

# Mild adrenal insufficiency due to a NROB1 (DAX1) gene mutation in a boy presenting an association of hypogonadotropic hypogonadism, reduced final height and attention deficit disorder

*Insuficiência adrenal leve causada por mutação do gene NROB1 (DAX1) em menino com uma associação de hipogonadismo hipogonadotrófico, baixa estatura final e transtorno de déficit de atenção*

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## SUMMARY

Mutation on NROB1 (DAX1) gene can cause different phenotypes of adrenal insufficiency in infancy. Long-term evolution of these patients shows that it is possible to have an association with hypogonadotropic hypogonadism. In this article we describe the evolution of a patient with NROB1 gene mutation, diagnosed with a mild form of adrenal insufficiency, and we highlight the presence of hypogonadotropic hypogonadism and short stature, besides the presence of attention deficit disorder. Such associations should make physicians aware during the follow-up of patients with this disease. *Arq Bras Endocrinol Metab.* 2013;57(7):562-5

## SUMÁRIO

Mutações no gene NROB1 (DAX1) podem levar a quadros de insuficiência adrenal com diferentes formas de apresentação na infância. A evolução a longo prazo desses pacientes mostra que pode haver associação com hipogonadismo hipogonadotrófico. Neste artigo, relatamos a evolução de um paciente com uma mutação do gene NROB1, diagnosticado com uma forma leve de insuficiência adrenal, na qual chamamos a atenção para a evolução com hipogonadismo hipogonadotrófico e baixa estatura final, além de apresentar transtorno de déficit de atenção. Tais associações devem ser motivo de atenção para os médicos no seguimento de pacientes portadores dessa alteração. *Arq Bras Endocrinol Metab.* 2013;57(7):562-5

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## INTRODUCTION

Congenital adrenal insufficiency is estimated to have an incidence of 1 in 12,500 births (1). Non-specific symptoms can begin in the first days of life or late in childhood; therefore, the disease may not be easily detected, delaying diagnosis and treatment. Congenital adrenal insufficiency can be caused by a number of diseases, including X-linked congenital adrenal hypoplasia (AHC) due to mutations in the NROB1 gene (2).

The NROB1 (DAX1) gene (dosage-sensitive sex reversal – congenital adrenal hypoplasia critical region on X chromosome) is located at the Xp21.3 region, and encodes an unusual orphan member of the nuclear hor-

mone receptor superfamily (HGNC:7960). All described NROB1 gene mutations alter the protein carboxy-terminal domain (ligand-binding domain), and impair the properties of bearing transcriptional repressor activity. This gene acts as a transcriptional repressor of genes involved in the steroidogenic pathway (2). The phenotype of the patients with different mutations is variable, and the presentation can occur in the newborn, during childhood, or even in adult life (3). Several mutations have been described in this gene, leading mainly to AHC, but also to hypogonadotropic hypogonadism. In the present report, we describe the long term evolution of a patient with primary adrenal insufficiency and hypogonadotropic hypogonadism due to a NROB1 gene mutation.

## CASE PRESENTATION

Patient MRD, male, born in June 17<sup>th</sup>, 1995.

Patient was referred to us for evaluation because he had two younger brothers who had been previously diagnosed with adrenal insufficiency due to NROB1 mutation (4). At that time, he was 8 years old, and there were no notable complaints. He had good general health, but was a hypoactive boy and easily fatigable. On physical examination, he had no alterations, except by mild gingival and skin hyperpigmentation. Pubertal stage was G1P1, testicular volume 2 cm<sup>3</sup>, penile length 4.5 cm. Height = 129.5 cm (p25-50), weight = 27, kg (p25-50). BP: 100/60 mmHg. TH = 180 cm.

Initial laboratory data: Na = 133 mEq/L; K = 3.8 mEq/L; ACTH = 4,000 pg/mL (10-60 pg/mL – chemoluminescence); cortisol = 3.9 µg/dL (5.4-25 µg/dL – RIA); aldosterone < 1.0 ng/dL (1-16 ng/dL – RIA).

Due to the fact that his brothers had confirmed familial primary adrenal insufficiency because of a NROB1 gene mutation, molecular evaluation was performed. The same novel mutation as his brothers was found in exon 1 of the NROB1 gene, consisting of a transition from C to T, determining a stop codon at position 359 (Q359X). The mutated gene encoded a truncated protein missing a large portion of the ligand-binding domain (C-terminal domain). This was the same mutation found in his younger brothers and in his mother (Figure 1).

