CASE REPORT

Generalized glucocorticoid resistance caused by a novel two-nucleotide deletion in the hormone-binding domain of the glucocorticoid receptor gene NR3C1

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Abstract

Objective: Generalized glucocorticoid resistance is characterized by impaired cortisol signaling, resulting from mutations of the glucocorticoid receptor (GR) gene NR3C1. The objective of our study was to identify the causative mutation in a patient with clinical manifestations compatible with generalized glucocorticoid resistance and to determine the functional consequences of the mutation. The possible occurrence of NR3C1 mutations in a selected group of hypertensive subjects with low plasma renin and aldosterone levels was also explored.

Patients: The proband, a male athlete, was diagnosed with hypertension associated with low plasma renin activity and low serum aldosterone concentration at the age of 27 years. Liddle’s syndrome was suspected and the patient was treated with amiloride with initial success. Subsequent examinations revealed elevated serum cortisol and ACTH levels, with resistance to suppression with low doses of dexamethasone. After identification of an NR3C1 mutation in the proband, the available family members and 51 nonrelated hypertensive subjects with low plasma renin and aldosterone concentrations were also studied.

Results: A two-nucleotide deletion in exon 9a, predicted to cause a frameshift mutation (p.L773VfsX25) in the hormone-binding domain of the GR, was identified in the patient in a heterozygous form. Affected brother and father died of premature coronary heart disease. Functional studies in COS-1 cells showed that this mutation eliminates both ligand-binding and transactivation ability of the receptor. No pathogenic NR3C1 mutations were identified in 51 unrelated hypertensive patients with low plasma renin and aldosterone levels.

Conclusion: We identified a novel frameshift mutation in NR3C1 as the cause of glucocorticoid resistance. The mutation eliminates the functional activity of the GR, as studied by in vitro experiments. Mutations in NR3C1 do not seem to be common causes for hypertension with low renin and aldosterone levels.

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Introduction

Generalized glucocorticoid resistance was first described by Vingerhoeds et al. (1976) (1) and by Chrousos et al. (1982) (2) and is caused by impaired cortisol signaling because of target tissue insensitivity to glucocorticoids (1, 2, 3, 4). This defect results in compensatory activation of the hypothalamic–pituitary–adrenal axis, which leads to increased secretion of hypothalamic corticotropin-releasing hormone (CRH) and elevated secretion of the circulating ACTH from the pituitary gland. The excess ACTH secretion, in turn, results in increased secretion of cortisol, the adrenal mineralocorticoids deoxycorticosterone and corticosterone, and adrenal steroids with androgenic activity (5).

The clinical expression of generalized glucocorticoid resistance is variable. Phenotypes commonly associated with generalized glucocorticoid resistance (3, 4) include hypoglycemia, hypertension, and metabolic alkalosis. The chronic fatigue observed in some subjects (6, 7) is believed to be caused by relative glucocorticoid deficiency. In females, overproduction of adrenal androgens has been associated with infertility, male-pattern baldness, hirsutism, and menstrual irregularities. The profound anxiety observed in some of the patients is believed to be caused by the increased CRH and arginine vasopressin secretion. Low renin and aldosterone levels associated with this condition are believed to be caused by inappropriate activation of the mineralocorticoid receptor by the excess cortisol (8).
Established causes for generalized glucocorticoid resistance are mutations in exons 4–9 of the NR3C1 gene encoding the DNA- and the hormone-binding domains of the human glucocorticoid receptor α isoform (hGRα) (3, 4). hGRα functions as a hormone-dependent transcription factor (9, 10) that upon glucocorticoid binding translocates from the cytoplasm to the nucleus and binds to glucocorticoid response elements in regulatory regions of glucocorticoid-responsive genes. The transcriptional activity of hGRα is regulated by another isoform of the GR, the hGRβ isoform, in which a portion of the protein is encoded by exon 9β, instead of exon 9α, of the NR3C1 gene (11).

