Familial/Sporadic Glucocorticoid Resistance

Authors: Dr Evangelia Charmandari¹, Dr Tomoshige Kino and Prof George P. Chrousos
Creation date: February 2004

Scientific editor: Prof Sebastiano Filetti

¹Pediatric and Reproductive Endocrinology Branch, National Institutes of Health, Building 10, Room 9D42, 10 Center Drive MSC 1583, Bethesda, MD 20892-1583, U.S.A. charmane@mail.nih.gov

Abstract
Glucocorticoids regulate a variety of biologic processes and exert profound influences on many physiologic functions. Their actions are mediated by the glucocorticoid receptor (GR), a ligand-dependent transcription factor. Glucocorticoid resistance is a rare condition characterized by generalized, partial, target tissue resistance to glucocorticoids. Compensatory elevations in circulating adrenocorticotropic hormone (ACTH) concentrations lead to increased production of adrenal steroids with mineralocorticoid and/or androgenic activity, and increased urinary free cortisol excretion without any clinical evidence of hypercortisolism. The clinical spectrum of the condition is broad, ranging from asymptomatic to severe cases of hyperandrogenism, fatigue and/or mineralocorticoid excess. The molecular basis of glucocorticoid resistance has been ascribed to mutations in the GR gene, which impair glucocorticoid signal transduction and alter tissue sensitivity to glucocorticoids. The study of functional defects of natural hGR mutants enhances our understanding of the molecular mechanisms of hGR action and highlights the importance of integrated cellular and molecular signalling mechanisms for maintaining homeostasis and preserving normal physiology.

Keywords
Glucocorticoid receptor (GR); Glucocorticoid resistance; Tissue sensitivity to glucocorticoids; Mutations in the hGR gene

Introduction
Glucocorticoids are steroid hormones synthesized and secreted by the adrenal cortex. They regulate a variety of physiologic functions and play an important role in maintaining basal and stress-related homeostasis (1-4). At the cellular level, the actions of glucocorticoids are mediated by the glucocorticoid receptor (GR), which belongs to the nuclear receptor family of ligand-dependent transcription factors. The present review focuses on the mechanisms of GR action, and the clinical manifestations and molecular mechanisms of familial/sporadic glucocorticoid resistance.

Background: Molecular Mechanisms of Glucocorticoid Action

Definition
The clinical syndrome of familial/sporadic glucocorticoid resistance is characterized by increased cortisol secretion without clinical evidence of hyper- or hypocortisolism, and...
manifestations of androgen and/or mineralocorticoid excess i.e. absence of stigmata of Cushing's syndrome. This condition results from partial failure of the glucocorticoid receptor (GR) to modulate transcription of its target genes.

Clinical Manifestations
Glucocorticoid resistance is a rare, familial or sporadic condition characterized by generalized, partial end-organ insensitivity to glucocorticoids (5-12). Affected subjects have compensatory elevations in circulating cortisol and adrenocorticotropic hormone (ACTH) concentrations, but no clinical evidence of hypercortisolism. However, the excess ACTH secretion results in increased production of adrenal steroids with mineralocorticoid activity, such as cortisol, deoxycorticosterone and corticosterone, and/or androgenic activity, such as androstenedione, dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS) (5-12). The former accounts for symptoms and signs of mineralocorticoid excess, such as hypertension and/or hypokalemic alkalosis. The latter accounts for manifestations of androgen excess, such as acne, hirsutism and infertility in both sexes, male-pattern hair-loss, menstrual irregularities (oligo-amenorrhea) and oligo-anovulation in females, and oligospermia and infertility in males. In children, the early and excessive prepubertal adrenal androgen secretion has been associated with ambiguous genitalia at birth and precocious puberty. The clinical spectrum of the disease is broad, ranging from completely asymptomatic to severe cases of hyperandrogenism, fatigue and/or mineralocorticoid excess (7-12) (Table 1). Fatigue has been considered a result of cortisol deficiency in tissues such as the central nervous system and the skeletal muscles.

Diagnosis
Increased serum cortisol concentrations and 24-hour urinary free cortisol excretion in the absence of clinical features of hypercortisolism are highly suggestive of the condition. The plasma concentrations of ACTH may be normal or high. The circadian pattern of ACTH and cortisol secretion and their responsiveness to stressors are preserved, albeit at higher concentrations, and there is resistance of the HPA axis to dexamethasone suppression. Thymidine incorporation and dexamethasone binding assays on peripheral blood mononuclear cells or cultured skin fibroblasts, as well as sequencing of genomic DNA or complementary DNA may be necessary to confirm the diagnosis (Table 1).

