

A Novel Pathogenic variant in *NR0B1* gene associated with Congenital Adrenal Hypoplasia

Nueva Variante Patogénica en el Gen *NR0B1* Asociada a Hipoplasia Adrenal Congénita

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What do we know about the subject matter of this study?

X-linked adrenal hypoplasia congenita (AHC) (OMIM # 300200) is a rare genetic disorder. It has three main features: primary adrenal insufficiency, hypogonadotropic hypogonadism, and infertility.

What does this study contribute to what is already known?

We identify a new "probably pathogenic" variant c.1275A>T; p.Arg425Ser in the *NR0B1* gene compatible with X-linked AHC, identified in a male patient with primary adrenal insufficiency associated with history of hypogonadism.

Abstract

X-linked adrenal hypoplasia congenita is a rare cause of primary adrenal insufficiency. Mutations in the *NR0B1* gene cause a loss of function in the DAX1 receptor, which activates genes involved in the development and function of the hypothalamic-pituitary-gonadal axis. **Objective:** To describe a case of adrenal hypoplasia congenita secondary to a mutation in the *NR0B1* gene and identified the differential diagnoses of the pediatric patient with adrenal insufficiency and hypogonadotropic hypogonadism. **Clinical Case:** A 4-year-old male patient with no relevant history and from a rural area was admitted to the emergency room due to a 15-days of emesis, asthenia, adynamia, myalgia, and ataxic gait. On the physical examination, hypotension, hyponatremia, and hyperkalemia, as well as mucosal hyperpigmentation and bilateral cryptorchidism were observed, therefore, adrenal crisis was diagnosed, starting fluid resuscitation with saline solution, hydrocortisone, and fludrocortisone, which stabilized the patient. Adrenal hyperplasia congenita, innate metabolic error, and infectious or

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autoimmune etiology were ruled out as etiology. A clinical exome test was performed which identified the variant c.1275A > T; p.Arg425Ser (Transcript ENST00000378970.5) in the *NR0B1* gene consistent with X-linked adrenal hypoplasia congenita. Management of the patient continued with glucocorticoids and mineralocorticoids with favorable clinical course at 7 years of follow-up. **Conclusion:** A novel pathogenic variant associated with X-linked adrenal hypoplasia is described. Variants in the *NR0B1* gene should be a differential diagnosis in a male patient with the association of primary adrenal insufficiency and hypogonadism.

Introduction

Primary adrenal insufficiency (PAI) is a rare, life-threatening disorder in childhood, characterized by inadequate synthesis and/or secretion of glucocorticoids in the adrenal cortex. In some cases, it may also affect the production of aldosterone and adrenal androgens. In contrast to the predominance of autoimmune etiologies in adults, most causes of PAI in childhood are of hereditary and monogenic origin¹⁻³. According to the pathophysiological mechanism, the genetic forms of PAI can be divided into 4 groups⁴: (a) Disorders in genes involved in the formation of adrenal organs resulting in hypoplasia (*NR0B1*, *NR5A1*) or dysgenesis (*CDKN1C*, *SAMD9*); (b) Inborn errors of metabolism affecting cholesterol (*DHCR7*) and steroid biosynthesis (such as all forms of congenital adrenal hyperplasia); (c) Familial glucocorticoid deficiency (*MC2R*, *MRAP*, *NNT*) and similar disorders (*MCM4*); (d) Adrenal destruction disorders (*LIPA*, *ABCD1/2*, *PEX1*, *AIRE*).

X-linked adrenal hypoplasia congenita (AHC) (OMIM # 300200) is a very rare genetic disorder. It occurs in less than 1:12,500 live births, caused by mutations in the *NR0B1* gene⁵. This gene codes for the DAX-1 receptor (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1), which is an "orphan" nuclear receptor, as no specific ligand has been identified. One of the actions of DAX-1 is to activate target genes involved in the development and function of the hypothalamic-pituitary-gonadal (HPG) axis. Paradoxically, however, most functional studies have shown that DAX-1 is an inhibitor of steroid biosynthesis that acts by repression of steroidogenic factor-1 (SF1), encoded by the *NR5A1* gene, mediating the transactivation of several genes in the steroid biosynthesis pathway, such as the steroidogenic acute regulatory protein (StAR), 3-hydroxysteroid dehydrogenase, and cholesterol desmolase genes^{6,7}. It also inhibits luteinizing hormone (LH) transcriptional activities and reduces gonadotropin-releasing hormone (GnRH) expression, therefore, mutations at this level affect the development and function of the adrenal gland^{7,8}.