Today, at least 14 mutations that impair or eliminate the intrinsic activity of hGRα have been identified in NR3C1 of patients with generalized glucocorticoid resistance (1, 2, 6, 7, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26). Eleven of these mutations represent point mutations that substitute amino acids in the DNA- or hormone-binding domain of the hGRα (6, 7, 12, 13, 14, 15, 16, 17, 18, 19). The remaining three mutations, all located in regions of the NR3C1 that encode the hormone-binding domain of the receptor, include a four-nucleotide deletion at the 3′-boundary of exon 6 (24) and two frameshift mutations, i.e. a two-nucleotide deletion in exon 9α (25) and a one-nucleotide deletion in exon 6 (26). Patients with generalized glucocorticoid resistance caused by mutations in the NR3C1 gene may benefit from treatment with high doses of synthetic glucocorticoids that overcome the biochemical defect and lead to suppression of the ACTH secretion (5).

We present here a new case of generalized glucocorticoid resistance associated with hypertension, low renin and low aldosterone levels, and familial occurrence of severe coronary heart disease. It is caused by a frameshift mutation in the hormone-binding domain of the hGR that eliminates ligand-binding and transactivation ability of the receptor.

**Subjects**

**Case report**

The proband, a nonsmoking olympic-level male athlete, was diagnosed with hypertension at the age of 27 years, with repeated measurements indicating high systolic (160–195 mmHg) and diastolic (105–125 mmHg) blood pressure. Subsequent examinations revealed unmeasurably low plasma renin activity (<0.2 μg/l per h, normal range 2–5) and low-normal serum aldosterone (203 pmol/l, normal range 183–940) and low-normal serum potassium (3.6 mmol/l, normal range 3.3–4.9) levels. The 24-h urinary aldosterone level was below the detection limit. Liddle’s syndrome was suspected and therapy with amiloride (2.5–10 mg amiloride hydrochloride daily) was commenced. Combination with a thiazide diuretic (25 mg hydrochlorothiazide daily) normalized the blood pressure. Exon 13 of the SCNN1B and SCNN1G genes encoding the β and γ subunits respectively of the epithelial sodium channel were sequenced without evidence of mutations causing Liddle’s syndrome (27, 28, 29). At subsequent follow-up visits during the following 6 years, during which the patient was treated with varying doses of amiloride and hydrochlorothiazide, his blood pressure was somewhat variable and not always desirable (systolic blood pressure (SBP) 114–158 mmHg and diastolic blood pressure (DBP) 75–104 mmHg). On admission, left ventricular hypertrophy was diagnosed by ultrasonography but was resolved during the antihypertensive treatment. Plasma renin activity (0.6 μg/l per h) and serum aldosterone (<69 pmol/l) remained low and plasma potassium (3.4–3.7 mmol/l) within low-normal levels during follow-up. Serum cholesterol level was 4.1 mmol/l (normal, <5 mmol/l), serum LDL-cholesterol 2.6 mmol/l (normal, <3 mmol/l), and HDL-cholesterol 1.2 mmol/l (normal, >1 mmol/l).

Six years after the initial admission (at the age of 33 years), strikingly high SBP (150–195 mmHg) and DBP (90–123 mmHg) levels were again measured on amiloride and thiazide therapy. An extensive number of examinations were performed, again showing low plasma renin activity (0.4 μg/l per h), low serum aldosterone (<69 pmol/l), and low plasma potassium (3.3 mmol/l) levels. The 24-h urinary aldosterone excretion rate was below the detection limit. A suspicion of glucocorticoid resistance was raised. Subsequent examinations revealed elevated 0800 h serum cortisol (991 nmol/l, normal range 150–650) and plasma ACTH (48 ng/l, normal range <46) levels. There was no hyperpigmentation of the skin. A low dose (1 mg at 1100 h) of dexamethasone failed to suppress 0800 h serum cortisol levels appropriately (results on two separate tests, 239 and 385 nmol/l), while a larger late evening dose (2 mg) of dexamethasone resulted in better but not completely normal suppression of the next-morning cortisol level (55 nmol/l). These findings were compatible with generalized glucocorticoid resistance, which was subsequently verified by DNA analysis that confirmed the presence of a mutation in the NR3C1 gene (see Results section).

Due to death from coronary artery disease of the proband’s father and brother (see below), the proband underwent coronary CT angiography that revealed caliber changes in all main coronary arteries, compatible with the presence of atherosclerosis, although none of the narrowings exceeded 50% of the lumen diameter. Aspirin and simvastatin were started, with subsequent decrease in serum LDL-cholesterol to 1.6 mmol/l.

