Bilateral adrenal incidentalomas and *NR3C1* mutations causing glucocorticoid resistance: is there an association?

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Abstract

Glucocorticoids signal through their cognate, ubiquitously expressed glucocorticoid receptor (GR), which influences the transcription of a large number of target genes. Several genetic defects, including point mutations, deletions or insertions in the *NR3C1* gene that encodes the GR, have been associated with familial or sporadic generalized glucocorticoid resistance or Chrousos syndrome. One of the clinical manifestations of this rare endocrine condition is bilateral adrenal hyperplasia due to compensatory elevations of plasma ACTH concentrations. In this commentary, we discuss the interesting findings of the recently published French MUTA-GR study and present our perspective on the evolving field of *NR3C1* pathology.

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Glucocorticoids in homeostasis and cacostasis

Secreted by the adrenal cortices, glucocorticoids are major effector molecules of the stress response (1). These steroid hormones contribute substantially to the maintenance of our resting and stress-related homeostasis by influencing almost all physiologic functions, including arousal and sleep, memory, maintenance of cardiovascular tone, intermediary metabolism, the immune response and many others (2). The myriad of glucocorticoid effects are mediated by a ubiquitously expressed intracellular protein, the human glucocorticoid receptor (hGR), which influences the transcription rate of approximately one in five genes of the human leukocyte expressed genome (3, 4). In addition, the crosstalk of hGR with several other fundamental transcription factors, such as the nuclear factor- κ B (NF- κ B), the activator protein-1 (AP-1), and the signal transducers and activators of transcription (STATs), allows indirect regulation of gene expression via their intracellular signaling systems (1, 2, 3). Furthermore, a growing body of evidence suggests that glucocorticoids

acting through their cognate receptor, may alter the methylation status of cytosine-guanine dinucleotides (CpG) located in the regulatory regions of several genes, leading to important epigenetic alterations (5).

The hGR is encoded by the *NR3C1* gene, which is located in the short arm of chromosome 5. This gene is composed of ten exons. Exon 1 contains several promoters of the gene, whereas exons 2–9 α or -9 β generate by alternative transcription of the two main receptor isoforms, the hGR α and the hGR β , respectively (6). Mutations, deletions or insertions in the *NR3C1* gene have been associated with familial or sporadic primary generalized glucocorticoid resistance or Chrousos syndrome (7, 8). This is a rare condition, characterized by decreased sensitivity to glucocorticoids of all their target tissues, including those responsible for the glucocorticoid negative feedback loops, and leading to compensatory elevation of ACTH and cortisol secretion (9). To date, 26 different genetic defects have been identified in the

NR3C1 gene (10, 11, 12). The compensatory increase in plasma ACTH concentrations has been considered responsible for adrenal hyperplasia and hypersecretion of cortisol, steroid precursors with mineralocorticoid activity (e.g. deoxy-corticosterone and corticosterone) and adrenal androgens, such as androstenedione, DHEA and DHEAS (Fig. 1). Patients with primary generalized glucocorticoid resistance (PGGR) may be asymptomatic with only biochemical alterations or may present with clinical manifestations of mineralocorticoid excess (hypertension and/or hypokalemic alkalosis) and/or adrenal androgen excess, such as ambiguous genitalia, precocious puberty, cystic acne, hirsutism and hypofertility in both sexes (Fig. 1) (10, 11).

We and others have identified novel NR3C1 genetic defects in patients with a broad clinical spectrum of PGGR (reviewed in (11)). The progress of molecular and cellular biology has allowed detailed investigation of the molecular mechanisms through which defective receptors cause glucocorticoid resistance both in *in vitro* assays and *in silico*. We demonstrated that all defective GRs impaired many steps of glucocorticoid signal transduction depending on the position of a missense mutation, insertion or deletion in the NR3C1 gene (11). Interestingly, a number of patients with clinical and/or biochemical findings suggestive of PGGR do not harbor a mutant GR, indicating that several other genes play important roles in defining tissue sensitivity to glucocorticoids (11).

Figure 1

Pathophysiology and clinical manifestations of PGGR syndrome. ACTH, adrenocorticotrophic hormone; AVP, arginine-vasopressin; CRH, corticotropin-releasing hormone; PGGR, primary generalized glucocorticoid resistance. Modified from Reference (10) with permission.

Lessons from the French MUTA-GR study

A recently published elegant French study provided interesting results on the association between bilateral adrenal hyperplasia and NR3C1 mutations (12). Vitellius *et al.* estimated the prevalence of NR3C1 mutations and polymorphisms in a cohort of patients with bilateral adrenal hyperplasia, high arterial blood pressure and/or biologic hypercortisolism without any stigmata of Cushing syndrome. This multicenter study was set up based on the results of two earlier studies, in which a heterozygous mutant GR was associated with bilateral adrenal hyperplasia both in a patient harboring the hGR α R469X mutation and in mouse GR^{+/-} model, respectively (13, 14). These findings led the authors to hypothesize that the NR3C1 mutations might be an unsuspected cause of bilateral adrenal hyperplasia. For this purpose, 100 patients with incidentally discovered bilateral adrenal hyperplasia, hypertension and/or hypercortisolism without any clinical manifestations of Cushing syndrome were recruited to participate in the study. The inclusion criteria and all clinical, biochemical, endocrine and imaging data were reported (12).

