Identification of a novel mutation in DAX1/NR0B1A gene in two siblings with severe clinical presentation of adrenal hypoplasia congenita

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ABSTRACT

Objective: To search for mutations in DAX1/NR0B1A gene in siblings to establish the molecular etiology of the adrenal hypoplasia congenita (AHC), a rare potentially life-threatening disorder.

Case report: We describe two siblings who presented with salt-wasting syndrome in the newborn period and received hormonal replacement for primary adrenal insufficiency. A diagnostic hypothesis of AHC was suspected because the children maintained, during hormonal treatment, low plasma 17-OH progesterone (17-OHP) and androgens, despite high ACTH levels. Results: DAX1 gene was studied by molecular analysis, which showed a mutation, confirming the diagnosis in the siblings and a heterozygous state in the mother. Direct sequencing of DAX1 revealed an insertion of an adenine base (c1382-1383 A ins), which lead to a pMet461Asp substitution.

Conclusion: A novel frameshift mutation of DAX1 gene, which established the molecular etiology of the AHC in the siblings, was identified. Obtaining a precise genetic diagnosis of this adrenal disorder, which, sometimes, cannot be confirmed only by clinical aspects, may have important implications for the long-term management of the disease.

Keywords: Congenital, hereditary, and neonatal diseases and abnormalities; adrenal insufficiency; frameshift mutation; genetic counseling

RESUMO

Objetivo: Pesquisar mutações no gene DAX1/NR0B1A em dois irmãos com suspeita de hipoplasia adrenal congênita (HAC), rara doença potencialmente fatal, para estabelecer sua etiologia molecular. Relato dos casos: São apresentados os relatos de dois irmãos com síndrome perdedora de sal no período neonatal que receberam terapia de reposição hormonal para insuficiência adrenal primária. O diagnóstico de HAC foi suspeitado porque as crianças mantiveram, durante o tratamento hormonal, níveis plasmáticos reduzidos de 17-OH-progesterona e andrógenos ao lado de níveis elevados de ACTH. Resultados: A análise molecular do gene DAX1 mostrou a mutação, confirmando o diagnóstico nos irmãos e o estado heterozigoto da mãe. No sequenciamento direto do DAX1 foi encontrada inserção de uma adenina (c1382-1383 A ins), levando à substituição pMet461Asp. Conclusão: Uma nova mutação da fase de leitura no gene DAX1 foi identificada, estabelecendo a etiologia molecular da HAC nos dois irmãos. Um diagnóstico genético preciso deste distúrbio adrenal, frequentemente não confirmado apenas pelos aspectos clínicos, pode ter importantes implicações para o maneuseio em longo prazo da doença.

Descritores: Doenças congênitas, hereditárias e neonatais e anormalidades; insuficiência adrenal; mutação da fase de leitura; aconselhamento genético
BACKGROUND

Adrenal hypoplasia congenita (AHC) is a rare potentially life-threatening disorder of adrenal gland development. The condition can be inherited as an autosomal-recessive or X-linked disease, both forms presenting different adrenal morphologies (1). Its precise incidence is unknown, although it has been quoted as approximately 1:12,000 live births (2). However, some authors believe that the true incidence of the X-linked form of AHC is much lower and might occur anywhere between 1:140,000 and 1:1,200,000 children (3).

The X-linked form of AHC (OMIM: 300200) is caused by mutations of DAX1 gene (dosage-sensitive sex reversal, AHC, critical region on the X chromosome, gene 1), also called NR0B1 gene (nuclear receptor, NR, superfamily 0, group B, member 1). It results in primary adrenocortical failure due to the lack of the permanent adult adrenal cortical zone, replaced by large cytomegalic vacuolated eosinophilic cells (1).

DAX1 gene contains two exons of 1,168 and 245 bp, separated by a 3,385 bp intron (4,5). It is expressed in many tissues, like skin, breast and prostate, as well as at all levels of the hypothalamic-pituitary-adrenal and gonadal axis (6). DAX1 encodes a 470-amino acid protein, which contains a C-terminal region showing homology to the ligand-binding domains of other members of the NR superfamily, but that lacks the typical zinc finger DNA-binding domain at the N-terminal region. Instead, it contains cysteines, positioned in the same region, to form a potential novel zinc finger (1,7-9).

Until 2003, about 112 mutations in DAX1 gene had been described in patients with the X-linked form of AHC, most of them being frameshift mutations and small deletions (10). In the last few years, many other DAX1 mutations have been described elsewhere (11-18). There is a wide phenotypic expression of DAX1 deficiency, ranging from severe salt-wasting with glucocorticoid and mineralocorticoid insufficiency presented in early infancy to more insidious and progressive onset of symptoms later in childhood or even in adulthood (14,18-20).