Table 1: Clinical Manifestations and Diagnostic Evaluation of Syndromes of Glucocorticoid Resistance

<table>
<thead>
<tr>
<th>Clinical Presentation</th>
<th>Diagnostic Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparently normal glucocorticoid function</td>
<td>Absence of clinical features of Cushing syndrome</td>
</tr>
<tr>
<td>Hypokalemic alkalosis</td>
<td>Normal or elevated plasma ACTH concentrations</td>
</tr>
<tr>
<td>Androgen excess</td>
<td>Elevated plasma cortisol concentrations, increased 24-hour urinary free cortisol excretion</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Maintenance of a normal circadian and stress-induced pattern of cortisol and ACTH secretion</td>
</tr>
<tr>
<td>Mineralocorticoid excess</td>
<td>Resistance of the HPA axis* to dexamethasone suppression</td>
</tr>
<tr>
<td>Acne, hirsutism, oligospermia and infertility, precocious puberty</td>
<td></td>
</tr>
<tr>
<td>Females: Ambiguous genitalia at birth, acne, hirsutism, male-pattern hair loss, menstrual irregularities, oligo-anovulation, infertility, precocious puberty</td>
<td></td>
</tr>
<tr>
<td>Males: Acne, hirsutism, oligospermia, infertility, precocious puberty</td>
<td></td>
</tr>
</tbody>
</table>

* HPA = Hypothalamic-pituitary-adrenal


Differential Diagnosis
The differential diagnosis includes:
1- mild forms of Cushing's syndrome, in which hypercortisolism is accompanied by normal or mildly elevated ACTH concentrations, preserved circadian pattern of ACTH and cortisol secretion, and lack of cortisol suppression by dexamethasone;
2- pseudocushing’s states, such as generalized anxiety disorder and melancholic depression;
3- conditions associated with elevated serum concentrations of cortisol-binding globulin;
4- other causes of mineralocorticoid-induced hypertension;
5- other causes of hyperandrogenism or virilization, such as idiopathic hirsutism, polycystic ovarian syndrome and congenital adrenal hyperplasia.

Treatment
The aim of treatment in glucocorticoid resistance is to suppress the excessive secretion of ACTH and, therefore, the increased production of...
mineralocorticoids and androgens from the adrenal cortex. Treatment involves administration of high doses of mineralocorticoid-sparing synthetic glucocorticoids, such as dexamethasone (1–3 mg/day), which activate the mutated and/or wild-type hGRα, and suppress the endogenous secretion of ACTH (7–12). Adequate suppression of the HPA axis is of particular importance in cases of severe impairment of hGRα function, because long-standing corticotroph hyperstimulation in association with decreased glucocorticoid negative feedback may lead to the development of an ACTH-secreting adenoma (13). Long-term dexamethasone treatment should be carefully titrated based on the clinical manifestations and biochemical profile (5). Asymptomatic, normotensive subjects with primary glucocorticoid resistance do not require any treatment.

Etiology
Molecular Mechanisms of Glucocorticoid Resistance
The molecular basis of glucocorticoid resistance has been ascribed to mutations in the hGRα gene, which impair one or more of the molecular mechanisms of glucocorticoid receptor function, altering tissue sensitivity to glucocorticoids. Abnormalities of several hGRα characteristics, such as cell concentration, affinity for ligand and translocation into the nucleus, have been associated with this condition (5,14-22). The molecular defects elucidated in the reported cases are summarized in Table 2, while the corresponding mutations in the hGRα gene are shown in both Table 2 and Figure 5. The propositus of the original kindred was homozygous for a single point mutation at nucleotide position 2054, which resulted in a nonconservative amino acid substitution at position 641, replacing aspartic acid with valine (15). Compared to the wild-type receptor, this mutant receptor exerted decreased transactivation effects on the glucocorticoid-responsive mouse mammary tumor virus (MMTV) promoter and had a three-fold reduction in the affinity for dexamethasone (15). In the absence of ligand, the mutant receptor was primarily localized in the cytoplasm of cells. Exposure to dexamethasone (10⁻⁶ M) induced a slow translocation into the nucleus, which took 22 min as opposed to the 12 min required for nuclear translocation of the wild-type receptor (22). Finally, the mutant receptor interacted with the amino-terminal but not with the carboxyl-terminal fragment or full-length GRIP1 in vitro (22).