The brother of the proband aged 38 years was invited to examinations after the diagnosis of glucocorticoid resistance had been established in the proband. He
practiced regular exercise and did not smoke. He was hypertensive but not hyperpigmented and used a combination of enalapril, hydrochlorothiazide, and amiodipine. Endocrinological studies disclosed elevated 0800 h serum cortisol levels (684–727 nmol/l) with plasma ACTH concentration in the normal range (33 ng/l). The urinary free cortisol excretion was 200 nmol/day (normal range <144). Serum potassium was 3.7 mmol/l, and serum aldosterone concentration was very low. Dexamethasone suppressed 0800 h serum cortisol level in a dose-dependent way: 1 mg to 188 nmol/l (insufficient suppression) and 2 mg to 25 nmol/l. Molecular genetic studies confirmed the diagnosis of glucocorticoid resistance (see Results section). Due to left ventricular hypertrophy, dexamethasone suppression therapy was considered justifiable and was instituted. After 2 months, he died unexpectedly. Before his death, a 24-h ambulatory blood pressure recording had revealed normotension with no hypertensive episodes. A forensic autopsy study disclosed coronary artery disease, with severe narrowing of two out of the three main arteries. According to the medical examiner’s statement, the cause of death was coronary artery disease.

The father of the proband, known to be hypertensive, died at the age of 50 years due to acute myocardial infarction. He had smoked 0–5 cigarettes/day and had an LDL-cholesterol level of about 4.5 mmol/l. An autopsy report documented severe calcification and narrowing of coronary arteries and concentric left ventricular hypertrophy. A DNA sample of the father was not available for studies. The mother of the proband was healthy and normotensive, and she was not a carrier of the mutant NR3C1 gene.

The proband has two healthy daughters aged 4 and 2 years. A pediatric consultation recommended DNA testing as a first line of studies. In both cases, the test was negative and other examinations were not performed accordingly.

Patients with hypertension, low plasma renin activity, and low serum aldosterone concentrations

In addition to the proband and his closest relatives, 51 hypertensive patients (20 males and 31 females; age 18–73 years) with low plasma renin activity (mean 0.3 μg/l per h, range 0.1–1.4 μg/l per h) and low serum aldosterone concentrations (mean 116 pmol/l, range 30–284 pmol/l) referred to examinations at the Hypertension Outward Patient Wards of University Hospitals throughout Finland were also studied. No dexamethasone tests were performed in these patients.

The study forms a part of a project on genetic causes of severe hypertension, approved by the Ethics Review Committee of the Department of Medicine, University of Helsinki (reference number 61/1998). In the case of the 51 hypertensive patients, informed consent was obtained for the DNA diagnostics of monogenic forms of hypertension. In the case of the family with glucocorticoid resistance, informed consent for molecular genetic and clinical studies was obtained from the adult members of the family and from the parents of the two children.

Methods

Molecular analysis of the NR3C1 gene

Genomic DNA was extracted from peripheral venous blood according to standard procedures. Exon 13 of the SCN1B and SCN1G genes and protein coding regions of exons 2–9α of the NR3C1 gene and areas flanking these regions were amplified using PCR and sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit and the ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Oligonucleotide sequences, annealing temperatures, and PCR product sizes are listed in Table 1.

Plasmid constructs

The pSG5-hGR expression vector has been described previously (30). The insert contains full-length hGRα cDNA, with 200 nucleotides of the 5′-UTR and 400 nucleotides of the 3′-UTR. The deletion present in the proband (c.2317_2318delCT) was introduced into the hGRα cDNA insert with PCR-assisted site-directed mutagenesis (QuickChange Lightning Site-Directed Mutagenesis, Stratagene, La Jolla, CA, USA) according to the manufacturer’s instructions with the following primers: 5′-GGAAATATCAAAAAACTTGTTCATCAA-AAGTGACTG-3′ and 5′-CAGTCACCTTTGATGAAA-CAAGTTTTTGATATTTCC-3′. After mutagenesis, the complete cDNA insert was sequenced to ensure that the mutation was introduced to the correct position. Oligonucleotide sequences and PCR conditions are available on request.