The classic form of X-linked AHC has three main features: PAI, hypogonadotropic hypogonadism, and infertility^{9,10}. Early recognition of clinical manifestations allows the initiation of timely treatment with glucocorticoids and hydroelectrolytic correction (with or without mineralocorticoids), which is essential to reduce the risk of mortality, while the diagnostic exercise and etiological search are carried out¹¹. Clinical signs and symptoms include typical features of adrenal insufficiency such as hyperpigmentation in gums and nail folds, vomiting, poor feeding, growth failure, seizures, cardiovascular collapse, and sudden death. Characteristic biochemical findings are hyponatremia, hyperkalemia, hypoglycemia, reduced serum cortisol, and aldosterone levels, and increased adrenocorticotrophic hormone (ACTH)¹².

Hypogonadism associated with X-linked AHC probably represents a combination of hypothalamic and pituitary defect. Many boys do not initiate puberty and others present with arrest of pubertal development around Tanner stage 3 or testicular volume of 6-8 ml. Surprisingly, there has been an increasing number of reports of exaggerated sexual maturation or precocious puberty in X-linked AHC. The mechanism of precocious puberty is unclear and may be multifactorial. Causes such as Leydig cell autonomy, persistent androgen production by the fetal adrenal gland, ACTH stimulation of steroidogenic cells in the testis via the melanocortin-1 receptor, and impaired negative feedback loop of the HPG axis have been postulated^{10,12}.

The correct diagnosis of PAI due to genetic disorders is essential because of the required monitoring of certain comorbidities and the different forms of inheritance in the different subtypes. Accurate molecular diagnosis is important not only to adequately guide parents about the probability of involvement in a future pregnancy but also to identify affected but clinically asymptomatic mutation carriers in the family⁸.

The objective of this article was to describe a case of AHC secondary to a mutation in the *NR0B1* gene that allows knowing the differential diagnoses of the pediatric patient with adrenal insufficiency and hypogonadotropic hypogonadism.

Clinical Case

Male patient from a rural area in the department of Antioquia, Colombia, fourth child, term newborn, with weight and height appropriate for gestational age, and without hospitalization in the neonatal period. Subsequently, he had no relevant history and normal psychomotor development. He was admitted to the emergency department at the age of 4 years due to 15 days of vomiting, asthenia, adynamia, myalgia, and ataxic gait. Physical examination on admission showed hypotension (blood pressure below the 5th percentile for age and height), hyperpigmentation of skin and mucous membranes (figure 1), and male genitalia with penis of normal size and thickness but with bilateral cryptorchidism. Additionally, he had alopecic plaques in the left frontotemporal region. Initial laboratory tests showed hyponatremia (serum sodium 128 mEq/L) and mild hyperkalemia (serum potassium 5.05 mEq/L) associated with low serum cortisol (figure 2).

Given clinical and laboratory findings, adrenal crisis was diagnosed and hydric reanimation with normal saline and hydrocortisone was started with an initial dose of 100 mg/m²/day, followed by a maintenance dose of 50 mg/m²/day every 6 hours. Due to persistent hyponatremia, fludrocortisone was added at a dose of 0.1 mg every 12 hours, achieving hemodynamic and electrolyte stability (figure 2).

In the neurological evaluation, there was no evidence of central or peripheral nerve involvement, and the ataxic gait was resolved, so it was assumed that it was secondary to hyponatremia.

