kr} \) channel, SQT2: gain-of-function mutation of the \( I_{Ks} \) channel and SQT3: gain-of-function mutation of the \( I_{K1} \) channel). SQT1 generates excessive \( I_{Kr} \) earlier during the AP, SQT2 produces excessive \( I_{Ks} \) during repolarisation and SQT3 generates excessive outward \( I_{K1} \) during terminal repolarisation. The excessive current leads to a shortening of the APD and ERP in the three mutations with an associated reduction in APD and ERP rate adaptation. The SQT variants shorten the QT interval on the ECG and alter the morphology of the T-wave, which is increased in amplitude, and becomes tall and peaked. All these characteristics make the SQT mutations pro-arrhythmic thus enabling them to stabilise, accelerate and perpetuate reentry.

Figure 10.1: Common mechanisms between SQT1-3 variants.
The increase in the T-wave height is due to the augmentation of the membrane potential differences ($\delta V$) between paired cells across the ventricular wall and amplification of the transmural dispersion of APD in localised regions. The mutations increased the temporal vulnerability of the tissue to the genesis of unidirectional conduction by a premature excitation at some localised regions and reduced the minimal tissue substrate size required to initiate and facilitate the maintenance of re-entry and accelerate re-entrant excitation waves. These augmentations of $\delta V$, transmural dispersion of APD, the increase in the temporal vulnerability window to premature stimulus and the reduction in the minimal substrate size required to initiate re-entry confer on the SQT1-3 mutations a pro-arrhythmic nature.

10.3 Potential Limitations of the Simulations

1. The TNNP human ventricular single cell model was used in the simulations and although it has been suggested to be well-suited to the study of re-entrant arrhythmia [38,305,306] and most of its constituent ion channel kinetic formulations have been derived from experimental data using human ventricular myocytes [439], it still has its shortcomings. For example, because of incomplete experimental data sets on the transmural heterogeneity of human ion channel current densities, incongruities between the simulated transmural APD dispersion and experimentally observed data are known [36,37,438,440]; the ENDO and EPI APD$_{90}$ from the model are very similar; the differences in APD between the MCELL and ENDO and EPI are smaller than those observed experimentally; the APD and APD restitution properties of the model were developed using a general knowledge of the influence of certain model parameters. These limitations are discussed extensively in [38,305].

2. In the multicellular simulations, due to insufficient experimental data regarding the proportion of EPI, MIDDLE and ENDO cell types in the ventricular wall, a cell type proportion was chosen to produce a positive T-wave and also a conduction velocity across the ventricular wall similar to that observed experimentally [337]. Nonetheless, this proportion is similar to that used in other studies [248,334–336].

3. The multicellular models assume the presence of M Cells in the human heart. This is a subject of disagreement and debate in the literature. Taggart et al. [439]
reported no transmural difference in APD in the left ventricular wall while other studies have reported the presence of M Cells in the human heart [36,37,438,440]. The most recent of these studies is that of Glukhov et al. [440] who found M Cells clustered in islands in the deep subendocardium of the human heart.

4. All the multicellular simulations assume a monodomain representation of tissue structure as opposed to a bidomain representation. Previous studies found extremely small differences in the solutions between the monodomain and bidomain models [320,323,441]. Potse et al. [323] found that propagation of activation was only 2% faster in the bidomain model [323] while they all found that the ECGs from both models were visually indistinguishable [320,323,441]. Bourgault and Pierre [326] carried out convergence analyses on both models and reported a relative error in activation time of order 1%. The only situation where the bidomain has been deemed absolutely necessary is in the presence of applied currents, e.g., a defibrillation shock [323].

5. While the 3D human ventricle incorporates a realistic stimulation sequence (tailored to that of the owner of the heart), it does not possess a purkinje fibre network. This may play a role in the genesis of arrhythmia in the SQTS.

6. The models do not consider uncertainty in parameters for a specific human heart, for example, pacing frequency and variations of model parameters, which can play significant roles in ventricular fibrillation genesis.

