Familial Glucocorticoid Resistance Caused by a Novel Frameshift Glucocorticoid Receptor Mutation

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Context: Familial glucocorticoid resistance is a rare condition with a typical presentation of women with hirsutism and hypertension, with or without hypokalemia.

Objective: The aim was to determine the cause of apparent glucocorticoid resistance in a young woman.

Patients and Methods: We studied a family with a novel glucocorticoid receptor (GR) mutation and a surprisingly mild phenotype. Their discovery resulted from serendipitous measurement of serum cortisol with little biochemical or clinical evidence for either hyperandrogenism or mineralocorticoid excess.

Results: The causative mutation was identified as a frameshift mutation in exon 6. Transformed peripheral blood lymphocytes were generated to analyze GR expression in vitro. Carriers of the mutation had less full-length GR, but the predicted mutant GR protein was not detected. However, this does not exclude expression in vivo, and so the mutant GR (Δ612GR) was expressed in vitro. Simple reporter gene assays suggested that Δ612GR has dominant negative activity. Δ612GR was not subject to ligand-dependent Ser211 phosphorylation or to ligand-dependent degradation. A fluorophore-tagged construct showed that Δ612GR did not translocate to the nucleus in response to ligand and retarded translocation of the wild-type GR. These data suggest that Δ612GR is not capable of binding ligand and exerts dominant negative activity through heterodimerization with wild-type GR.

Conclusion: Therefore, we describe a novel, naturally occurring GR mutation that results in familial glucocorticoid resistance. The mutant GR protein, if expressed in vivo, is predicted to exert dominant negative activity by impairing wild-type GR nuclear translocation. (J Clin Endocrinol Metab 95: E490–E499, 2010)
zyme’s barrier function and so illicitly activate the mineralocorticoid receptor (1).

The syndrome of familial glucocorticoid resistance is rare and has been ascribed to mutations in the glucocorticoid receptor (GR) gene. Inactivating mutations in the ligand binding domain, DNA binding domain, and a splice site mutation have been described (2–15). Familial glucocorticoid resistance may be inherited as an autosomal dominant or recessive trait. Dominant inheritance may be due to dominant negative activity of the expressed mutant GR or to haploinsufficiency (2, 5, 8). However, the heterozygous parents of a child presenting with complete generalized glucocorticoid resistance due to homozygous carriage of a null GR mutation have no endocrine abnormality, suggesting phenotype heterogeneity (15).

We describe a family with glucocorticoid resistance due to a frameshift mutation in the GR gene, which results in expression of a truncated GR protein. The three affected women have a mild phenotype, because indeed the family was only discovered as a result of a serendipitous serum cortisol assay. The mutation introduces a premature stop codon after insertion of 15 novel amino acids. In vitro studies demonstrated that the truncated receptor was unresponsive to ligand, but exerted modest ligand-independent anti-nuclear factor kB (NFkB) activity and template-specific dominant negative action on transactivation by the wild-type GR. The truncated receptor remained cytoplasmic before and after ligand addition and delayed nuclear translocation of the wild-type receptor, suggesting heterodimerization.

Subjects and Methods

Clinical diagnosis of familial glucocorticoid resistance

A 20-yr-old female presented to her primary care physician complaining of fatigue. A 0900 h serum cortisol concentration of 1636 nmol/liter was obtained, raising the possibility of Cushing’s syndrome; hence, she was referred for an endocrine opinion (Table 1). On examination, she had no clinical features of Cushing’s syndrome. Treatment with a combined oral contraceptive pill in the form of Cilest (ethinylestradiol 35 µg/norgestimate 250 µg) was thought to be responsible, but despite stopping Cilest for 6 wk, a repeat 0900 h cortisol remained elevated at 1003 nmol/liter.

After obtaining informed consent, we further investigated the patient (index case), her 18-yr-old sister, and her parents (Table 1). The index’s sister and mother had both received laser treatment for facial hirsutism. The only possible relevant family history was that of facial hirsutism in the deceased maternal grandmother.