Cell culture and transfections

COS-1 cells, devoid of endogenous GR gene expression, were grown in DMEM supplemented with 10% fetal bovine serum (FBS), 25 U/ml penicillin, and 25 U/ml streptomycin in a humidified atmosphere of 5% CO2 at 37 °C. The cells were seeded onto six-well plates (200 000 cells/well for quantitative RT-PCR, immunoblotting and dexamethasone-binding assay) or 12-well plates (50 000 cells/well for transactivation assay) 24 h before transfection. For GR transactivation and dexamethasone-binding assays, culture medium was replaced with DMEM containing 10% charcoal-stripped FBS 4 h before transfection. Transfections were carried out with FuGENE 6 transfection reagent (Roche Applied Science), according to the manufacturer’s instructions.
Table 1 Oligonucleotide sequences, annealing temperatures, and fragment sizes used for amplification and sequencing of coding regions of exons 4–9 of the NR3C1 gene and exon 13 of the SCNN1B and SCNN1G genes.

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Oligonucleotide sequences</th>
<th>Annealing temperature (°C)</th>
<th>Fragment size</th>
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<tr>
<td>1. NR3C1 exon 1F</td>
<td>5'-TCGGATCGAGAATATGGTG-3'</td>
<td>57</td>
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<td>2. NR3C1 exon 1R</td>
<td>5'-TGCGAGCTTGGGGTCT-3'</td>
<td>57</td>
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<td>3. NR3C1 exon 2F</td>
<td>5'-TGGAGAAGAGCTTGGGACA-3'</td>
<td>57</td>
<td>586</td>
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<tr>
<td>4. NR3C1 exon 2R</td>
<td>5'-ACTTGGGCAGTATCTTTAGGGCCTGCT-3'</td>
<td>57</td>
<td>471</td>
</tr>
<tr>
<td>5. NR3C1 exon 3R</td>
<td>5'-AAATCTCTTCTGACCATGAGAG-3'</td>
<td>57</td>
<td>404</td>
</tr>
<tr>
<td>6. NR3C1 exon 4F</td>
<td>5'-ACCGGAAAACAGAGAAGAGG-3'</td>
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<td>292</td>
</tr>
<tr>
<td>7. NR3C1 exon 4R</td>
<td>5'-TTTGCTGGAACACACGTTTC-3'</td>
<td>57</td>
<td>397</td>
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<tr>
<td>8. NR3C1 exon 5F</td>
<td>5'-GGCAATTCGAGACAGAGTG-3'</td>
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<td>9. NR3C1 exon 5R</td>
<td>5'-GGAAAAATCAGCTGTTAAAAGGAG-3'</td>
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<td>10. NR3C1 exon 6F</td>
<td>5'-CCAAGTGCAGGAAAGTTGAA-3'</td>
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<td>11. NR3C1 exon 6R</td>
<td>5'-TTTGGAGAGGGCCACTAAACCTT-3'</td>
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<td>12. NR3C1 exon 7F</td>
<td>5'-TCGAATCTCCTCCAAGGAAATTTCA-3'</td>
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<td>13. NR3C1 exon 7R</td>
<td>5'-CTGTGTAGCGTCTTTAACTTCC-3'</td>
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<tr>
<td>14. NR3C1 exon 8F</td>
<td>5'-CTTTGGTTCTTCCATGATGCT-3'</td>
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<tr>
<td>15. NR3C1 exon 8R</td>
<td>5'-GGAACACTCCAGGGCGCC-3'</td>
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<td>16. NR3C1 exon 9F</td>
<td>5'-GGAAGGAAACTCCAGCCA-3'</td>
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<td>17. NR3C1 exon 9R</td>
<td>5'-TTAAGCTTTTCTAAGGCGGGACG-3'</td>
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<td>18. SCNN1B exon 13F</td>
<td>5'-GCCTGGTCTCTCTCTCACCCAGG-3'</td>
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<td>19. SCNN1B exon 13R</td>
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<td>20. SCNN1G exon 13F</td>
<td>5'-GCCTGGTCTCTCTCTCACCCAGG-3'</td>
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<td>21. SCNN1G exon 13R</td>
<td>5'-TTTGGGAGAGGCCACTAAACCTT-3'</td>
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**Immunoblot analysis**

