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CASE REPORT

Primary adrenal insufficiency caused by DAX1 gene deletion

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Abstract

Primary adrenal insufficiency (PAI) is a potentially serious disease. Early diagnosis and treatment are required to reduce its morbidity and mortality. This article reports the case of a newborn who started showing signs and symptoms suggestive of congenital PAI at 2 days of age. However, adequate treatment was started only at 3 months of age, after an evaluation by pediatric endocrinologists. Later, a genetic analysis showed a mutation in the DAX1 gene. In PAI, the initial clinical presentation may be nonspecific and mistaken for several common diseases of the neonatal period, which delays the administration of adequate therapy. Genetic analysis is increasingly important for the management of such cases, not only in family counseling but also because it allows an earlier diagnosis, which can prevent the serious clinical manifestations of this disease by administering adequate therapy.

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INTRODUCTION

Primary adrenal insufficiency (PAI) is defined as an inability of the adrenal cortex to produce sufficient amounts of glucocorticoids or mineralocorticoids.^{1,2} It is a rare condition, but it is serious and potentially lethal. In children, the congenital form is more common, and congenital adrenal hyperplasia (CAH) is its leading cause. The incidence is estimated to be 1:10,000 to 1:15,000 live births.³ The highest prevalence is associated with mutations in the CYP21A2 gene, leading to a deficiency of the 21 β hydroxylase enzyme. Less frequently, other genes are also affected such as those that encode 11 β hydroxylase, 3 β hydroxysteroid dehydrogenase, and 17 α hydroxylase.³ All these defects have an autosomal recessive inheritance.

In addition to genetic defects affecting steroidogenesis, there are other causes of PAI, such as adrenal hypoplasia, familial glucocorticoid deficiency, resistance to adrenocorticotrophic hormone (ACTH), and gland destruction from infectious or autoimmune causes.^{4,5} The initial PAI clinical presentation may be nonspecific, and affected children may die from a seizure, respiratory distress, or salt-losing crisis even before reaching a hospital. They may also be misdiagnosed with sepsis or other diseases, delaying the administration of adequate treatment.

In particular, in CAH cases, children may present with different clinical manifestations, depending on the degree of adrenal steroidogenesis impairment and of residual enzymatic activity.³ In classic CAH forms, excess androgens may be present since birth, causing virilization of male genitals in the first years of life and ambiguous genitals in girls.

Early recognition and treatment are determining factors in PAI morbidity and mortality. Recently, several genetic mutations have been identified that are valuable for use in genetic counseling, while making diagnosis and treatment possible before symptoms appear.⁵

CASE REPORT

K.G.S.S., a male born on September 19, 2015. This was the mother's fourth pregnancy, with three previous births and no miscarriages; she presented with pregnancy-induced hypertension and was medicated with alpha-methyl dopa. No gestational diabetes or other morbid conditions were observed in the prenatal period. The patient was born by cesarean section at 41 weeks and 1 day. He weighed 3,030 g at birth, his height was 49 cm, and his Apgar score was 9/9.

On the second day of life, the newborn started with vomiting, gaze shift, myoclonic contractions, hypoactivity, and feeble sucking reflex and had to be admitted to a neonatal intensive care unit (NICU). Laboratory tests were performed and showed a peripheral capillary glycemia of 29 mg/dL, serum sodium of 123 mg/dL, and serum potassium of 6.7 mg/dL.

While at NICU, hydroelectrolytic imbalance persisted despite adequate therapy, including electrolyte replacement. Other clinical alterations were diagnosed, with elevated urea

and transaminases, and the newborn was treated for late neonatal sepsis and kidney failure.

At 3 months of age, the infant was transferred to a pediatric hospital unit, and the pediatric endocrinology sector was asked to evaluate him. At that moment, he was in normal clinical condition, hypoactive, reactive to handling, hypotonic, moderately dehydrated, and with no jaundice or cyanosis. The anterior fontanelle was depressed and the skin was dry, rough, and hyperpigmented.