The results of a dexamethasone suppression test revealed failure of cortisol suppression in all three women (Table 1), fulfilling the criteria for diagnosis of familial glucocorticoid resistance. Investigations on the father were normal.

The only apparent phenotypical abnormality was mild facial hirsutism in the sister of the index case, as evidenced by a minimally raised Ferriman-Gallwey score. The mother reported previous hirsutism but did not have a raised Ferriman-Gallwey score, possibly as a result of the combined oral contraceptive pill that she had taken for over 10 yr, stopping only 6 wk before investigation.

All three women had raised androstenedione concentrations and minimally elevated calculated free testosterone (Table 1). One sister, the proband, had suppressed renin concentration, despite stopping Cilest for 6 wk, a repeat 0900 h cortisol suppression test revealed a failure of cortisol suppression in all three women (Table 1), fulfilling the criteria for diagnosis of familial glucocorticoid resistance. Investigations on the father were normal.

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### TABLE 1. Biochemical investigations

<table>
<thead>
<tr>
<th>Investigations</th>
<th>Mother</th>
<th>Sister 1 (index)</th>
<th>Sister 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>58.8</td>
<td>55.2</td>
<td>64.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24</td>
<td>23.5</td>
<td>24.5</td>
</tr>
<tr>
<td>Hirsutism score (Ferriman-Gallwey)</td>
<td>3 (&lt;8)</td>
<td>5 (&lt;8)</td>
<td>11 (&lt;8)</td>
</tr>
<tr>
<td>0900 h ACTH (ng/liter)</td>
<td>912 (7–63)</td>
<td>891 (7–63)</td>
<td>48 (7–63)</td>
</tr>
<tr>
<td>0900 h cortisol (nmol/liter)</td>
<td>1126 (176–536)</td>
<td>915 (176–536)</td>
<td>780 (176–536)</td>
</tr>
<tr>
<td>ACTH/cortisol profile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0900 h</td>
<td>33.2/853</td>
<td>43.1/811</td>
<td>24.2/1165</td>
</tr>
<tr>
<td>1100 h</td>
<td>37.9/672</td>
<td>21.8/412</td>
<td>7.2/594</td>
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<tr>
<td>1400 h</td>
<td>28.6/520</td>
<td>31.0/436</td>
<td>17.0/708</td>
</tr>
<tr>
<td>1600 h</td>
<td>22.3/350</td>
<td>19.0/393</td>
<td>22.2/711</td>
</tr>
<tr>
<td>Testosterone (nmol/liter)</td>
<td>1.5 (&lt;2.9)</td>
<td>2.4 (&lt;2.9)</td>
<td>2.7 (&lt;2.9)</td>
</tr>
<tr>
<td>Androstenedione (nmol/liter)</td>
<td>14.1 (4.0–10.2)</td>
<td>16.3 (4.0–10.2)</td>
<td>22.5 (4.0–10.2)</td>
</tr>
<tr>
<td>DHEAS (µmol/liter)</td>
<td>6.0 (0.96–6.95)</td>
<td>5.5 (4.02–11)</td>
<td>9.5 (1.77–9.99)</td>
</tr>
<tr>
<td>17-Hydroxyprogesterone (nmol/liter)</td>
<td>3.4 (&lt;20)</td>
<td>2.6 (&lt;20)</td>
<td>6.3 (&lt;20)</td>
</tr>
<tr>
<td>Urinary free cortisol (nmol/24 h)</td>
<td>212 (&lt;290)</td>
<td>207 (&lt;290)</td>
<td>460 (&lt;290)</td>
</tr>
<tr>
<td>Plasma renin activity (nmol/liter · h)</td>
<td>2.2 (1.5–3.5)</td>
<td>0.8 (1.5–3.5)</td>
<td>1.8 (1.5–3.5)</td>
</tr>
<tr>
<td>Aldosterone (pmol/liter)</td>
<td>285 (100–450)</td>
<td>320 (100–450)</td>
<td>490 (100–450)</td>
</tr>
<tr>
<td>SHBG (nmol/liter)</td>
<td>42 (26.1–110)</td>
<td>83.6 (26.1–110)</td>
<td>33.6 (26.1–110)</td>
</tr>
<tr>
<td>FAI</td>
<td>3.57 (&lt;7.5)</td>
<td>2.87 (&lt;7.5)</td>
<td>8.04 (&lt;7.5)</td>
</tr>
<tr>
<td>Free testosterone (pmol/liter)</td>
<td>23.1 (2.82–21.86)</td>
<td>22.7 (2.82–21.86)</td>
<td>47.8 (2.82–21.86)</td>
</tr>
<tr>
<td>Bioavailable testosterone (nmol/liter)</td>
<td>0.506</td>
<td>0.545</td>
<td>1.17</td>
</tr>
<tr>
<td>1-mg dexamethasone test 0900 h cortisol (nmol/liter)</td>
<td>129 (&lt;50)</td>
<td>272 (&lt;50)</td>
<td>321 (&lt;50)</td>
</tr>
</tbody>
</table>

