Glucocorticoid resistance syndrome caused by a novel NR3C1 point mutation

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Abstract. Glucocorticoid resistance syndrome (GRS) is a rare genetic disorder caused by inactivating mutations of the NR3C1 gene which encodes the glucocorticoid receptor. The phenotypic spectrum is broad but typically include symptoms of adrenal insufficiency, mineralocorticoid excess and hyperandrogenism. We report a new case associated with a novel NR3C1 mutation. A 55-year-old woman with lifelong history of low body weight, hyperandrogenism and anxiety was seen at the endocrine clinic after left adrenalectomy and salpingoophorectomy for lesions suspicious of ovarian cancer and adrenal metastasis. The tumors turned out to be a 3.5 cm benign ovarian serous adenofibroma and a 3.5 cm multinodular adrenal mass. She complained of worsened fatigue and inability to recover weight lost with surgery. Pre-operative serum and urinary cortisol were elevated, but she had no stigma of Cushing’s syndrome. Plasma ACTH was elevated and a 1-mcg cosyntropin stimulation test was normal. Her fatigue persisted over ensuing years and ACTH-dependent hypercortisolemia remained stable. Low dose oral dexamethasone failed to suppress endogenous cortisol. A pituitary MRI was normal but revealed incidental brain aneurysms. Bone densitometry showed profound osteoporosis. On the bases of this contradictory clinical picture, glucocorticoid resistance syndrome (GRS) was suspected. Using next generation sequencing technology, a novel heterozygous pathogenic variant in the NR3C1 gene was detected. We speculate that vascular malformations and profound osteoporosis, findings associated to cortisol excess, reflect in our patient a variable tissue sensitivity to glucocorticoids. In conclusion, in patients with clinically unexpected ACTH-dependent hypercortisolemia, primary glucocorticoid resistance (GRS) should be considered.

Key words: Glucocorticoid resistance, Chrousos syndrome, Glucocorticoid receptor, NR3C1 gene
an ovarian mass. CT and MRI raised the suspicion of adrenal metastasis and therefore she underwent salpingo-oophrectomy and left adrenalectomy. Pre-operatively a morning serum cortisol and a 24-hour urinary free cortisol (UFC) were mildly elevated (Table 1). The surgery was uneventful with no need for perioperative glucocorticoids. Histopathology showed a 3.5 cm left paraovarian benign serous adenofibroma and a 3.5 cm benign adrenal multinodular mass, with background changes suggestive of adrenocortical hyperplasia (multinodular pattern of proliferation of adrenal cortical cells with limits of proliferation less defined than usual for typical adrenal cortical adenomas).

When seen by endocrinology, 2 months after surgery, she was complaining of severe fatigue, anorexia and inability to regain weight lost during the perioperative period. Review of her past medical history revealed a longstanding anxiety disorder controlled with benzodiazepines. She was also known for chronic fatigue, severe acne and hirsutism since puberty. Pubertal development was reported as normal; she had regular menses with hypomenorrhea. Her only pregnancy resulted in first trimester miscarriage and, despite unprotected intercourse and several ovarian stimulation cycles, she could not conceive again. She was an only child. Her mother had three first trimester miscarriages and was not able to carry another pregnancy to term. A maternal aunt and possibly a maternal uncle were infertile (Fig. 1).

Her exam revealed a minute, slim woman with minimal subcutaneous or abdominal fat. Blood pressure was normal. Her weight remained stable at 40–41 kg; height 156 cm (BMI 16.4–17 kg/m²). Skin complexion was fair, facial skin was oily with healed acne scars. She had no ecchymosis, striae or proximal muscle weakness. A 1 mcg-Cosyntropin stimulation test was normal (basal cortisol 281 nmol/L; peak 812 nmol/L, normal response >400 nmol/L). The patient was reassured and instructed to return to the clinic if symptoms worsened.

