

amniocentesis, which allow for taking fetal cells on which genetic studies of OI may be performed. If the fetus carries the mutation, no cure exists. The only possibility is therapeutic abortion.

Non-invasive procedures such as the detection of fetal circulating DNA in maternal blood, are promising.

Other options for having a child include natural pregnancy accepting the risks entailed, or adoption.

Our patient is currently considering the pros and cons of these alternatives.

Women with OI who receive treatment with bisphosphonates during childbearing age also worry about their safety in future pregnancies, because these drugs remain for years in the bone matrix and cross the placental barrier. Bone turnover suppression may cause fetal hypocalcemia. Data in females are sparse, but no serious adverse effects have so far been reported.¹¹ However, the safety period has yet to be established.

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Glucocorticoid resistance syndrome caused by two novel mutations in the *NR3C1* gene



Síndrome de resistencia a los glucocorticoides causado por dos nuevas mutaciones en el gen *NR3C1*

Glucocorticoid resistance (GCCR, OMIM #615962) is a rare condition characterized by generalized, partial, target tissue resistance to glucocorticoids. Cortisol action mediated by the glucocorticoid receptor (GR) is decreased, with a compensatory elevation of circulating ACTH. This increases the production of glucocorticoids, mineralocorticoids and adrenal androgens.¹ The diurnal rhythm of cortisol is maintained but in an elevated level and no adequate cortisol suppression is observed after 1 mg dexamethasone (DXM) test.¹

The molecular basis of GCCR has been related to mutations in the *NR3C1* gene, located on 5q31–q32, which encodes for the GR (Fig. 1A).

In this letter, we report two novel heterozygous mutations in the *NR3C1* gene in two unrelated patients in whom glucocorticoid resistance was suspected.

Biochemical values obtained from the patients are shown in Table 1. In both cases, these pointed to an ACTH-dependent Cushing's syndrome. No pituitary lesion was observed in either patient's magnetic resonance imaging (MRI). Bone densitometry was also normal in both cases.

Patient 1 was a 12-years old girl referred to the endocrinologist after her pediatrician observed a clinically irrelevant white stretch mark on the right thigh and, afterwards, a high basal cortisol level. Menarche occurred at the age of 11 and she referred regular periods. Physical evaluation was normal (weight in percentile 75, height in percentile 90 and Tanner stage IV for pubertal development). Her blood pressure was normal. She presented mild hirsutism. Physiological causes for elevated cortisol levels were excluded. As she remained asymptomatic, no therapeutic attitude was taken. Subsequent evaluations showed no physical or biochemical changes. Hormone studies were performed in the patient's parents. Her mother had slightly elevated cortisolemia (27 µg/dl), with normal DHEA-S, testosterone and 17-Hydroxyprogesterone values. Plasma cortisol level after 1 mg oral DXM administration overnight was 3.7 µg/dl. Father's results were normal.

Patient 2 was a 41-years-old woman referred to the endocrinologist for hirsutism in 1998. She presented regular menses with no other symptoms or signs of Cushing's

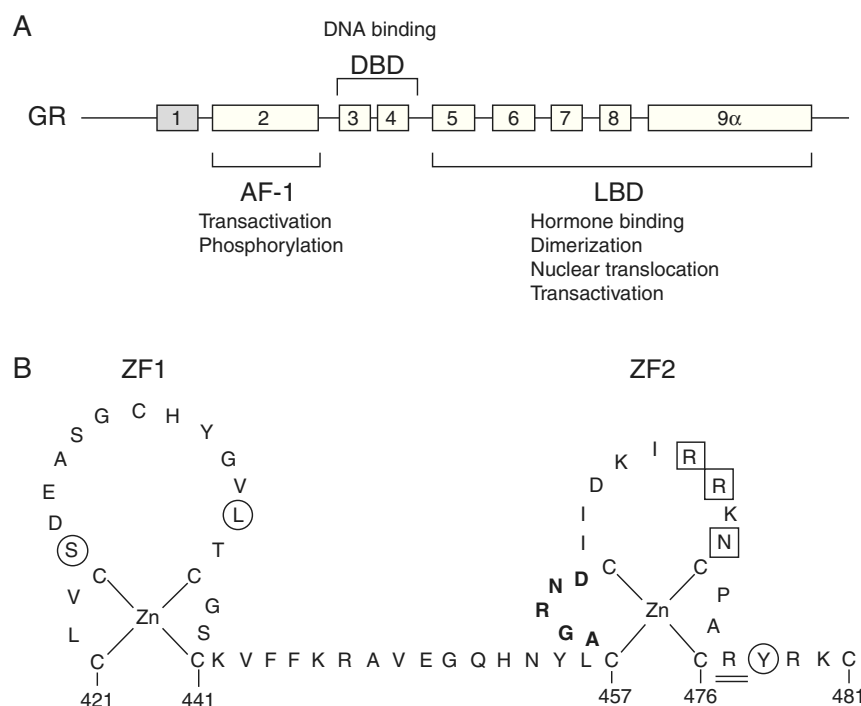


Figure 1 (A) Schematic representation of the structure and functions of the human glucocorticoid receptor (GR). Exon 1 is not translated. AF-1, activation function-1 domain; DBD, DNA-binding domain; LBD, ligand-binding domain. (B) Schematic diagram of the zinc finger structure of the DBD of the human GR. Several residues at these zinc fingers are required for dimerization (aminoacids 458–462; highlighted in bold), transactivation (aminoacids 469, 470, 472; boxed) or to repress other transcription factors, such as AP-1 (aminoacids 425, 436, 478; circled). Aminoacid 477, affected in Case 1, is represented double-underlined.

syndrome. She was depressed after her mother's terminal disease. She chose electrolysis for permanent hair removal after unsuccessful medical treatment. In 2012, she claimed an increment of the hirsutism, chronic fatigue and anxiety. Physical examination was normal except for the hirsutism (weight 50.6 kg, height 154 cm, BMI 21.3 kg/m², BP 125/80 mmHg). Diabetes was ruled out (fasting glucose, 85 mg/dl; HbA1c 5%). Hormonal tests persisted altered.