The characteristics and relevant biochemical investigations for the index family are shown with normal range. Abnormal results are highlighted in bold. DHEAS, dehydroepiandrosterone sulfate; FAI, free androgen index.
raised cortisol, but all three were normotensive and normokalemic, and none were taking any medication (Table 1).

**Research design**

Anti-GR (clone 41) was obtained from BD Biosciences (Oxford, UK); anti-phospho-(Ser111)-GR, from Cell Signaling Technology (Danvers, MA); horseradish peroxidase conjugated antimouse and antirabbit, from GE Healthcare (Buckinghamshire, UK); dexamethasone, from Sigma (Dorset, UK); and mifepristone (RU486), from Sigma. AH3-Luciferase and NRE-luciferase have been previously described (16, 17).

**Amplification of genomic DNA by PCR**

PCR of patient DNA was performed in a final volume of 25 μl containing 500 ng genomic DNA, 50 pmol of each forward and reverse oligonucleotide primer, 0.25 μl of each 25 μM deoxyribonucleotide triphosphate (Bioline, London, UK), 2.4 μl x 10 NH4 buffer (Bioline), 0.75 μl of 50 mM MgCl2 (Bioline), and 1.25 U Taq polymerase (Bioline).

The PCR consisted of 30 cycles, with each cycle made up of a denaturation step of 1 min at 94 C, an annealing step of 1-min gradient between 54 or 60 C, and a primer extension step of 1 min at 72 C. Primer sequences are listed in the Supplemental Data, published on The Endocrine Society’s Journals Online website at http://jcem.endojournals.org. Before the initial cycle, the temperature was increased to 95 C for 4 min; after the final cycle, an extension step of 8 min was added. Each PCR was placed in the gradient at a temperature that corresponded to the annealing temperature of the primers (exon 4, 53 C; exon 7, 55 C; exon 3, 56 C; exon 9, 57 C; exon 8, 58 C; and exon 5, 59 C). Single products were confirmed by agarose gel electrophoresis.

**Sequencing and cloning**

A Quick Spin PCR Purification Kit (QIAGEN, Crawley, UK) was used to purify PCR product according to the manufacturer’s instructions. Sequencing was carried out at the University of Manchester core facility using the corresponding forward or reverse primer with Big Dye Terminator cycle sequencing chemistry (Applied Biosystems, Foster City, CA) and an Applied Biosystems 3730 DNA Analyzer. The obtained sequencing data were analyzed using Applied Biosystems (Foster City, CA) and an Applied Biosystems 3730 DNA Analyzer. The obtained sequencing data were analyzed using Chromas Lite 2.01 (www.techneylism.com/techneo/lite.html). The amplified PCR product of exon 6 was cloned into a pGEM-T–EcoRI (Roche Diagnostics, Burgess Hill, UK) and subcloned into pcDNA3 GR plasmid using a Quick Change Site-Directed Mutagenesis Kit from Stratagene (La Jolla, CA) according to the manufacturer’s instructions (primers: forward, ttgctctggggtggagatatatagacaatcaagtgc; reverse, gcacttgattgtctagctatcgctatcctgccacccagacga).