At subsequent follow-ups, she complained of recurrent infections, worsened chronic fatigue and anxiety. She

### Table 1  Representative laboratory tests results

| Test (units) | Reference range | 2010 Pre-operatively | 2010 Post-operatively | 2013 | 2014 | 2015 (pre CPAP) | 2016 (On CPAP) | 2017 | 2018 |
|-------------|----------------|----------------------|----------------------|------|------|----------------|----------------|------|------|
| Electrolytes |                |                      |                      |      |      |                |                |      |      |
| Na (mmol/L)  | 133–143        | 141                  | 139                  | 141  | 138  | 137            | 140            | 138  | 142  |
| K (mmol/L)   | 3.5–5.0        | 3.9                  | 4.2                  | 4.7  | 4.4  | 4.0            | 4.4            | 4.7  | 4.5  |
| AM Cortisol (nmol/L) | 120–535   | 790                  | 364                  | 741  | 806  | 677            | 746            | 631  | 851  |
| Urinary Free Cortisol (nmol/day) | <120 | 126                  | 159                  | 213  | 264  | 223            | 172            |      |      |
| ACTH (pmol/L) | (<=10)²       | 9                    | 11                   | 14   | 10   | 13.89, 17.17²  | 21.59          | 27.07 |
| Aldosterone (pmol/L) | 111–860  |                      |                      |      |      |                | 278            |      |      |
| Plasma Renin activity (ng/L/s) | 0.21–1.06 |                      |                      |      |      |                | 0.15           |      |      |
| Aldosterone Renin Ratio | <4.000   |                      |                      |      |      |                | 1.853          |      |      |
| Total testosterone (nmol/L) | 0.35–2.70 |                      |                      | 1.09 | 1.19 |                |                |      |      |
| Androstenedione (nmol/L) | 2.6–11.0  |                      |                      |      |      |                | 6.69           |      |      |
| DHEAS (μmol/L) | 0.1–2.7    | 1.3                  | 1.2                  | 1.3  |      |                |                |      |      |
| 17-OH progesterone (nmol/L) | 0.4–1.5   |                      | 6.5                  |      |      |                | 3.9            |      |      |
| TSH (mIU/L)  | 0.4–4.4       | 0.93                 | 1.2                  | 1.7  | 1.89 | 2.27           | 2.71           |      |      |
| Free T4 (pmol/L) | 8–18       | 12.7                 | 11.7                 | 10   | 12.1 | 12.6           | 12.7           |      |      |
| Prolactin (μg/L) | 3.3–26.7   | 4.9                  |                      | 6.15 |      |                |                |      |      |
| LH (IU/L)    | 29.7–72.4    | 45.5                 | 43.3                 |      | 39.3 |                |                |      |      |
| FSH (IU/L)   | 28–99.7      | 141.6                | 126.8                |      | 148.4|                |                |      |      |

¹ CPAP, Continuous positive airway pressure.  
² Reference range changed in 2015 to (1.6–13.9 nmol/L).
remained underweight. Morning serum cortisol, ACTH and UFC remained elevated and did not suppress during a 1 mg-dexamethasone suppression test (Table 1). MRI of the sella turcica showed a normal pituitary but revealed a 16-mm vertebral artery aneurysm, and a smaller right middle cerebral artery aneurysm. MRI scan showed right adrenal hyperplasia, small hepatic hemangiomas, hepatic focal nodular hyperplasia, and non-obstructive kidney stones. Bone mineral density showed severe osteoporosis (T-score L1–L4: –4.1, femoral neck –3.3).

At ensuing visits the possibility of anorexia nervosa, major depression or anxiety status was excluded. She did not drink alcohol and had never been on estrogen or selective estrogen receptor modulators therapy. There was no evidence of additional tumors on extensive imaging and her clinical picture remained stable over a 5-years follow-up period. Sleep studies revealed obstructive sleep apnea for which she was started on continuous positive airway pressure treatment. However, ACTH, serum cortisol and 24-hour UFC remained elevated after appropriate OSA treatment (Table 1).

The combination of ACTH-dependent hypercortisolemia, clinical hyperandrogenism with no clinical evidence of Cushing’s syndrome, and intermittent symptoms of adrenal insufficiency led to the suspicion of GRS.