The authors found that 5 in 100 patients harbored novel heterozygous NR3C1 mutations. Two of them, the R469X and the R477S, were located in the DNA-binding domain of hGR α , whereas the R491X, the Q501H and the L672P mutations were found in the ligand-binding domain of the receptor. These findings indicate a prevalence of 5% of NR3C1 mutations in patients with bilateral adrenal hyperplasia (12). Two of the patients harboring the mutant GRs had high blood pressure, whereas four of them met the diagnostic criteria of PGGR (increased urinary free cortisol excretion, normal or high plasma ACTH concentrations and resistance of the HPA axis to the 1 mg dexamethasone suppression test). The authors also showed that patients with defective hGRs had lower potassium levels, lower or normal renin levels and lower aldosterone concentrations, than those without NR3C1 mutations (12). These results may be explained by the elevated secretion of steroids with mineralocorticoid activity, such as cortisol itself, 11-deoxycorticosterone and corticosterone. In *in vitro* experiments, the investigators exposed fibroblasts obtained from the patients with NR3C1 mutations to dexamethasone for 6 h and demonstrated that the expression of a glucocorticoid target gene coding for the FKBP5 protein, was lower, suggesting defective glucocorticoid sensitivity (12). Moreover, the authors searched for a possible association between known NR3C1 polymorphisms and bilateral adrenal

hyperplasia. They only found a high representation of the *BclII* polymorphism in the 100 participants. Finally, the authors concluded that sequencing of the *NR3C1* should be performed in selected patients with bilateral adrenal hyperplasia, endocrinologic findings compatible with PGGR, decreased aldosterone concentrations and decreased potassium levels below 4 mmol/L (12).

This study provides an estimation of the prevalence of *NR3C1* mutations in bilateral adrenal hyperplasia. Although only 26 *NR3C1* genetic defects have been identified so far, the percentage of 5% is indeed a high prevalence, indicating that many cases of *NR3C1* neutralizing mutations, deletions or insertions in the general population might be present and missed. Therefore, clinicians should search the *NR3C1* for such genetic defects in patients with bilateral adrenal hyperplasia who also have low aldosterone concentrations, serum potassium below 4 mmol/L and increased urinary free cortisol excretion or resistance of the HPA axis to the 1 mg dexamethasone suppression test. This might be extremely helpful for clinicians to distinguish such cases from preclinical Cushing syndrome or pseudo-Cushing conditions.

The French MUTA-GR study showed that all patients with *NR3C1* mutations had low aldosterone concentrations combined with low or normal renin in the presence or absence of hypertension, suggesting an impairment of aldosterone secretion (12). Interestingly, in three of the mutated patients that DOC concentrations were measured, the latter were normal, but this may be due to the known pulsatile and/or marked ultradian variability of steroid hormone secretion. Carriers of R469X had impaired activity of renal 11- β hydroxysteroid dehydrogenase type 2 (11- β HSD2), probably a result of saturation of the enzyme by the elevated steroids with salt-retaining activity (Fig. 2) (13, 14). Although these results are valid, more studies are needed to justify a role of impaired aldosterone secretion and/or signaling in PGGR.

Another important issue raised in this study was the role of ACTH hyperstimulation on the adrenal glands. In the MUTA-GR study, most of the patients harboring *NR3C1* mutations had normal plasma ACTH concentrations, indicating lack of ACTH hyperstimulation on the adrenal cortex, but this could be due to a reset of the HPA axis as that reported in melancholic depression (15) or, again, could be a methodologic issue, granted that the secretion of ACTH is markedly circadian and pulsatile (12). Older studies had demonstrated that ACTH alone may not promote adrenocortical cell growth; yet, simultaneous

Figure 2

Possible molecular mechanisms leading to activation of mineralocorticoid receptor signaling in the kidney distal convoluted tubule. The presence of markedly elevated levels of salt-retaining corticoids, such as cortisol, 11-deoxycorticosterone and corticosterone, led to the impairment of renal 11- β hydroxysteroid dehydrogenase type 2 (11- β HSD2) activity and increased activation of the mineralocorticoid receptor in the kidney distal convoluted tubule. 11- β HSD2, 11- β hydroxysteroid dehydrogenase type 2; ACTH, adrenocorticotrophic hormone; FKBP, immunophilins; HSPs, heat shock proteins; MR, mineralocorticoid receptor; PGGR, primary generalized glucocorticoid resistance.

activation of the adrenal medulla through secretion of NPY or other peptides might synergize in promoting growth of *zona fasciculata* cortisol-secreting cells (16).

Beyond GR: bilateral adrenal hyperplasia and glucocorticoid resistance in the era of next generation sequencing

In the French MUTA-GR study, the authors found a significant prevalence of *NR3C1* mutations in patients with bilateral adrenal hyperplasia (12). Clinicians should keep in mind that not all cases of PGGR are attributed to *NR3C1* mutations, suggesting a possible role of other molecules (GR partners, co-factors, etc.) in the pathogenesis of primary generalized glucocorticoid resistance (11). Novel technologies, such as whole exome/genome sequencing, could undoubtedly unravel the complex pathways involved in the regulation of tissue sensitivity to glucocorticoids. The 'new' GR interactome is to be extensively investigated.

Declaration of interest

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

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