To evaluate the NROB1 (DAX) gene, the following protocol was used: DNA samples were extracted from peripheral blood lymphocytes, and the entire coding region of the DAX1 gene was amplified by PCR using specific primers. Exon 1 was amplified with primers 1F - 5' - cac tgg gca gaa ctg ggc tac - 3' and 1R - 5' - cgc ccc tag ata ggc act ggc - 3' (Invitrogen™

Life Technologies, Carlsbad, CA, USA), using an initial denaturation step at 94°C for 5 min, followed by 40 cycles consisting of 94°C for 1 min, 65°C for 1.5 min and 72°C for 2 min, with a final extension step at 72°C for 10 min. Exon 2 was amplified with primers 2F - 5' - gct agc aaa gga ctc tgt ggt - 3 and 2R - 5' - cag ctc ttt att ctt ccc tca - 3', using an initial denaturation step at 94°C for 5 min, followed by 35 cycles consisting of 94°C for 1 min, 60°C for 1 min and 72°C for 2 min, with a final extension step at 72°C for 10 min. Both PCR reactions included 20 pmol of each primer, 200 µmol of each deoxynucleotide, 0.5 U of the *Taq* DNA polymerase, 10X buffer, 50 mmolar MgCl<sub>2</sub> (cat# N801-0055, GeneAmp – PCR reagent kit with AmpliTaq DNA polymerase Perkin Elmer, Branchburg, NJ, USA) and ddH<sub>2</sub>O to obtain a final volume of 25 µL in a thermocycler (GeneAmp PCR System 9700, Applied Biosystem, Forster City, CA, USA). PCR products were, respectively, 1275 and 364 bp.

Direct sequencing analysis was performed by fluorescent dideoxynucleotides on an ABI PRISM 310 automatic sequencer, using internal primers for exon 1 (1iF - 5' - ggt aaa gag gcg cta cca ggc - 3' and 1iR - 5' - cgc ttg att tgt gct cgt gg - 3') and the same primers used for the PCR reaction for the exon 2 sequencing, following the manufacturer's protocol (ABI PRISM BigDye Terminator, version 3.1, Cycle Sequencing Kit, Applied Biosystem, Forster City, CA, USA).

The diagnosis of congenital adrenal hypoplasia due to a NROB1 gene mutation was confirmed, and treatment with glucocorticoids and mineralocorticoids was initiated.

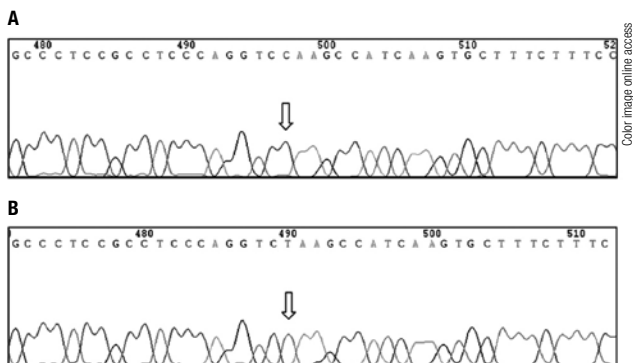
Introduction of medication resulted in significant improvement in physical aspects, increased resistance in sports and other activities, alleviation of hyperpigmentation, and also psychological benefits.

During the period of infancy and adolescence, he received prednisolone, doses varying between 3 – 5 mg/m<sup>2</sup>/day, associated to fludrocortisone, 50 mcg/d.

At 11 years of age, he complained about the size of his penis. He had G1P1 Tanner Stage of puberty, with a testicular volume of 2 cm<sup>3</sup>, and penile length of 4.5 cm; BP = 90/60 mmHg.

Aiming to increase of penile size, he received 2 cycles of 3 monthly doses of testosterone esters, separated by a 6-months interval between them, with satisfactory results in patient's view, penile length grew to 5.5 cm.

At 12.5 years of age, he still had no pubertal development and was complaining about genital devel-



**Figure 1.** Sequence analysis of exon 1 of the NROB1 gene. (A) Control – normal individual; (B) Index case. Arrows show the mutation site.

opment. Initial laboratory investigation showed: Na = 142 mEq/L; K = 3.2 mEq/L; DHEA Sulfate < 7 mcg/dL; total testosterone = 9 ng/dL; androstenedione = 30 ng/dL; LH < 0.1 mU/L; FSH = 0,3 mU/L; bone age = 12 years.

Although he had no pubertal delay at that moment, hypogonadotropic hypogonadism was suspected, and cycles of reposition with testosterone esters were initiated, mainly due to patient's request. After each 3-month cycle with testosterone, medication was suspended, and no signs of puberty appeared spontaneously. Initial dose of testosterone esters was 75 mg monthly, and it was increased slowly to 250 mg. There was genital development and increase in pubic hair growth. During this period, bone age was always concordant with chronological age.