It was considered compatible with primary adrenal insufficiency and, within the etiological possibilities, adrenal hyperplasia congenita was ruled out due to 21-hydroxylase deficiency because of normal levels of 17-hydroxyprogesterone. Given the possibility of adrenoleukodystrophy due to age, sex, and history of ataxia, brain MRI and very-long-chain fatty acids test were performed, which ruled them out. Autoimmune

etiology was ruled out with negative anti-21-hydroxylase antibodies. An adrenal CT scan showed no lesions or hemorrhage. Infectious etiologies were ruled out with negative studies for *Mycobacterium tuberculosis*, Cytomegalovirus, and Human Immunodeficiency Virus. No drugs were found to be involved in the genesis of adrenal insufficiency, nor other causes of secondary adrenal insufficiency.

Given the onset of PAI with no established etiology, it was considered necessary to rule out genetic cause due to probable adrenal hypoplasia congenita, and clinical exome sequencing was requested which identified a “probably pathogenic” variant c.1275A>T; p.Arg425Ser in the *NR0B1* gene¹³.

At the date of the report, the patient was 11 years old, the cryptorchidism had been corrected, the testicular volume was 2 to 3 ml bilaterally, continuing follow-up by pediatric endocrinology, in treatment with hydrocortisone at a dose of 13 mg/m²/day and fludrocortisone 0.1 mg every 12 hours, with adequate clinical evolution, without new adrenal crisis, and with adequate growth pattern. Laboratory studies showed normal values of electrolytes and plasma renin. Still without clinical onset of puberty and with Luteinizing Hormone (LH) values within the prepubertal range.

Discussion

Childhood PAI is a rare disorder which is predominantly hereditary and monogenic (80% of cases)¹⁴⁻¹⁶. Molecular advances have expanded knowledge about the etiology of PAI, but clinical diagnosis may still be delayed until an acute illness or stressful event precipitates a severe cardiovascular collapse resulting in an adrenal crisis^{8,10}. This case describes a male patient with childhood-onset adrenal insufficiency associated with bilateral cryptorchidism secondary to X-linked adrenal hypoplasia due to a novel, probably pathogenic variant in the *NR0B1* gene.



Figure 1. Gum and flexural chronic hyperpigmentation.

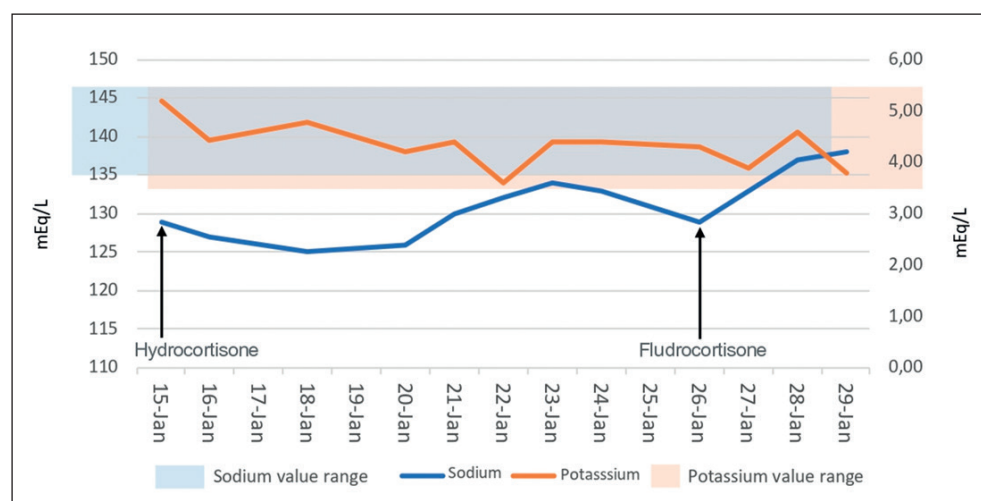


Figure 2. Follow-up of the electrolyte imbalance.

Currently, there are no studies to make an early neonatal diagnosis and usually, patients with X-linked adrenal hypoplasia present with adrenal crisis in the first months of life, while 40% present it between 1-9 years of age, similar to what occurred in this patient¹⁷. As observed in this case, it is usual that the adrenal crisis is accompanied by various degrees of hyperpigmentation related to the increased production of proopiomelanocortin. In some cases, the identification of hyperpigmentation and its early study would allow a more opportune diagnosis⁵.