COS-1 cells transfected with 2 µg of the wild-type or the c.2317_2318delCT hGRα plasmid or a control plasmid (vector without hGRα cDNA insert) were washed with PBS and PBS supplemented with 20 nM protease inhibitor cocktail (PIC; Roche Applied Science). Cells were transfected into tubes and centrifuged at 15 000 g for 30 s. The pellets were resuspended into radioimmunoprecipitation assay (RIPA-2) buffer supplemented with a dilution of 1:100 of PIC, 10 mM N-ethylmaleimide (Sigma–Aldrich), and 1 mM dithiothreitol, incubated on ice for 10 min, and cleared by centrifugation. Samples were boiled in Laemmli buffer for 5 min at 95 °C, resolved by SDS–PAGE, and transferred onto nitrocellulose membranes for immunoblot analysis. A rabbit polyclonal antiglucocorticoid receptor antibody (ab3579) (Abcam, Cambridge, UK) was used to detect the GR. α-Tubulin, detected by a mouse monoclonal α-tubulin antibody (sc-5286) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), was used as loading control. Goat polyclonal anti-mouse IgG antibody (178–2504) (Serotec, Düsseldorf, Germany) was used as the secondary antibody.

**Quantitative RT-PCR (qRT-PCR)**

Total RNA was isolated from COS-1 cells transfected with 2 µg of the wild-type or the c.2317_2318delCT hGRα plasmid or control (vector without hGRα cDNA insert) using Nucleospin RNA II kit (Macherey-Nagel GmbH, Düren, Germany) according to the manufacturer’s instructions. cDNA was synthesized from 2 µg of total RNA using oligo-dT20 primers and the iScript Select cDNA synthesis kit (Bio-Rad). qRT-PCR was performed with LightCycler 480 Real-Time PCR System (Roche Diagnostics) in 20 µl reactions containing 10 µl LightCycler 480 SYBR Green I Master (Roche Diagnostics) and the following primers at a final concentration of 1 µM: 5'-GAAAGGAAAATCCAGCCAGGATCAGGAAGAAGCAGG-3' and 5'-TGTTTGGAAGCAATAGTTAAGGA-3'. PCR started with 5 min at 95 °C followed by 40 cycles of 10 s at 95 °C, 5 s at 60 °C, 10 s at 72 °C, and 5 s SYBR Green signaling measurement at 72 °C. hGRα mRNA levels were normalized using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels.

**GR transactivation assay**

COS-1 cells were transfected with 30 ng of the wild-type or the c.2317_2318delCT hGRα plasmid. In addition, to determine whether the mutated receptor exerts a dominant negative effect on the wild-type receptor, COS-1 cells were cotransfected with 30 ng of wild-type and 30 ng c.2317_2318delCT hGRα plasmids. For each transfection, 50 ng β-galactosidase expression plasmid (pCMVβ; Clontech Laboratories, Inc.), used to monitor transfection efficacy, and 200 ng of the pMMTV-Luc reporter construct (Promega), used as the reporter in transactivation assays, were cotransfected. Transfected cells received a fresh medium containing 2% charcoal-stripped FBS and 100 nM dexamethasone (Sigma–Aldrich) or vehicle (ethanol) 24 h after transfection.
After a 24-h cultivation, the cells were harvested and LUC and β-galactosidase activities were determined as described previously (31).

Dexamethasone-binding assay

COS-1 cells were transfected with 2 μg of the wild-type or the c.2317_2318delCT hGRα plasmid. Nontransfected COS-1 cells were used as controls. After receiving a fresh medium containing 10% charcoal-stripped FBS, the cells were treated with DMEM containing 100 nM [1,2,4,6,7-3H]dexamethasone (Amersham) in the presence or absence of a 100-fold molar excess of nonradioactive dexamethasone for 1 h at 37°C. To remove unbound steroid, the cells were washed three times with PBS supplemented with 1% BSA (Sigma–Aldrich). Cells were transferred into tubes and dexamethasone was extracted using 1 ml absolute ethanol. Samples were centrifuged at 15,000 g for 1 min and the supernatant was recovered. The extraction was repeated once, and the supernatants were transferred into scintillation vials containing 10 ml Optiphase Hisafe 3 (PerkinElmer, Waltham, MA, USA). The radioactivity was measured using the Liquid Scintillation Counter 1409 (Wallac, Turku, Finland).