He was treated until he was 17 years old. At this point, he weighed 58 kg, measured 169 cm, and growth velocity was 1.5 cm/year. Physical examination showed penile development equivalent to Tanner stage 4; pubic hair 4; testicular volume = 2 cm<sup>3</sup>; BP = 110/80 mmHg.

Testosterone was withdrawn and the gonadotropic axis was re-evaluated, in use of glucocorticoids and mineralocorticoids. Laboratory data confirmed the hypogonadotropic hypogonadism, as follows: total testosterone = 9 ng/dL; LH < 0.1 mU/L; FSH = 0.4 mU/L; Na = 143 mEq/L; K = 3.9 mEq/L; glycemia = 90 mg/dL; aldosterone < 1.0 ng/dL (VR:1-16); renin plasmatic activity = 0.9 ng/mL/h (0.5-2.5); bone age = 16 years; bone mineral density (DEXA) = 1,136 g/cm<sup>2</sup> (Z=+0.2); fat content = 30%.

### Psychosocial evolution

The patient always had difficulties at school, failing two years (1<sup>st</sup> and 2<sup>nd</sup> year) in high school. Evaluated by a neurologist and a psychologist, they reported that he had impaired executive functions associated with depression and anxiety. He was started on long-action methylphenidate (20 mg), with good response regarding school development. Against his parents' will, due to his learning difficulties, he changed schools and will quit studying after graduating in high school.

### DISCUSSION

Although there are many reports of adrenal insufficiency due to NROB1 gene mutation, only a few articles

refer to the long term evolution of these patients. The present case has some aspects of medical interest.

The patient had no important complaints, and the investigation was done only because of the familial history – his brothers had severe symptoms of adrenal insufficiency in the first months of life and had been diagnosed as having NROB1 mutation. This difference highlights the discrepancy between genotype and phenotype, and the importance of the investigation of this disease even with mild symptoms.

The main important fact during his follow-up was the lack of pubertal development. Clinical evolution and laboratory exams confirmed a hypogonadotropic hypogonadism (HH), which is a combination already described in other cases of NROB1 gene mutation, associated with adrenal insufficiency. Muscatelli and cols. (5) found that mutations in the NROB1 gene can determine both AHC and hypogonadotropic hypogonadism, confirming that this gene is essential for the development of a functioning hypothalamus-pituitary-gonadal axis. Not only adrenal symptoms can be variable with the same mutation. Habiby and cols. (6) studied the secretion of LH and FSH, and found a heterogeneous pattern of response to GnRH, suggesting that NROB1 mutations impair gonadotropin production acting at both hypothalamic and pituitary levels. In Brazil, there is one report of a missense mutation (A300V) causing AHC and hypogonadotropic hypogonadism in a boy (7).

Interestingly, Calvari and cols. reported two siblings, very similar to our family, with different ages of adrenal symptoms, but both with HH (8). Regarding pubertal development, there is only one description of precocious puberty associated to NROB1 gene mutation, bringing more discussion about the mechanisms involved (9).

The induction of puberty was done at 12-13 years of age, due to psychological aspects, referred by the mother as social maladjustment and behavior changes. After puberty, he reached a near final height of 169 cm, lower than the familial target height. Final stature of patients with NROB1 mutation is scarcely reported in the literature, but there is a case report of Perez Rodriguez and cols. (10), who describe a boy, very much like our patient, with adrenal hypoplasia, hypogonadotropic hypogonadism and short stature, final height of 150 cm, versus target height of 160 cm.

It is important to notice that, if the diagnosis of the younger brothers had not been done, maybe this

patient would go until pubertal age without knowing about his condition. He would probably be evaluated due to pubertal delay, and the diagnosis of idiopathic hypogonadotropic hypogonadism would be the most probable suspicion. NROB1 gene mutation is normally not cited as a possible cause of HH, so it would be difficult to think about adrenal insufficiency without the brother's diagnosis. These facts suggest that maybe some of the patients with HH could have NROB1 gene mutation with oligosymptomatic adrenal insufficiency.

Other important fact to be noted was the psychological evolution of this patient. He was diagnosed as having executive function alterations associated with depression and anxiety, besides important learning difficulties. This called our attention, especially because the other two brothers probably have the same problem and are under evaluation. Although the diagnosis of attention deficit disorder is not uncommon nowadays, it is possible that this condition might be associated with the mutation itself, to the chronic reduction of cortisol or even secondary to previous unnoticed moments of dehydration and hyponatremia.

In conclusion, we described the evolution of a teenager with NROB1 gene mutation diagnosed due to an oligosymptomatic adrenal insufficiency, who later developed hypogonadotropic hypogonadism, short stature and attention deficit disorder. This report should encourage other investigators to look for these associations in patients with NROB1 gene mutation.

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