Gonadotropic axis involvement is usually severe with delayed puberty, although cases of arrested puberty have been described and some patients even develop gonadotropin deficiency between 15 and 18 years of age. Patients present alteration in spermatogenesis with gradual worsening, oligospermia, and endocrine dysfunction of Sertoli cells^{17,18}. In this case, the phenotype gives evidence of bilateral cryptorchidism, indicating impairment of the gonadal axis function since the fetal period. Absent or delayed pubertal development would be expected, but this was not yet identifiable because of the patient's age at the time of the report.

Genetic testing is useful and, in the last 15 years, has contributed to the diagnosis in children with PAI of unknown etiology or to confirming it in those with a history¹⁸. It is important to investigate consanguinity, family history of PAI or unexplained deaths in maternal brothers or uncles, and evidence of hypogonadotropic hypogonadism. The diagnostic performance of the *NR0B1* gene study in male patients with PAI with adrenal crisis is as high as 40% if other common conditions such as adrenal hyperplasia congenita have been excluded⁶.

As of 2006, about 80 to 100 different *NR0B1* gene mutations had been described, but to date, more than

200 mutations have been identified¹⁹. The variant c.1275A > T; p.Arg425Ser in the *NR0B1* gene has not been previously described, but it is believed that it is a possible pathogenic missense variant¹³. In our patient, the location of the codon mutation is a highly conserved amino acid position in the ligand-binding domain of the receptor and constitutes the codon with the most reported mutations (along with codon 291)^{6,20}. The mutation reported in this patient is the fourth at codon 425, suggesting that this is a hot spot mutation in this gene. Our report is about the codon change AGA > AGT (X:30'304,717A > T), corresponding to one of the few exceptions to the degeneracy characteristic of the genetic code¹³. This exception occurs because the change in the third codon position leads to a change in the encoded amino acid.

The reported variant is classified as probably pathogenic as supported by In Silico functional predictions using the sequence recorded in UniProtKB (P51843). Since this is an exonic variant, we considered the functional predictors SIFT (P = 0) and Polyphen2 (P = 1.0) which predicted that this variant is harmful or probably deleterious, respectively.

Although in most cases they do not establish a genotype-phenotype correlation, there are exceptions such as variants in the amino-terminal nonsense region or variants in the ligand-binding region around the hydrophobic core that are associated with the late onset of the disease^{5,19,21}. Additionally, most cases associated with nonsense or frameshift mutations establish an absent or truncated protein with a severe secondary phenotype. Missense mutations are reported in 20% of cases with a variable phenotype. This case suggests that the new variant would be associated with a late evolution and development of the disease¹⁷.

Management of X-linked AHC includes glucocor-

ticoid and mineralocorticoid replacement, in order to mimic the physiological circadian rhythm of cortisol secretion and to avoid the effects of long-term glucocorticoid overexposure, including growth suppression, obesity, metabolic syndrome, diabetes, and osteoporosis²¹. Hormone levels should be monitored during the pubertal period and, if there is no spontaneous development, initiate sex steroid replacement therapy for pubertal induction¹². It is currently unknown if the previous use of recombinant Follicle Stimulating Hormone (FSH) has any long-term benefit in case assisted reproductive techniques are used. Psychotherapeutic support for young people and their families is important, especially to discuss puberty and fertility issues. Additionally, education on stress patterns should be established to prevent decompensation and death from acute adrenal insufficiency^{6,21}.

Conclusion

This case extends the pathogenic variants associated with the spectrum of X-linked adrenal hypoplasia. In those male patients with the presence of PAI independent of the age of clinical manifestation, especially if it is associated with findings suggestive of hypogonadotropic hypogonadism, the possibility of variants in the *NR0B1* gene should be evaluated. Its adequate treatment and recognition are essential to avoid the associated morbidity and mortality.

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Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the parents (tutors) of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

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