Results

Mutation analysis of the NR3C1 gene

Sequencing of the protein coding regions of exons 2–9α of NR3C1 and the areas flanking these regions revealed a two-nucleotide deletion at positions 2317 and 2318 (c.2317_2318delCT or g.121423_121424, reference sequence GenBank ID: X032235) in exon 9α, present in a heterozygous form in the proband (Fig. 1A). This deletion represents a frameshift mutation (p.L773VfsX25) in the wild-type sequence, with the deleted nucleotides indicated with a box, has been included (bottom). For comparative reasons, a wild-type sequence, with the deleted nucleotides indicated with a box, has been included (bottom). The glucocorticoid receptor, leading to substitution of the last five amino acid residues at codons 773–777, loss of the stop codon at position 778, addition of 19 amino acids to the carboxyl terminus and a new stop codon (*) at position 797. The substituted and added amino acids are highlighted in the figure (top). For comparative reasons, a wild-type sequence, with the deleted nucleotides indicated with a box, has been included (bottom).
Expression studies of the p.L773VfsX25 receptor  

Immunoblot analysis confirmed expression of both wild-type and p.L773VfsX25 hGRα in COS-1 cells transfected with the corresponding plasmids. The three hGRα isoforms seemed to be expressed to a slightly lower level for the mutated receptor than the corresponding wild-type hGRα isoforms (hGRα-A, 94 kDa; hGRα-B, 91 kDa; and hGRα-C, 82 kDa), resulting from utilization of different N-terminal methionines for translation initiation (11) (Fig. 2), perhaps due to decreased stability of the p.L773VfsX25 hGRα protein. We were unable to detect differences in mRNA expression between wild-type and mutant hGRα. The mRNA expression level of the mutant hGRα in relation to that of wild-type hGRα was 0.98 ± 0.16 (mean ± s.d.; n = 6). The experiment was repeated twice with similar results.

Transactivation ability of the p.L773VfsX25 receptor  

In order to study the transactivation properties of the mutant hGRα, COS-1 cells were transfected with the wild-type or the c.2317_2318delCT hGRα plasmid, alone or in combination, along with the pMMTV-LUC reporter construct. The presence of p.L773VfsX25 mutation in the receptor eliminated the 16-fold increase in dexamethasone-induced luciferase activity observed in cells transfected with the wild-type receptor construct (Fig. 3). To study whether the p.L773VfsX25 mutant acts as a dominant negative receptor, wild-type and p.L773VfsX25 plasmids were transfected together into COS-1 cells. Cotransfection of an equal amount (not shown) of the mutant receptor failed to influence transactivation ability of wild-type hGRα, suggesting that the p.L773VfsX25 mutant does not display significant dominant negative action on the wild-type receptor.

Ligand-binding ability of the p.L773VfsX25 receptor  

The ligand-binding ability of the mutant p.L773VfsX25 hGRα receptor was compared with that of the wild-type receptor using a whole-cell binding assay in COS-1 and a saturating concentration of 3H-dexamethasone, without (to measure total binding) or with (to measure nonspecific binding) the presence of a 100 molar excess of nonradioactive dexamethasone. Nontransfected COS-1 cells showed little if any specific 3H-dexamethasone binding (Fig. 4). In contrast to the wild-type hGRα, the p.L773VfsX25 mutant did not display any specific dexamethasone binding (Fig. 4).

Discussion  

We present here a new case of generalized glucocorticoid resistance associated with hypertension, low plasma renin activity, low serum aldosterone concentration, and a family history of severe coronary heart disease. Glucocorticoid resistance in our patient was caused by a heterozygous frameshift mutation in the hormone-binding domain of hGRα. Our functional studies in cultured cells indicate that this mutation eliminates both ligand-binding and transactivation ability of the receptor. Mutations in the hormone or the DNA-binding domain of hGRα do not seem to constitute a common cause for hypertension with low renin plasma and aldosterone levels in Finland, at least as judged by the absence of these mutations in the protein-coding regions of the NR3C1 gene of our 51 hypertensive patients with this phenotype.