NR3C1 molecular analysis was offered to the patient who agreed and provided written informed consent. Gene sequencing and analysis was performed at Fulgent Genetics (Los Angeles, CA) a CLIA certified lab (www.fulgentgenetics.com). Genomic DNA was isolated from blood specimen collected in EDTA tubes. The DNA was barcoded and enriched for the coding exons of the NR3C1 gene using hybrid capture technology. Prepared DNA libraries were then sequenced using a clinically validated next generation sequencing technology. Following alignment, 100% of coding regions and splicing junctions of the NR3C1 gene were sequenced with coverage at 20X. By this method, potentially clinically significant variants are confirmed by additional coverage (>100 reads) and a positive quality score (500 or more) as previously defined [4]. Variants are interpreted manually using locus specific databases and literature searches. Only variants classified as pathogenic, likely-pathogenic, or unknown significance which are thought to be related to the patient’s phenotype or test indication are reported. The NR3C1 gene was evaluated for large deletions and/or duplications. Single exon deletions or duplications are not detected by this assay.

This analysis revealed a novel heterozygous frameshift
alteration (NM_001018077.1:c.1392del, p.Ile465Serfs*22) located in exon 4 of the 9 exon transcript. This variant is predicted to lead to a truncated, non-functional protein, therefore predicted to be pathogenic. This variant is located in the zinc finger nuclear hormone receptor-type domain and was not found in the Broad ExAC database. This specific variant had a 157 X coverage and a quality score of over 500 [4]. No other clinically significant variants, deletions or duplications were identified in the specimen. Patient declined having her relatives tested for the same variant.

Discussion

We report a case of a postmenopausal woman with ACTH-dependent hypercortisolism due to glucocorticoid resistance, who presented with worsened fatigue, weight loss and anorexia after left adrenalectomy for a benign lesion.

In primary GRS, first described by George P. Chrousos in 1982, tissue resistance to circulating GC leads to activation of the hypothalamic-pituitary-adrenal axis and hypersecretion of ACTH [5]. As a result, affected individuals have hyperplasia of adrenal cortex and increased secretion of GC, mineralocorticoids (deoxycorticosterone and corticosterone) and adrenal androgens. Cortisol hypersecretion is resistant to suppression by dexamethasone [5]. It is caused by mutations in the NR3C1 gene located on chromosome 5q31.3 and coding for the hGR, a steroid hormone receptor from the nuclear receptor superfamily of transcription factors. The pattern of inheritance is autosomal dominant.

NR3C1 contains 10 exons of which exons 2–9 are responsible for protein expression [1]. Alternative splicing in exon 9 generates two highly homologous receptor isoforms, hGRα and hGRβ, identical through amino acid 727 (out of 777 amino acids in hGRα, or 742 in hGRβ). In humans, hGRα represents the classic hGR that functions as a ligand-dependent transcription factor. Meanwhile, hGRβ does not bind glucocorticoids but appears to exert a dominant-negative effect on the transcriptional activity of hGRα in a dose-dependent manner [6]. Therefore, alterations in the molecular structure, level of expression or tissue distribution of either GR may result in GC resistance or hypersensitivity [3]. GRS is exceedingly rare with only 23 index cases (43 individuals) reported to date worldwide [5, 7-25] (Table 2).

A diagnosis of GRS is suggested by unexplained sustained hypercortisolemia without Cushing’s syndrome but rather, at times, manifestations of adrenal insufficiency instead. Serum cortisol and urinary free cortisol has been reported to reach up to 7 and 50 folds the upper limit in their reference range, respectively [26]. Plasma ACTH may be normal or high.

A review of the published cases of GRS [5, 7-25] due to mutations in the NR3C1 gene (Table 2) shows that the clinical features of this rare condition can be summarized as follows:

1) Hyperandrogenism and impaired fertility (63% of cases): ambiguous genitalia, hirsutism and oligomenorrhea in females; precocious puberty and oligospermia in males.
2) Hyperaldosteronism, with or without hypertension and/or hypokalemia (50% of cases).
3) Non-specific symptoms including fatigue (in 38%) and anxiety (in 21%).