The proposita of the second family was heterozygous for a 4-base deletion at the 3’-boundary of exon and intron 6. The deletion removed a donor splice site in one allele, affecting the last two bases of the exon 6 and the first two bases of the intron 6. This resulted in complete ablation of the expression of one of the hGRα alleles and a decrease in GRα protein by 50% in affected members of the family (16). The propositus of the third kindred was homozygous for a point mutation at nucleotide position 2317, which results in substitution of valine for isoleucine at amino acid 729 of the ligand-binding domain of hGRα (17). This mutation resulted in decreased transcriptional activity of the receptor and a four-fold reduction in the affinity for dexamethasone (17). The mutant receptor was localized primarily in the nucleus of cells in the absence of ligand, while further translocation from the cytoplasm into the nucleus required longer (120 min) exposure to dexamethasone (10⁻⁶ M), and demonstrated a weak, ligand-dependent interaction with the full-length and carboxyl-terminal fragment but not with the amino-terminal fragment of GRIP1 in vitro (22).

The first sporadic case of glucocorticoid resistance was due to a de novo, germ-line, heterozygous mutation at nucleotide position 1808, resulting in substitution of isoleucine for asparagine at amino acid 559 in the hormone-binding domain of hGRα. This mutation reduced the transcriptional activity of hGRα significantly and was associated with the development of an ACTH-secreting adenoma (13). Although the affinity for ligand was preserved in the patient studied, there was a 50% decrease in the hGR binding sites (13). Furthermore, the mutant receptor had a markedly delayed nuclear translocation (180 min) and a dominant negative activity upon the wild-type receptor, i.e. it decreased the transcriptional activity of hGRα in a dose-dependent manner (19). The latter may account for manifestation of the disease at the heterozygotic state. There was no interaction between the mutant receptor and the p160 coactivator GRIP1 (22).

The fifth and sixth cases of glucocorticoid resistance were due to heterozygous mutations at nucleotide positions 1430 and 2035, resulting, respectively, in substitution of arginine for histidine at amino acid 477 and glycine for serine at amino acid 679 (18). The former mutation is located in the second zinc finger of the DNA-binding domain. This mutant receptor had no transactivation activity due to impaired binding to GREs but had the same affinity for ligand as the wild-type receptor. The latter mutation is located in the ligand-binding domain, outside the ligand-binding pocket, and resulted in a 50% reduction both in the transcriptional activity and the ligand-binding affinity of the receptor (18). The proposita of the seventh case was homozygous for a point mutation at nucleotide
position 1844, which results in a valine to alanine substitution at amino acid 571 in the ligand-binding domain of hGRα (20). This mutation caused up to 50-fold decrease in the transcriptional activity of the receptor and a six-fold reduction in the affinity for ligand (20). The nuclear translocation of the mutant receptor was delayed (25 min), while its interaction with the GRIP1 coactivator occurred mostly via its AF-1 domain (22).

The eighth case of glucocorticoid resistance was due to a heterozygous mutation at nucleotide position 2373, which causes an isoleucine to methionine substitution at amino acid 747 in the ligand-binding domain of the receptor (21). This mutation is located at the carboxyl-terminus of the ligand-binding domain, close to helix 12, which plays a pivotal role in the formation of AF-2, a domain that interacts with p160 and other coactivators. The mutant receptor had a 20- to 30-fold decrease in the transactivation of the MMTV promoter, two-fold reduction in the affinity for dexamethasone and delayed nuclear translocation. It also exerted a dominant negative effect upon the wild-type hGRα and interacted with the GRIP1 coactivator in vitro only through its intact AF-1 domain. Overexpression of GRIP1 restored the transcriptional activity and reversed the dominant negative activity of the mutant upon the wild-type receptor (21).

We have demonstrated that the mutant receptors hGRα I559N, hGRα V571A, hGRα D641V, hGRα V729I and hGRα I747M preserve their ability to bind to DNA, and that their ligand-binding domains have decreased intrinsic transcriptional activity (22). Therefore, the process through which hGRα mutant receptors studied thus far impair the physiologic mechanisms of glucocorticoid action at the molecular level is multifactorial, and involves impaired ability to bind ligand, aberrant nucleocytoplasmic trafficking, and abnormal interaction with the p160 coactivators. These variable functional defects of the mutant receptors upon the glucocorticoid signaling pathway may explain the genetic transmission and the variable clinical phenotype of glucocorticoid resistance.