A fragment spanning the region containing the deletion (C1835) was excised from pcDNA3 Δ612GR using EcoRI (Roche Diagnostics, Burgess Hill, UK) and subcloned into pcDNA3-green fluorescent protein (GFP) using a rapid ligation kit (Roche Diagnostics). Correct orientation was confirmed by DNA sequencing using tiled primers to cover the entire 2.5-kb sequence (primer sequences available on request).

**Cell culture and maintenance**

Human embryonic kidney (HEK) 293 cells (American Type Culture Collection, Tedsington, UK) were cultured in low glucose (1 g/liter) DMEM from PAA Laboratories (Yeovil, UK) with stable glutamine (2 mM; PAA) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen, Paisley, UK) or 10% charcoal dextran stripped fetal calf serum (sFCS; Invitrogen) in a humidified atmosphere of 5% carbon dioxide at 37 C. The human osteosarcoma cell line (U20S; American Type Culture Collection) was cultured in DMEM with stable glutamine 2 mM supplemented with 10% fetal bovine serum or 10% sFCS in a humidified atmosphere of 5% carbon dioxide at 37 C (16, 17).

Lymphocyte cultures were immortalized using Epstein-Barr virus infection in the regional clinical genetics laboratory after full patient consent.

**Immunoblot analysis**

Cell lysates (20 μg protein) in RIPA buffer [50 mM Tris Cl (pH 7.4), 1% Nonidet P-40 (Igepal), 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA] containing protease and phosphatase inhibitors were electrophoresed on Tris/Glycine 4–12% gels (Invitrogen) and transferred to 0.2-μm nitrocellulose membranes (Bio-Rad Laboratories, Hertfordshire, UK) overnight at 4 C. Membranes were blocked for 1 h (0.15 M NaCl, 2% dried milk, 0.1% Tween 20) and incubated with primary antibodies (diluted in blocking buffer) overnight at 4C. After three 10-min washes (88 mM Tris (pH 7.8), 0.25% dried milk, 0.1% Tween 20), membranes were incubated with a species-specific horseradish peroxidase-conjugated secondary antibody (diluted in wash buffer) for 1 h at room temperature, and washed three more times for 10 min each. Immunoreactive proteins were visualized using enhanced chemiluminescence (ECL Advance; GE Healthcare) (16, 17).

**Reporter gene assay**

Cells were cotransfected with 3 μg AH3-luciferase or NFkB-luciferase reporter gene construct together with 0.1 μg cytomegalovirus-Replica luciferase (to correct for transfection efficiency) and either 3 μg full length GR or 3 μg Δ612GR using Fugene 6 (Roche Diagnostics), as described before (16). Twenty-four hours after transfection, cells in DMEM containing sFCS were treated as specified before lysis, then assayed for luciferase activity using a dual-luciferase reporter assay system, following the manufacturer’s instructions (Promega, Southampton, UK) as we have previously described (16–18).

**Immunofluorescence**

After 24 h in DMEM containing sFCS, U20S cells transfected (Fugene 6) with GR-GFP, GR-ASRed, or Δ612GR-GFP were treated as specified. Cells were fixed with 4% paraformaldehyde for 30 min at 4 C, and subsequently the cells were stained with Hoechst (Sigma) in PBS (2 μg/ml) for 20 min at 4 C. After three washes in PBS, coverslips were mounted using Vectamount AQ (Vector Laboratories, Peterborough, UK). Images were acquired on a Delta Vision RT (Applied Precision Inc., Issaquah, WA) restoration microscope using a 40X/0.85 Uplan Apo objective and the Sedat Quad filter set (Chromag 860000v2). The images were collected using a CoolSnap HQ (PhotoMetrics Inc., Huntington Beach, CA) camera with a Z optical spacing of 0.5 μm. Raw images were then deconvolved using the Softworx software, and average intensity projections of these deconvolved images.
were processed using Image J. Images for Hoeschst and GFP were excited with the 405 and 488 nm laser lines, respectively, as previously described (16).