The cardiovascular system had a regular heart rate of 124 beats per minute in two stages, normal sounds, no murmurs, good peripheral capillary perfusion, and full, symmetrical pulse. The respiratory system examination showed slight subcostal depression and suprasternal retraction, vesicular murmur audible throughout, no adventitious sounds, and a respiratory rate of 24 breaths per minute.

The abdomen was flaccid, peristaltic, depressible, and painless, with no masses or visceromegaly. The genitals were typically male, testes in the inguinal canal were bilaterally palpable, and the Tanner score was G1P1. Laboratory tests showed sodium 115 mg/dL, potassium 6.9 mg/dL, urea 135 mg/dL, SGOT 431 mg/dL, and SGPT 521 mg/dL; arterial gasometry indicated metabolic acidosis.

Other tests were also performed at maternity, such as karyotype, which was 46XY, 17hydroxyprogesterone 136.4 ng/dL (reference value: 200 ng/dL), ACTH 1,250 pg/mL (reference value: 6-76 pg/mL), and serum cortisol below 1 μ g/dL. An abdominal ultrasound showed no changes and no visible adrenals.

Because congenital adrenal insufficiency was suspected in addition to the therapy for hydroelectrolytic imbalance control, hydrocortisone was started at a loading dose (100 mg/m²/day, administered intravenously every 6 h), followed by a stress dose (50 mg/m²/day, divided into four daily doses), and sodium replacement at 2 mEq/kg/day.

Although still at the hospital, because the patient clinically improved, the corticosteroid dose was adjusted, switching to oral prednisolone and fludrocortisone. On February 24, 2016, the infant was discharged from the hospital, with a prescription for prednisolone 3 mg/m²/day, fludrocortisone 0.3 mg/day, and salt, all taken orally.

A genetic and molecular analysis of the patient's peripheral blood was performed using the multiplex ligation probe-dependent amplification (MLPA) technique to detect any changes in the number of copies of genes involved in gonad development. The analysis showed a deletion region of more than 78 megabases long, involving genes NR0B1 (known as DAX1) and CXorf21 (Table 1), thus confirming the PAI diagnosis.

This analysis method is based on the hybridization of DNA to two specific probes for each region to be studied, totaling six genes (PHEX, NR0B1, CXorf21, PQBP1, GJB1, and COL4A5), which are later joined by a ligase enzyme. Then the joined products are amplified by PCR using universal primers,

Table 1. Results of DNA analysis by the MLPA technique, using the MRC-Holland P185 v.C2 test kit. The deleted region is framed in red.

Gene	Genomic position in the X chromosome	Patient
<i>PHEX</i>	X-022.106365	+
<i>NROB1</i> -Exon 2	X-030.232695	-
<i>NROB1</i> -Exon 1B	X-030.236440	-
<i>NROB1</i> -Exon 1A	X-030.237398	-
<i>NROB1</i> - region A	X-030.255082	-
<i>NROB1</i> - region B	X-030.363644	-
<i>CXorf21</i> -2	X-030.505510	-
<i>CXorf21</i> -1	X-030.525214	-
<i>PQBP1</i>	X-048.644183	+
<i>GJB1</i>	X-070.360638	+
<i>COL4A5</i>	X-107.725394	+

and the results are read by genotyping software on sequencing devices.

DISCUSSION

CAH is a rare developmental disorder of the adrenal gland.⁶ It has an incidence estimated to be 1:12,500 live births and is inherited in both autosomal recessive and X-linked forms.⁶ The X-linked form is caused by deletions or mutations in the *DAX1* gene, which is located in the short arm of the X chromosome at position 21. Its incidence is estimated to be 1:140,000 to 1:200,000 live births.^{7,8}

The *DAX1* gene encodes a 470-amino acid protein that is part of a large family of nuclear receptors and has an important role in the development and function of hormone-producing tissues in glands such as the adrenals, pituitary, hypothalamus, and gonads.⁹ The patient in question had a hemizygous microdeletion in the genomic region of the short arm of the X chromosome, as described above. In this case, for purposes of genetic counseling, an investigation of the mother regarding the presence of a heterozygous deletion is indicated.⁹