In addition to mutations in the hGRα gene, steroid receptor coactivator defects may also account for generalized glucocorticoid resistance and/or resistance to other steroid hormones (23-24). Affected subjects present with the clinical and biochemical manifestations of glucocorticoid resistance, however, no defect of the hGR gene has been identified.

Figure 5: Location of the known mutations of the human glucocorticoid receptor gene (upper panel) and protein (lower panel).

The latter may account for manifestation of the disease at the heterozygotic state. There was no interaction between the mutant receptor and the p160 coactivator GRIP1 (22).

The fifth and sixth cases of glucocorticoid resistance were due to heterozygous mutations at nucleotide positions 1430 and 2035, resulting, respectively, in substitution of arginine for histidine at amino acid 477 and glycine for serine at amino acid 679 (18). The former mutation is located in the second zinc finger of the DNA-binding domain. This mutant receptor had no transactivation activity due to impaired binding to GREs but had the same affinity for ligand as the wild-type receptor. The latter mutation is located in the ligand-binding domain, outside the ligand-binding pocket, and resulted in a 50% reduction both in the transcriptional activity and the ligand-binding affinity of the receptor (18).

The proposita of the seventh case was homozygous for a point mutation at nucleotide position 1844, which results in a valine to alanine substitution at amino acid 571 in the ligand-binding domain of hGRα (20). This mutation caused up to 50-fold decrease in the transcriptional activity of the receptor and a six-fold reduction in the affinity for ligand (20). The nuclear translocation of the mutant receptor was delayed (25 min), while its interaction with the GRIP1 coactivator occurred mostly via its AF-1 domain (22).

The eighth case of glucocorticoid resistance was due to a heterozygous mutation at nucleotide position 2373, which causes an isoleucine to methionine substitution at amino acid 747 in the ligand-binding domain of the receptor (21). This mutation is located at the carboxyl-terminus of the ligand-binding domain, close to helix 12, which plays a pivotal role in the formation of AF-2, a domain that interacts with p160 and other coactivators. The mutant receptor had a 20- to 30-fold decrease in the transactivation of the MMTV promoter, two-fold reduction in the affinity for dexamethasone and delayed nuclear translocation. It also exerted a dominant negative effect upon the wild-type hGRα and interacted with the GRIP1 coactivator in vitro only through its intact AF-1 domain. Overexpression of GRIP1 restored the transcriptional activity and reversed the dominant negative activity of the mutant upon the wild-type receptor (21).

We have demonstrated that the mutant receptors hGRα I559N, hGRα V571A, hGRα D641V, hGRα V729I and hGRα I747M preserve their ability to bind to DNA, and that their ligand-binding domains have decreased intrinsic transcriptional activity (22). Therefore, the process through which hGRα mutant receptors studied thus far impair the physiologic mechanisms of glucocorticoid action at the molecular level is multifactorial, and involves impaired ability to bind ligand, aberrant nucleocytoplasmic trafficking, and abnormal interaction with the p160 coactivators. These variable functional defects of the mutant receptors upon the glucocorticoid signaling pathway may explain the genetic transmission and the variable clinical phenotype of glucocorticoid resistance.

In addition to mutations in the hGRα gene, steroid receptor coactivator defects may also account for generalized glucocorticoid resistance and/or resistance to other steroid hormones (23-24). Affected subjects present with the clinical and biochemical manifestations of glucocorticoid resistance, however, no defect of the hGR gene has been identified.
Table 2: Mutations of the human glucocorticoid receptor gene causing glucocorticoid resistance