Clinical presentation of generalized glucocorticoid resistance is variable (3, 4), ranging from mild (26) to severe (25), up to potentially lethal forms. Phenotypes may vary even among patients and relatives with the same disease-causing mutation (18, 20, 26). The characteristically low plasma renin and aldosterone levels in glucocorticoid resistance have been ascribed to the high cortisol level. Excess cortisol is proposed to saturate the enzyme 11β-hydroxysteroid dehydrogenase type 2 (HSD11B2) (8) that under normal circumstances converts cortisol into cortisone, thus preventing cortisol binding to the mineralocorticoid receptor and assuring an aldosterone-specific regulation of this receptor. In glucocorticoid resistance,
inappropriate binding of cortisol to the mineralocorticoid receptor causes increased sodium reabsorption and extracellular volume expansion, resulting in low renin and aldosterone levels and contributing to the elevated blood pressure.

At least 14 mutations in \textit{NR3C1} in regions that encode the DNA- or hormone-binding domain of hGR\textsubscript{α} have been previously reported in patients with generalized glucocorticoid resistance (1, 2, 6, 7, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26). Eleven of these mutations represent amino acid substituting point mutations in exons 4, 5, 7, 8, and 9\textsubscript{α}. Three of them were homozygous from, this mutation was found to be a heterozygous deletion of the second and third nucleotides of exon 6 (24) and two frameshift mutations, i.e. a heterozygous one-nucleotide deletion in exon 9\textsubscript{α} (25). The deletion in exon 9\textsubscript{α} (25) and a homozygous and a heterozygous amino acid substituting mutation, i.e. p.V571A (16) and p.R714Q (17) respectively, were associated with a particularly severe form of glucocorticoid resistance.

These patients were diagnosed under the age of 10 (16, 17, 25) and had a number of endocrine or metabolic problems, including GH deficiency, profound hypoglycemia (25), generalized seizures, premature pubarche (17), and pseudohermaphroditism (16). In two of these cases, the glucocorticoid resistance was caused by homozygous mutations associated with a complete (25) or severe (16) loss of the hormone-binding ability of hGR\textsubscript{α}.

One of the more severe forms of glucocorticoid resistance recently reported by McMahon \textit{et al.} (25) is caused by a hGR\textsubscript{α} mutation that is very similar to that of our study. While our mutation represents a heterozygous deletion of the first and second nucleotides of codon 773, the mutation described by McMahon \textit{et al.} was a homozygous deletion of the second and third nucleotides of the same codon. When present in a homozygous from, this mutation was found to be associated with severe neonatal glucocorticoid resistance and GH deficiency (25). The mutations described both by us and by McMahon are predicted to result in hGRs with similar elongated carboxy termini, differing only by the nature of the amino acid at codon 773. In fact, our \textit{in vitro} functional studies are in line with those performed by McMahon \textit{et al.} (25). By studying dexamethasone-induced suppression of interleukin-6 (IL6) expression, they demonstrated that the lymphocytes of the patients were insensitive to dexamethasone. In addition, the mutant receptor had lost dexamethasone-dependent transactivation ability and was unable

![](https://www.eje-online.org/data/figs/fig3.png)

**Figure 3** Transactivation activity of the wild-type and mutated GR\textsubscript{α}. The mean luciferase activity is expressed as relative light units (RLU) \(\pm\) s.e.m. of three independent transfections. The cells were treated with 100 nM dexamethasone (Dex (+)) or vehicle (ethanol) (Dex (−)). A significant difference was observed in the dexamethasone-mediated luciferase activity between cells with p.L773VsX25 hGR\textsubscript{α} and cells with wild-type hGR\textsubscript{α} (Student’s \(t\)-test, \(P = 0.003\)). A 16-fold increase in dexamethasone-mediated mean luciferase activity was observed in cells with wild-type hGR\textsubscript{α} whereas dexamethasone did not affect the luciferase activity in cells with p.L773VsX25 hGR\textsubscript{α}. No difference was observed between cells with wild-type hGR\textsubscript{α} and cells with equal amounts of both wild-type and p.L773VsX25 hGR\textsubscript{α}. The experiment was repeated twice with identical results.