The clinical presentation of CAH is variable. Approximately 60% of male children with a *DAX1* mutation will develop early PAI, with salt-losing crises in the first 2 months of life.^{6,8} The disease can also have a more insidious presentation, with symptoms triggered by stress or during puberty with hypogonadotropic hypogonadism.^{6,8} In girls, heterozygous *DAX1* mutations are associated with a late menarche, whereas homozygous mutations lead to hypogonadotropic hypogonadism.⁸

In the case described herein, the patient already presented with clinical manifestations of adrenal insufficiency in his first days of life. In newborns and infants, PAI signs and symptoms may be nonspecific and cause difficulties for diagnosis, delaying the introduction of adequate treatment. In general, manifestations of cortisol deficiency may appear, with hypoglycemia, weight loss, vomiting, anorexia, anemia,

prolonged jaundice, and postural hypotension. Signs and symptoms of mineralocorticoid deficiency are also common, with salt loss, hyponatremia, hyperkalemia, metabolic acidosis, hypotension, dehydration, and hypovolemic shock.^{2,3,7,10}

Hyperpigmentation of the skin and mucosae occurs due to the absence of a negative feedback for ACTH production. The low cortisol concentration in plasma stimulates ACTH hypersecretion, and other peptides from the pro-opiomelanocortin molecule are also produced, including several forms of melanocyte-stimulating hormones.⁷

A diagnosis of PAI is confirmed by a low serum cortisol level associated with a high ACTH level because in such cases the corticotropin-releasing hormone (CRH)-ACTH system is intact.^{1,2} In the congenital form of the insufficiency, adrenal androgens must also be assessed. More usual forms of CAH, such as 21 β hydroxylase deficiency, lead to elevated 17hydroxyprogesterone and adrenal androgens, whereas in glandular hypoplasia these hormones have physiologically low levels before puberty.² In the case described herein, the patient had low cortisol levels, elevated ACTH, and normal 17hydroxyprogesterone, which primarily indicated a diagnosis of adrenal hypoplasia.

A wide gamut of diseases is associated with PAI in children. Measuring the levels of anti-adrenal cortex and anti-21 β hydroxylase antibodies may help diagnose autoimmune adrenalitis.² Metabolic tests may also be necessary, measuring very long-chain fatty acids for adrenoleukodystrophy or 7-dehydrocholesterol for Smith-Lemli-Opitz syndrome.² However, these diseases manifest themselves later in childhood.^{1,2,3} In congenital forms, genetic testing is fundamental to confirm the precise etiology of adrenal insufficiency.²

The treatment of PAI is based on the supplementation of glucocorticoids and mineralocorticoids. Hydrocortisone is the corticosteroid of choice in children because it has a shorter half-life, which minimizes side effects compared with long-acting glucocorticoids.^{2,3,10} The indicated daily dose is in the 8-10 mg/m²/day range, which must be divided into 2 or 3 doses.

Oral hydrocortisone is not available in Brazil; thus, an equivalent dose of prednisolone may be used, but the patient must be monitored because of the greater risk of growth impairment and weight gain associated with prednisolone.³ In patients with aldosterone deficiency, mineralocorticoid supplementation must also be given. Fludrocortisone is indicated at a dose of 100-150 μ g/m²/day.^{3,10} Newborns and infants, particularly until the sixth month of life, may also need oral sodium supplementation, 1-2 g/day, distributed in the diet. All children undergoing treatment for PAI must be evaluated for growth and development like any child while paying special attention to blood pressure.