Genetic analyses were performed after written informed consent and local ethical committee endorsement were obtained. Briefly, PCR amplification and direct sequencing of

the nine *NR3C1* gene exons were performed and sequences were compared with the reference sequence (Ensembl identifier: ENSG00000113580) using specific software. Primer sequences and conditions are available on request.

We identified two novel heterozygous mutations in the *NR3C1* gene. Patient 1 and her mother presented the p.Arg477Cys (c.1429C > T) mutation, located in exon 4, in the second zinc finger of the DNA binding domain of the protein. Its '*in silico*' functional effect was assessed using pathogenicity prediction software, being characterized as pathogenic. Despite this mutation is novel, another naturally

Table 1 Biochemical results of the patients. Biochemical values obtained in the different tests for the two patients. Normal values at the local laboratory are indicated in brackets in the first column. DXM, dexamethasone. DHEA-S, dehydroepiandrosterone sulfate.

Normal values and units	Case 1		Case 2	
	1st determination	2nd determination	1998	2012
24 h urine cortisol (<100 µg)	417	300	167	336 150
Plasma cortisol (5–25 µg/dl)	30.8	38.1	26.7	
Salivary cortisol 23.00–24.00 h (<0.15 µg/dl)	–	0.46	–	0.28
Plasma cortisol after 1 mg DXM overnight (<1.8 µg/dl)	19.3	–	14.7	–
Plasma cortisol after DXM 2 mg/6 h/48 h (µg/dl)	1.3	–	–	–
24 h urinary cortisol after DXM 2 mg/6 h/48 h (µg).	–	–	<11	127
ACTH (4.7–48.8 pg/ml)	108	121	45	30
DHEA-S (50–1500 ng/ml)	1430	–	1500	1590
Testosterone (20–50 ng/dl)	80	–	88	102.6

occurring point mutation has been previously reported at the same amino acid position.² The clinically severe p.Arg477His was related with the elimination of the transactivating activity and primary cortisol resistance, still presenting intact ligand binding capacity. DNA binding capacity was decreased. It also presented a negative effect on the transcriptional activity. It is probable that the p.Arg477Cys mutation, located in the same amino acid, behaves the same way as p.Arg477His, affecting the transactivating activity and/or DNA binding capacity.

Patient 2 carried the p.His588Leufs*5 (c.1762_1763insTTAC) mutation, in exon 6, in the ligand binding domain (LBD) of GR. This frameshift mutation determines a substitution of four amino acid residues at codons 588–591 and a premature stop codon at position 592, thus leading to a truncated protein. The alteration is located at the second charge clamp of the steroid binding domain, and point mutations in this region have been described to reduce activation mediated by the LBD.³ In this case, lack of the C-terminal domain of the protein is expected to alter some functions of the LBD. Another similar truncated protein already described was predicted to have a dominant negative activity by impairing wild-type GR nuclear translocation. Similar features may be expected from the p.His588Leufs*5 mutation.

In summary, in this article we report two novel pathogenic mutations in the glucocorticoid receptor gene in two unrelated patients with GCCR. The studied patients presented an ACTH-dependent hypercortisolism with no symptoms, except hirsutism and, more recently, anxiety and chronic fatigue in Patient 2. Other causes of ACTH-dependent hypercortisolism were ruled out and the persistence in time of elevated cortisol and ACTH levels without any other clinical symptom drove us to the diagnosis of GCCR.

Generalized glucocorticoid resistance can have a variable clinical presentation, ranging from absence of symptoms to the presence of clinical features due to an excess of mineralocorticoids and/or adrenal androgens.^{1,4} Severity of the disease cannot be inferred from the type of mutation in the *NR3C1* gene, as there is not a clear phenotype-genotype relationship, with clinical symptoms varying even among patients and relatives with the same disease-causing mutation.⁵ Therefore, GCCR may be suspected when hypercortisolism is present and there is no other associated clinical feature. Molecular analysis of the *NR3C1* gene is

recommended in order to confirm the diagnosis and provide a better genetic counseling to the affected families.

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Conflict of interest

The authors have nothing to disclose.

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Medullary thyroid carcinoma as manifestation of the loss of heterozygosity in a patient with MEN1



Carcinoma medular de tiroides como manifestación de la pérdida de heterocigosidad en un paciente con MEN1

Multiple endocrine neoplasia type 1 (MEN1) is a rare inherited disease characterized by hyperplastic and neoplastic

disorders mainly affecting parathyroids, anterior pituitary and gastroenteropancreatic endocrine tissues.¹ In most subjects with MEN1 phenotype a heterozygous germline mutation in the *MEN1* gene is identified. In addition, somatic mutations and large deletions of the *MEN1* locus and its surrounding regions in chromosome 11, known as loss of heterozygosity (LOH), are found in MEN1 associated tumors.² We hereby present a MEN1 patient who developed a thyroid neuroendocrine carcinoma, and analyze the potential involvement of *MEN1* gene in the development of this tumor.

A 44 year-old Caucasian woman with MEN1 familial background was identified as a carrier of the mutation c.549G>A (p.Trp183*) in exon 3 of *MEN1* gene in 2008. Previously,