Only 29% of reported index cases have both hyperandrogenism and mineralocorticoid excess. About 1 in 5 patients have hyperaldosteronism without hyperandrogenism, while 1 in 3 patients display only hyperandrogenism without hypertension (Table 2). Hypoglycemia and hypoglycemic seizures, poor feeding, and increased susceptibility to infections have been reported only in an infant and a young child [17, 18]. Additional manifestations include adrenal adenomas and/or hyperplasia [16, 20, 24], testicular adrenal rest tumors and, in one case, a corticotropoma [10]. The heterogeneity in the clinical presentation of GRS reflects both, the different degrees of hormonal resistance resulting from specific NR3C1 gene mutations (a function of the mutated receptor expression levels, its nuclear translocation ability, and its ligand, corepressors and coactivators as well as DNA binding affinity); but also the variable tissue sensitivity to the steroid hormones involved (a function of tissue specific receptors and cofactors expression, as well as post-translational modifications). Many patients with milder clinical presentations may remain undiagnosed.

In keeping with this inhomogeneous clinical picture in GRS, our patient had a longstanding history of low body weight, chronic fatigue, an anxious diathesis, mild hyperandrogenism, and infertility. These symptoms triggered a more in-depth endocrine assessment because of the incidental finding of an adrenal mass and hyperplasia while serum and urinary cortisol were elevated. Her chronic fatigue was noticed to aggravate after left adrenalectomy and after any moderate or major stress (such as after coiling of her brain aneurysm) and was associated with anorexia and muscle weakness, suggestive of relative
Severe osteoporosis and vascular abnormalities have not been previously reported in association with GRS. Although this association may be coincidental, we speculate that they may be secondary to tissue heterogeneity in glucocorticoid sensitivity, as seen in other nuclear hormone resistance syndromes. As seen in cases of Cushing’s syndrome presenting with vascular aneurysms, these vascular malformation are thought to be linked to the effect of hypercortisolemia on blood vessels [27]. In mice, injection of hydrocortisone has been showed to lead to induction of ectasia, aneurysms and, in
gluocorticoid deficiency.

Table 2  Reported cases of glucocorticoid resistance syndrome, mutation and reported clinical features

| Publication first author [Reference] | Number of patients per report | Sex | Mutation Position | Clinical Manifestations |
|--------------------------------------|-------------------------------|-----|-------------------|------------------------|
|                                      |                               |     |                   |                         |
| Chrousos et al. [5]                  | 2                             | 2M  | c.1922A>T         | X                      |
| Karl et al. [8]                      | 2                             | 1F  | c.2185G>A         | X                      |
| Karl et al. [9]                      | 4                             | 1F  | c.1891_1894delGAGT|                         |
| Hurley et al. [7]                    | 1                             | 1M  | c.1676T>A         |                         |
| Malchoff et al. [8]                  | 1                             | 1F  | c.2035G>A         |                         |
| Karl et al. [10]                     | 1                             | 1M  | c.1430G>A         |                         |
| Kino et al. [11]                     | 1                             | 1M  | c.1712T>C         |                         |
| Ruiz et al. [12]                     | 1                             | 1F  | c.1667C>T         |                         |
| Ruiz et al. [13]                     | 1                             | 1M  | c.2318T>C         |                         |
| Ruiz et al. [14]                     | 1                             | 1M  | c.2209T>C         |                         |
| Ruiz et al. [15]                     | 1                             | 1F  | c.2318_2319delTG  |                         |
| McMahon et al. [16]                  | 1                             | 1M  | c.2318_2319delTG  |                         |
| Votto et al. [17]                    | 1                             | 1F  | c.2318_2319delTG  |                         |
| Mendonca et al. [13]                 | 1                             | 1M  | c.2141G>A         |                         |
| Votto et al. [18]                    | 1                             | 1F  | c.2141G>A         |                         |
| Votto et al. [19]                    | 1                             | 1M  | c.1667C>T         |                         |
| Chamous et al. [20]                  | 1                             | 1M  | c.1268T>C         |                         |
| Damien et al. [21]                   | 1                             | 1M  | c.1724T>G         |                         |
| Nicolai et al. [22]                  | 3                             | 2F  | c.1724T>G         |                         |
| Nicolai et al. [23]                  | 1                             | 1F  | c.1724T>G         |                         |
| Zhu et al. [24]                      | 1                             | 1M  | c.1429C>T         |                         |
| Roberts et al. [21]                  | 1                             | 1M  | c.1429C>T         |                         |
| Nicolai et al. [22]                  | 1                             | 1M  | c.1429C>T         |                         |
| Nicolai et al. [23]                  | 1                             | 1M  | c.1429C>T         |                         |
| Vitellius et al. [24]                | 5                             | 1F  | c.1392_1392delC   |                         |
| Velayos et al. [25]                  | 3                             | 2F  | c.1392_1392delC   |                         |
| Malchoff et al. [8]                  | 1                             | 1M  | c.1392_1392delC   |                         |
| This report                          | 1                             | 1M  | c.1392_1392delC   |                         |
| Total Number                         | 44                            | 20M | 24F               | 21                     |