Results

Identification of a GR truncation mutant, Δ612GR

Blood samples were taken from the index case, and genomic DNA was extracted. Exons 2 through to 9 of the GR gene were amplified and sequenced. Analysis revealed that exon 6 did not correlate with the wild-type sequence (Fig. 1A), which was confounded due to heterozygous peaks within the sequence trace indicative of a heterozygous genotype (Fig. 1B). Subcloning of the PCR products into a pGEM-T–Easy vector followed by a second round of sequencing revealed deletion of a single base (C1835), causing a frameshift at amino acid 612 and introducing a stop codon at position 627 (Fig. 1, C and D). The predicted GR truncation lacks a significant portion of the ligand binding domain, with an additional, novel 15-amino acid sequence (Fig. 1D). Site-directed mutagenesis removed C1835 from pcDNA3 GR forming pcDNA3 Δ612GR. U2OS cells, which are deficient in endogenous GR expression, were transfected with Δ612GR to determine whether the mutant GR is produced. In U20S cells transfected with GR, an intense band with the same migration as the endogenous GR was seen, and those cells transfected with Δ612GR had a new protein species with lower apparent

FIG. 1. Sequencing of GR exon 6 with subsequent identification of deletion mutation Δ612GR. A–C, GR exon 6 was amplified from DNA isolated from patient blood samples using PCR. The PCR product was subsequently sequenced. The sequence trace is shown for GR (A) and the initial heterozygous Δ612GR exon 6 (B). C, After cloning into the pGEM–T–Easy vector, the sequence trace of the Δ612GR is shown with the deletion mutation highlighted in red. D, Schematic demonstrating that a receptor truncation is caused by introduction of a stop codon at residue 627. E, U2OS cells were transfected with GR or Δ612GR. After treatment with 100 nM dexamethasone for 1 h, cells were lysed in RIPA buffer containing phosphatase and protease inhibitors and analyzed by immunoblotting for GR abundance and phosphorylation on Ser211 (as indicated). β-Actin was used as a loading control. Wild-type and truncated GR is indicated with arrows. Representative images are shown.
molecular mass (Fig. 1E). After 1-h treatment with 100 nM dexamethasone, there was a significant reduction in steady-state protein expression, an expected consequence of ligand-dependent protein degradation. There was no ligand-dependent reduction in \( H9004 \)612GR expression (Fig. 1E). The wild-type GR undergoes rapid ligand-dependent phosphorylation on multiple residues, but the best defined is Ser211. After dexamethasone treatment, untransfected U2OS or those expressing GR showed enhanced phosphorylation of the GR at Ser211. In contrast, \( H9004 \)612GR showed no change (Fig. 1E). This suggests that \( H9004 \)612GR is not activated by ligand.

**Screening for the \( H9004 \)612GR mutation**

Screening of other family members, including the index case’s sister, mother, father, maternal uncle, and aunt, identified the same mutation in two other subjects, the index’s sister and mother (Fig. 2A). Consistent with the clinical data outlined in Table 1, the father was not affected.

**Steady-state expression of GR and ligand activation**

Blood samples were subsequently taken from the index case, her sister, and both parents, and immortalized lymphocyte cell lines were generated. Cells were lysed and immunoblotted for GR (Fig. 2B). There was no detectable expression of \( \Delta612GR \) in any of the three affected individuals. Quantification of GR expression did reveal that the index, sister, and mother expressed only approximately half the amount of GR compared with the father (Fig. 2C).

Expression of GR was down-regulated by ligand treatment in all family members tested (Fig. 3A), and GR was S211-phosphorylated by ligand (Fig. 3B). Basal phosphorylation was higher in the unaffected father, and the lower molecular weight bands seen are alternatively translated isoforms.

**Glucocorticoid-induced gene regulation by \( \Delta612 \)**

Although we were unable to detect expression of the truncated GR protein isoform in transformed lymphocytes, the protein may be expressed below the limit of detection or in other tissues, and so its function was determined. In GR-deficient HEK293 cells cotransfected with GR and the glucocorticoid-activated mouse mammary tumor virus reporter plasmid (AH3-luc), treatment with dexamethasone induced a significant and robust response, whereas \( \Delta612GR \)-transfected cells were unresponsive (Fig. 4A). In cells cotransfected with GR and \( \Delta612GR \), there was significant inhibition of GR transactivation (Fig. 4B). Similar effects were observed using the partial GR agonist RU486 (Fig. 4, C and D).