![](https://www.eje-online.org/data/figs/fig4.png)

**Figure 4** \(^{3}\text{H}\)-dexamethasone binding in COS-1 cells. The cells were transfected with the mutant or wild-type hGR\textsubscript{α} expression vectors, and the steroid-binding activity is expressed as disintegrations per minute (dpm) \(\pm\) s.d. of three independent transfections. The cells were treated with 100 nM radioactive dexamethasone \(^{3}\text{H}\)-Dex) or, to determine nonspecific binding, with 100 nM radioactive dexamethasone in the presence of a 100-fold molar excess of nonradioactive dexamethasone \(^{3}\text{H}\)-Dex + 100×Dex). Control corresponds to COS-1 cells not transfected with GR expression vectors.
to bind dexamethasone (25). Compared with the homozygous patient and controls, heterozygous relatives showed intermediate dexamethasone-induced suppression on IL6 expression and intermediate ligand-binding ability (25). All these data are in accordance with our functional studies, and considering the fact that our p.L773VfsX25 mutation does not appear to exert a dominant negative effect on the wild-type receptor, our heterozygous patients probably expressed half the amount of normal functional hGRα. It is of note that the homozygous frameshift mutation causing the severe form of neonatal complete glucocorticoid resistance is the only one that thus far has been associated with a completely abolished hGRα activity in a patient (25).

In addition to these two deletions ((25) and this study), a missense mutation substituting the second thymine of codon 773 to cytosine has been identified in a heterozygous patient with a severe phenotype of glucocorticoid resistance (7). This mutation results in the substitution of a single amino acid, i.e. proline for leucine, at this position (7). This region of hGRα appears to be important for the ligand-induced conformational change in helix 12 of the receptor, which is required for a proper interaction between the receptor and a coactivator, such as GRIP1 (7). Considering the rare nature of glucocorticoid resistance, the occurrence of three disease-causing mutations at the exactly same codon is striking and supports the idea that this DNA region represents a mutation hot spot for unknown reasons.

The mechanisms by which a heterozygous mutation, i.e. decrease in biologically active receptor level by one-half, causes generalized glucocorticoid resistance are not known. Similar phenotypic presentations have not been described for other steroid receptors (estrogen, progesterone, and mineralocorticosteroid), the genes of which are located on autosomal chromosomes. Our cell culture experiments failed to show any dominant negative regulation by the mutant receptor of the wild-type GRα activity. However, we cannot formally exclude the possibility that it would not happen in vivo, for example, due to aberrant activation of the mutant receptor. Nevertheless, the genes or genetic networks affected by GR haploinsufficiency must require high loading of the glucocorticoid-occupied receptor onto the regulatory regions and be, therefore, in relative terms, less sensitive to glucocorticoids. This concept is supported by the finding that the relative glucocorticoid resistance in our patient – lack of suppression of cortisol secretion by a low dose of dexamethasone – was overcome by a higher dose of dexamethasone. As formation of dexamethasone–GR complex follows the law of mass action, the higher steroid dose supports the formation of a higher amount of glucocorticoid–GR complexes.

A striking feature in the family that we studied was the early-onset severe coronary artery disease. While there is paucity of information on the possible association of generalized glucocorticoid resistance with heart disease, there is some evidence that population-prevalent polymorphisms of hGRα may play a role in this respect. A number of common hGRα polymorphisms, including the ER22/23EK, Bcl1, GR-9-β, and N363S polymorphisms (for review see (32)), have been identified, and their possible relationship to metabolic and inflammatory diseases have been explored in a large number of studies, with somewhat conflicting results (reviewed in (32)). It is of interest, however, that a recent very large population-based study by van den Akker et al. (33) reported a twofold increased risk of myocardial infarction and an almost threefold risk of coronary artery disease in subjects homozygous for the haplotype 3, determined by a 3′-UTR variation of the GR. The same haplotype was also associated with a 1.4-fold risk of cardiovascular disease in men with familial hypercholesterolemia, a disease as such entailing an elevated risk of vascular disease (34). Manenschijn et al. (32) speculate that this haplotype may lead to higher risk of cardiovascular disease, due to a relative glucocorticoid resistance and resulting lifelong defect in glucocorticoid suppression of the proinflammatory system.

In conclusion, we present here a new case of generalized glucocorticoid resistance associated with hypertension, low plasma renin activity, low serum aldosterone concentration, and a familial occurrence of severe coronary heart disease. It is caused by a heterozygous frameshift mutation in the hormone-binding domain of hGRα that eliminates the functional activity of the GR in vitro.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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