1 Genebank transcript ID: NM_001018074.
2 Includes ambiguous genitalia, precocious puberty, advanced bone age, infertility, amenorrhea, clitoromegaly, oligospermia.
3 Glucocorticoid-resistant acute lymphocytic leukemia.
4 Affected daughters of index case had mild hirsutism.
genetically susceptible animals, aortic rupture [28].

In our patient, sequencing and deletion/duplication analysis revealed a novel heterozygous variant at nucleotide position 1392 resulting in a frameshift and the introduction of a premature stop codon at amino acid residue 487; only the 23rd different mutation in 44 primary GRS patients reported to date (Table 2). In keeping with current guidelines for the interpretation of sequence variants [29], several factors point to the pathogenicity of this novel mutation: its position (in the same exon and upstream of NR3C1 variants previously recognized as pathogenic, at a site where all protein isoforms would be affected), its null nature (expected to lead to a truncated, disabled protein) in a gene where loss of function is a known mechanism of disease, consistent with the established dominant inheritance pattern; and its absence from large population databases (suggesting that this variant is not compatible with health). Additionally, two similar cases where GRS was associated with null mutations would allow us to infer on the functional impact of this mutation. The first GRS case was found to harbor a cytosine to thymidine substitution at nucleotide position 1405, also in exon 4 of NR3C1, resulting in a premature stop codon at amino acid residue 469. Sequencing of cDNA in a fibroblast culture failed to detect any mutated transcripts and the GR species was undetectable by western blot. Administration of a Nonsense-mediated mRNA Decay (NMD) inhibitor led to the detection of the predicted truncated protein product [30]. The second report on a GRS family, showed a single base deletion at nucleotide position 1835 resulting in a frameshift and protein truncation at amino acid residue 627. Assessment of lymphocyte cell lines showed no detectable expression of the truncated protein in 3 affected family members [20]. Given these similar findings, it seems likely that premature stop codons in NR3C1 are selectively degraded through NMD [31]. Therefore, our germline NR3C1 variant likely also results in NMD of the mRNA transcript and haploinsufficiency of GR, previously shown to result in disease without the requirement of dominant negative antagonisation [25].

Concerning treatment approach for patients with GRS, we conclude that it must be individualized. In recent reviews, it has been suggested that the goal of therapy would be to suppress the high levels of ACTH hence suppressing mineralocorticoids and androgens over secretion, and reducing the risk adrenal adenomas, testicular adrenal rest tumors and pituitary adenomas. This objective can be achieved by using synthetic potent glucocorticoid with minimal intrinsic mineralocorticoid activity such as dexamethasone (1–3 mg daily) [32]. Aldosterone antagonists may also be particularly helpful in such scenarios because of its anti-androgen effect [33]. In our patient we opted to not use any of these strategies because there was no evidence of mineralocorticoid excess and because of the risk that dexamethasone would aggravate her severe osteoporosis, assuming a lesser degree of GC insensitivity in her bones.

**Conclusion**

Increasing awareness of the clinical heterogeneity of primary glucocorticoid resistance may result in uncovering more cases of this syndrome. Clinicians need to be reminded to consider GRS when facing patients with ACTH-dependent hypercortisolism without Cushing’s syndrome especially if associated with adrenal adenomas or hyperplasia, hyperandrogenism and/or mineralocorticoid excess. Our case questions the wisdom of recommending treatment with ACTH-reducing doses of dexamethasone for all GRS patients. An individualized approach to treatment seems more appropriate.

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The authors have nothing to disclose.

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