Modulation of target gene expression by non-DNA binding or tethering mechanisms is important in physiology. Therefore, \( \Delta612GR \) effects on NFkB-driven gene expression were measured. In cells expressing GR and NRE-luc, treatment with dexamethasone significantly inhibited TNF induction of the NFkB reporter (Fig. 5A). In comparison to GR, \( \Delta612GR \) had no effect (Fig. 5A). Cotransfection of \( \Delta612GR \) with GR had no significant effect when compared with GR-only transfected cells (Fig. 5B). Treatment of GR-expressing cells with RU486 induced significant repression of NFkB (Fig. 5C), whereas RU486 was
without effect in Δ612GR-expressing cells (Fig. 5D). Interestingly, expression of Δ612GR consistently potentiated the TNFα transactivation of an NFκB reporter gene (Fig. 5E).

**Subcellular localization of Δ612**

GFP-tagged GR and Δ612GR were also expressed in U2OS cells and migrated with molecular weights of 30 kDa higher than their untagged counterparts.

![Diagram](https://example.com/diagram.png)
To determine whether Δ612GR was constitutively cytoplasmic, U20S cells were transfected with Δ612GR-GFP. In untreated cells, the GR-GFP localizes predominantly to the cytoplasm (Fig. 6A). Dexamethasone induced near complete nuclear translocation of GR-GFP by 30 min, which was sustained over the 120-min assay period (Fig. 6A). In contrast, transfected Δ612GR-GFP was only observed in the cytoplasm of transfected cells (Fig. 6B).

To determine whether Δ612GR influenced wild-type GR trafficking when coexpressed, U20S cells were cotransfected with Δ612GR-GFP. When coexpressed with GR-GFP, GRAsred was nuclear in 50% of the cells after 10-min treatment with 100 nM dexamethasone (Fig. 6C). Near complete nuclear translocation of GR-Asred was evident by 20 min (Fig. 6C). In contrast, in cells coexpressing Δ612-GFP, dexamethasone induced nuclear translocation of GR-Asred in 50% of the cells after 30 min, whereas near complete nuclear translocation was not evident until 60 min (Fig. 6D).

**Discussion**

Familial glucocorticoid resistance due to mutations in the GR gene is thought to be rare and to present with a distinct clinical phenotype (1, 19). Therefore, it is important that the range of clinical manifestation, as in the kindred reported here, is broadened to include apparently normal women with normal reproductive potential. In the kindred described here, we were unable to document the presence of the mutation in the grandparents of the index case because they were deceased, and her mother’s surviving siblings were mutation negative. However, there was no menstrual irregularity, nor were problems with conception reported by the mother of the index case.
There was clear biochemical evidence of glucocorticoid resistance with raised serum cortisol concentrations after dexamethasone suppression. In addition, androstenedione and testosterone levels were high normal or high. However, these biochemical changes were not accompanied by significant hirsutism, as measured by Ferriman-Gallwey score, supporting the importance of other genetic background effects in determining the phenotype of mild androgen excess in women, which is a frequent clinical observation in women with polycystic ovarian syndrome.

The clinical diagnosis prompted sequencing of the GR gene, which identified a novel mutation in exon 6. This led to a frameshift mutation. This would be predicted to induce nonsense mediated RNA decay, and so result in GR haploinsufficiency. However, haploinsufficiency has previously been reported to be without endocrine phenotype in the parents of the first reported complete glucocorticoid resistance patient (15), or with hirsutism and hypertension in an affected young woman (2), indicating phenotypic heterogeneity. In addition, alternative splicing in the GR 3’ region has previously been reported to result in expression of truncated GR proteins capable of affecting glucocorticoid sensitivity (GR-P, or GRA) (20), and so further analysis was undertaken.