School age children should make frequent visits to the physician.¹⁰ Patients on chronic corticosteroid therapy should

always be aware of the need to adjust the dose in stressful periods, such as febrile disease, surgical stress, or trauma.¹⁰

If an adrenal crisis is suspected, the child must be given intravenous hydrocortisone at 50 mg/m²/day as well as fluid replacement. Patients with glandular hypoplasia must be clinically followed up regarding puberty and the possible need for androgen supplementation.⁷ Hearing function must also be monitored because hearing impairment is common in affected adolescents.¹¹

The early diagnosis and treatment of PAI are the only ways to reduce the morbidity and mortality associated with this disease. Boys with CAH usually respond well to glucocorticoid and mineralocorticoid supplementation therapy, but they tend to need testosterone to induce puberty, and spontaneous fertility is rare in males who have DAX1 mutations.⁷

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REFERENCES

1. Donohoue PA. Diagnosis of adrenal insufficiency in children. Uptodate; 2016. [acesso 2016 Abr 18]. Disponível em: <https://www.uptodate.com/contents/diagnosis-of-adrenal-insufficiency-in-children>
2. Malikova J, Flück CE. Novel insight into etiology, diagnosis and management of primary adrenal insufficiency. *Horm Res Paediatr.* 2014;82(3):145-57. DOI: 10.1159/000363107 DOI: <http://dx.doi.org/10.1159/000363107>
3. Webb EA, Krone N. Current and novel approaches to children and young people with congenital adrenal hyperplasia and adrenal insufficiency. *Best Pract Res Clin Endocrinol Metab.* 2015;29(3):449-68. DOI: 10.1016/j.beem.2015.04.002 DOI: <http://dx.doi.org/10.1016/j.beem.2015.04.002>
4. Guran T, Buonocore F, Saka N, Ozbek MN, Aycan Z, Bereket A, et al. Rare Cases of Primary Adrenal Insufficiency: Genetic and Clinical Characterization of a Large Nationwide Cohort. *J Clin Endocrinol Metab.* 2016;101(1):284-92. DOI: <http://dx.doi.org/10.1210/jc.2015-3250>
5. Chan LF, Campbell DC, Novoselova TV, Clark AJ, Metherell LA. Whole-Exome Sequencing in the Differential Diagnosis of Primary Adrenal Insufficiency in Children. *Front Endocrinol (Lausanne).* 2015;6:113.
6. Seminara SB, Achermann JC, Genel M, Jameson JL, Crowley WF Jr. X-linked adrenal hypoplasia congenital: a mutation in DAX-1 expands the phenotypic spectrum in males and females. *J Clin Endocrinol Metab.* 1999;84(12):4501-9.
7. Miller WL, Flück CE. Adrenal cortex and its disorders. In: Sperling MA. *Pediatric Endocrinology.* 4th ed. Philadelphia: Saunders-Elsevier; 2014. p. 471-532.
8. Evliyaoglu O, Dokurel İ, Bucak F, Özcabi B, Ercan Ö, Ceylaner S. Primary adrenal insufficiency caused by a novel mutation in DAX1 gene. *J Clin Res Pediatr Endocrinol.* 2013;5(1):55-7. DOI: <http://dx.doi.org/10.4274/jcrpe.895>
9. Rojek A, Krawczynski MR, Jamsheer A, Sowinska-Seidler A, Iwaniszewska B, Malunowicz E, et al. X-Linked Adrenal Hypoplasia Congenita in a Boy due to a Novel Deletion of the Entire NROB1 (DAX1) and MAGEB1-4 Genes. *Int J Endocrinol.* 2016;2016:5178953. DOI: 10.1155/2016/5178953 DOI: <http://dx.doi.org/10.1155/2016/5178953>
10. Bornstein SR, Allolio B, Arlt W, Barthel A, Don-Wauchope A, Hammer GD, et al. Diagnosis and Treatment of Primary Adrenal Insufficiency: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab.* 2016;101(2):364-89. DOI: <http://dx.doi.org/10.1210/jc.2015-1710>
11. Zachmann M, Fuchs E, Prader A. Progressive high frequency hearing loss: an additional feature in the syndrome of congenital adrenal hypoplasia and gonadotrophin deficiency. *Eur J Pediatr.* 1992;151(3):167-9. PMID: 1601004 DOI: <http://dx.doi.org/10.1007/BF01954375>