7. The left-ventricular 2D sheet used for the mechanical simulations is fixed along the left edge in order to avoid rigid body rotation but in a realistic setting, this edge would move in tandem with the ventricular wall. It is also assumed that the active tension force generated by the ventricular cells acts in the x-direction only (which was considered the fibre direction for those simulations). Realistically, the force would act orthotropically in the fibre, sheet and cross-sheet directions.

8. There is no consideration of cardiac mechanics on realistic tissue geometries, which may have important consequences for the genesis and maintenance of reentry. Since repolarisation occurs during tension development, the effect of cardiac mechanics on tissue geometry and stretch-activated channels could be significant. There is also evidence of altered electromechanical coupling in some SQT patients [437]. However, it is notable that electrophysiological changes due to the altered kinetics of $I_{K1}$ by the D172N mutation appear to be sufficient to increase the risk of arrhythmogenesis.
Whilst it is important to enumerate the potential limitations of the models used in these simulations, they do not adversely affect the conclusions that can be drawn on the substrates and the likely mechanisms by which the SQT1, SQT2 and SQT3 mutations facilitate, stabilise and perpetuate arrhythmia.

10.4 Future Developments

10.4.1 Drug Actions

An important extension to the work carried out in this thesis is the investigation of the effects of channel-blocking drugs on the SQTS. Using the SQTS models developed in this thesis, it is possible to investigate or mimic the action of several drugs on the SQT-mutated channel. Three possible questions to investigate would be:

1. Does mimicking drugs that block hERG/IKr, IKs and IK1 terminate re-entrant activity in SQT1, SQT2 and SQT3 respectively?
2. Does blocking one of hERG/IKr, IKs and IK1 affect SQT variants involving the other channel types?
3. To what extent does the mutated channel need to be inhibited to normalise the QT interval?
4. In blocking the channel current, is there a cut-off point or limit at which re-entry is terminated and yet the QT interval is still not normalised? In other words, what is the lower limit of channel block required to terminate reentry?

This is all the more pertinent, given the growing interest in determining if acquired QT interval shortening via drugs (as for drug-induced forms of the LQTS) renders the patient more susceptible to arrhythmia [442,443]. There are currently insufficient data on this issue and no regulatory guidelines regarding drug candidates that result in QT interval shortening.
10.4.2 Mechanical Function

The simulations in Chapter 9 suggest that abbreviated repolarisation alters mechanical function in the SQTS but that there could be compensatory mechanisms such as stretch-activated channels (SACs) which maintain the normal amplitude of the contractile force in SQTS patients. Simulations without stretch-activated current \( I_{\text{sac}} \) profoundly impaired ventricular contraction whereas incorporation of \( I_{\text{sac}} \) maintained contractile force at an adequate level but still below that of WT. The results depend on the presence/absence of SACs in human ventricular myocytes and with SACs, it also likely depends (i) on the magnitude of the current incorporated and (ii) the extent to which \( \text{Ca}^{2+} \) permeability is incorporated into the SAC simulation equations.

However, at present, there are insufficient data (experimental and \textit{in silico}) to reach a definite conclusion. The most notable work on mechanical function in the SQTS is by Schimpf \textit{et al.} [437] in which no significant difference in end systolic volume, end diastolic volume and ejection fraction was seen between control subjects and SQTS patients. It is anticipated that the electromechanics simulation results of the present thesis form a basis for further research by clinicians, experimentalists and mathematical modellers, out of which, over the next few years, a clearer picture will emerge.

10.5 Closing Words

Three novel \textit{in silico} models of the variants of the SQTS that affect potassium channels have been developed; Markov models for SQT1 and SQT2 and a Hodgkin-Huxley model of SQT3. These models have been use to investigate the functional consequences of SQT1-3 in the human ventricle at the single cell, 1D, 2D and 3D levels. The findings in this thesis provide a comprehensive explanation for clinical consequences of these forms of the SQTS in terms of abbreviation of repolarisation and susceptibility to arrhythmia. The multiscale ventricular models developed and employed also have further utility for probing the basis of arrhythmia in other forms of the SQTS and other repolarisation disorders and in the design and investigation of therapeutic interventions for the SQTS.
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