<table>
<thead>
<tr>
<th>Author (Reference)</th>
<th>cDNA</th>
<th>Amino acid</th>
<th>Biochemical Phenotype</th>
<th>Present Study</th>
<th>Genotype</th>
<th>Transmission</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrousos et al. (5)</td>
<td>2054</td>
<td>A→T</td>
<td>Affinity for ligand ↓</td>
<td>Transcriptional activity of LBD ↓</td>
<td>Homozygous</td>
<td>Autosomal</td>
<td>Hypertension</td>
</tr>
<tr>
<td></td>
<td>641</td>
<td>D→V</td>
<td>Transactivation ↓</td>
<td>Transdominance(−)</td>
<td></td>
<td>Recessive</td>
<td>Hypokalemic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nuclear translocation ↓</td>
<td></td>
<td></td>
<td>alkalosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DNA binding (+)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Abnormal interaction with GRIP1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karl et al. (16)</td>
<td>4 bp deletion in exon-6</td>
<td>hGRx number: 50% of control</td>
<td>Inactivation of the affected allele</td>
<td>Heterozygous</td>
<td>Autosomal</td>
<td>Dominant</td>
<td>Hirsutism</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male-pattern</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>hair-loss</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Menstrual</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>irregularities</td>
</tr>
<tr>
<td>Malchoff et al. (17)</td>
<td>2317</td>
<td>G→A</td>
<td>Affinity for ligand ↓</td>
<td>Transcriptional activity of LBD ↓</td>
<td>Homozygous</td>
<td>Autosomal</td>
<td>Precocious</td>
</tr>
<tr>
<td></td>
<td>729</td>
<td>V→I</td>
<td>Transactivation ↓</td>
<td>Transdominance(−)</td>
<td></td>
<td>Recessive</td>
<td>puberty</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nuclear translocation ↓</td>
<td></td>
<td></td>
<td>Hyperandrogenism</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DNA binding (+)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Abnormal interaction with GRIP1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karl et al. (13)</td>
<td>1808</td>
<td>T→A</td>
<td>Affinity for ligand ↓</td>
<td>Transcriptional activity of LBD ↓</td>
<td>Homozygous</td>
<td>Autosomal</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Kino et al. (19)</td>
<td>559</td>
<td>I→N</td>
<td>Transactivation(+)</td>
<td>Transdominance(+)</td>
<td></td>
<td>Sporadic</td>
<td>Oligospermia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nuclear translocation ↓</td>
<td></td>
<td></td>
<td>Infertility</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DNA binding (+)</td>
<td></td>
<td></td>
<td>Hyperandrogenism</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Abnormal interaction with GRIP1</td>
<td></td>
<td></td>
<td>Hypokalemia</td>
</tr>
<tr>
<td>Mendonca et al. (20)</td>
<td>1844</td>
<td>C→T</td>
<td>Affinity for ligand ↓</td>
<td>Transcriptional activity of LBD ↓</td>
<td>Homozygous</td>
<td>Autosomal</td>
<td>Ambiguous</td>
</tr>
<tr>
<td></td>
<td>571</td>
<td>V→A</td>
<td>Transactivation(+)</td>
<td>Transdominance(+)</td>
<td></td>
<td>Recessive</td>
<td>genitalia at birth</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nuclear translocation ↓</td>
<td></td>
<td></td>
<td>Hypertension</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DNA binding (+)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Abnormal interaction with GRIP1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voltero et al. (21)</td>
<td>2373</td>
<td>T→G</td>
<td>Affinity for ligand ↓</td>
<td>Transcriptional activity of LBD ↓</td>
<td>Heterozygous</td>
<td>Autosomal</td>
<td>Cystic acne</td>
</tr>
<tr>
<td></td>
<td>747</td>
<td>I→M</td>
<td>Transactivation(+)</td>
<td>Transdominance(+)</td>
<td></td>
<td>Dominant</td>
<td>Hirsutism</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nuclear translocation ↓</td>
<td></td>
<td></td>
<td>amenorrhoea</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DNA binding (+)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ruiz et al. (19)</td>
<td>1430</td>
<td>G→A</td>
<td>Transactivation ↓</td>
<td>Heterozygous</td>
<td>Sporadic</td>
<td>Hirsutism, Fatigue,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>477</td>
<td>R→H</td>
<td></td>
<td></td>
<td></td>
<td>Hypertension</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2035</td>
<td>G→A</td>
<td>Affinity for ligand ↓</td>
<td>Heterozygous</td>
<td>Sporadic</td>
<td>Hirsutism, Fatigue,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>679</td>
<td>G→S</td>
<td>Transactivation ↓</td>
<td></td>
<td></td>
<td>Hypertension</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Conclusion
Mutations in the human glucocorticoid receptor gene impair one or more of the molecular mechanisms of glucocorticoid action, affecting glucocorticoid signal transduction and altering tissue sensitivity to these hormones. A subsequent increase in the activity of the HPA axis compensates for the reduced sensitivity of peripheral tissues to glucocorticoids at the expense, however, of ACTH hypersecretion-related pathology. The study of the functional defects of natural hGR mutants sheds light to the molecular mechanisms of hGR action, including hGR-mediated transactivation of target genes, ligand-binding, nuclear translocation, DNA binding and interaction with coactivators, and highlights the importance of integrated cellular and molecular signaling mechanisms for maintaining homeostasis and preserving normal physiology.

References