Epstein-Barr-transformed B lymphoblasts were established from the family members to allow measurement of GR protein expression. These studies showed reduced GR protein in the carriers of the mutation, with impaired basal S211 phosphorylation. However, in these studies no truncated GR protein was observed. The process of nonsense-mediated RNA decay is complex, and it relies on cellular expression of key proteins, which may vary between cell types (reviewed in Ref. 21). Indeed, previous studies have shown significant expression of proteins even in the presence of premature stop codons (22). Therefore, the predicted protein product of the mutant allele was expressed, and its function was analyzed.

The predicted GR has 15 novel amino acids and a premature stop codon, giving rise to a 75-kD protein that did not undergo ligand-dependent phosphorylation on Ser211, nuclear translocation, or acute ligand-dependent protein degradation, in contrast to wild-type GR (16, 18, 23). However, the lack of evidence of a ligand-dependent action does not exclude biologically relevant activity. Therefore reporter gene studies were undertaken initially using a transactivation reporter (16, 17).

The Δ612GR did not show any ligand-dependent activation of AH3-luc, as would be predicted based on the disrupted ligand binding domain and lack of evidence of ligand activation of GR. However, Δ612GR consistently inhibited the transactivation seen with wild-type GR. This was an unexpected finding, possibly reflecting a cytoplasmic effect on wild-type GR because Δ612GR was constitutively cytoplasmic. Previous reports suggest that cytoplasmic GR can exert an anti-NFκB effect, possibly through protein kinase A (24, 25). Therefore, the effect of Δ612GR on TNFα-driven NFκB transactivation was measured. There was no ligand-independent inhibition of
NFkB activity and no evidence of ligand-dependent repression. The small inhibition seen at higher ligand concentrations was similar to that seen in vector-transfected cells and is attributable to the low level endogenous expression of GR in these cells.

In contrast to the dominant negative action on wild-type GR seen with the transactivation assay, there was no impact of Δ612GR expression on repression of NFkB by the wild-type GR. Therefore, the effects of Δ612GR are mechanism specific. Δ612GR expression resulted in consistent, significant potentiation of TNFα transactivation of an NFkB reporter gene, suggesting opposition to endogenous limiting factors, but the mechanism and implications remain uncertain.

The dominant negative effects of Δ612GR on wild-type GR transactivation were interesting because previous reports have suggested that the GRβ splice isoform of GR, similarly incapable of binding glucocorticoid agonists, can exert dominant negative effects on GR transactivation (26–32), although this is controversial (33, 34). A further exert dominant negative effects on GR transactivation similarly incapable of binding glucocorticoid agonists, can cations remain uncertain.

In the absence of ligand binding (20). This suggests the presence of modulating activity in exons 5 and 6, possibly by competing with wild-type GR for binding to heat shock protein complexes in the cytoplasm, required for ligand binding competency (18, 35–38). A further point mutation in the GR C terminal has been reported to cause dominant negative activity by interference with coactivator recruitment in the nucleus, not a feasible mechanism for the Δ612GR because it is retained in the cytoplasm (5), and another potential mechanism is regulation of wild-type GR nuclear translocation (19). Indeed, in cotransfection studies, we show that the truncated GR significantly slows the rate of nuclear accumulation in response to ligand, potentially a mechanism for dominant negative action.

In conclusion, we report a family with glucocorticoid resistance due to a novel mutation in exon 6. The phenotype is mild and is intermediate between carriers of ligand binding domain missense mutations, who present with features of androgen excess and hypertension, and carriers of some null or hypofunctioning mutations, who are apparently without any endocrine phenotype (9, 11, 15). The expressed mutant GR exhibited dominant negative activity on wild-type GR, which may explain the difference between the family reported here and that recently described from Australia (15). It is clear that the spectrum of clinical manifestation in heterozygous carriers of deleterious mutations in the GR gene is broad, but it is possible that dominant negative activity of the mutant GR plays a role in clinical manifestation.

Acknowledgments

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This work was supported by GSK (to P.T.), The Wellcome Trust, NIHR Manchester Biomedical Research Centre (to D.R.), The Medical Research Council (to J.B.), and University of Manchester Stepping Stones Fellowship (to L.M.).

Disclosure Summary: The authors have no conflict of interest to declare.

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