This variability of phenotypic expression of DAX1 deficiency underlines the importance of genetic counseling for known carrier mothers. Prenatal or early postnatal genetic analysis can identify asymptomatic affected males before occurrence of adrenal crisis, decreasing mortality risk and allowing adequate hormonal replacement (24,25).

The objective of this study was to search for mutations in DAX1 in two siblings to establish the molecular etiology of the AHC.

SUBJECTS AND METHODS

Case report

The proband was a term newborn, adequate for gestational age (weight: 2,820 g; length: 47 cm) without history of perinatal complications. At 30 days of life, he was admitted with vomiting, dehydration, failure to thrive and generalized skin hyperpigmentation. He presented with severe hyponatremia (Na+: 103 mEq/L) and normal potassium levels (K+: 3.6 mEq/L), which later on had increased (5.6 mEq/L). Plasma 17-OH progesterone (17-OHP) (440 ng/dL; RV: 53-186 ng/dL) and testosterone concentrations (520 ng/dL; RV: 6-496 ng/dL) were mildly elevated. Androstenedione levels (6.5 ng/mL; RV: <1.6 ng/mL) were found to be significantly elevated. Plasma ACTH levels were very high (2,437 pg/mL; RV: 12-70 pg/mL) as well as plasma renin activity (PRA) (27 ng/mL/h; RV: 0.3-1.6 ng/mL/h). The diagnosis of congenital adrenal hyperplasia, due to 21-OH deficiency (21-OHD) was suspected and replacement with hydrocortisone acetate (20 mg/m²/day) and fludrocortisone (0.1 mg/day) was initiated. After clinical improvement and normalization of blood tests, 20 days later (Na+: 140 mEq/L; K+: 4.8 mEq/L; 17-OHP: 31 ng/dL; androstenedione: 0.3 ng/mL), treatment was maintained and the child was discharged.

From that moment on, his blood analyses showed normal-to-low androgen and 17-OHP levels. Throughout his childhood, he maintained high ACTH (ACTH: 2,437 to 3,940 pg/mL) and variable basal cortisol levels (cortisol: 0.5 to 8.2 µg/dl). Besides this, he didn’t show dehydration or any other unexpected events. At two years of chronological age (CA), the bone age (BA) was 1.5 years (Greulich-Pyle - GP). On his last visit, at 11 years old, he was on steroid replacement (hydrocortisone 14 mg/m²/day plus fludrocortisone 0.05 mg/day), with good adherence to treatment. Physical ex-
amination revealed overweight (40.9 kg; SD = 0.82), and adequate stature (height: 139.8 cm; SD = 0.3). The body mass index (BMI) was 20.9 kg/m², above the 90th centile. He had prepubertal testes, at Tanner I stage. There was hyperpigmentation and dry skin in both cervical areas and internal thighs. Again, ACTH levels were high (670 pg/mL), and basal cortisol levels were in normal range (6.6 µg/dL). Plasma electrolytes were normal.

His younger brother was a term newborn, adequate for gestational age (weight: 2.680 g; length: 47 cm), with no history of prenatal complications. Because he needed treatment for urinary tract infection, he remained in the hospital for 45 days. His initial hormonal profile was not available, as the child stayed in an Intensive Care Unit (ICU), where the diagnosis of adrenal insufficiency was suspected, following his brother pattern. He presented hyponatremia, which could be promptly managed with proper electrolyte and fluid support followed by adequate hormonal replacement with hydrocortisone acetate (20 mg/m²/day) and fludrocortisone (0.1 mg/day). During his first year of life, he showed low levels of plasma 17-OHP (maximum: 20 ng/dL), testosterone (maximum: 294 ng/dL), and androstenedione (maximum 0.7 ng/mL). PRA levels (maximum: 2.3 ng/mL/h) were mildly elevated and ACTH levels were very high (38.901 pg/mL).

In spite of adequate treatment, this child presented an episode of adrenal insufficiency at seven months, but no other unexpected events. When he was four years old, he had 14.3 mg/m²/day of hydrocortisone acetate and fludrocortisone (0.05 mg/day). Physical examination revealed overweight (22.5 kg; SD = 1.72), adequate statural height (106.5 cm; SD = 0.34) and BMI of 19.1 kg/m² (above the 97th centile). His BA was four years (GP). As for his brother, ACTH levels remained very high (1,201 pg/mL); plasma basal cortisol levels were in normal range (12.3 µg/dL) and there were normal electrolyte concentrations.

During treatment, the patients maintained low 17-OHP and plasma androgens, despite very high ACTH levels, so that we searched for mutations in DAX1 gene in order to establish the etiology of AHC.

Molecular analysis

Children’s parents were informed about the importance of establishing the molecular etiology of AHC, in order to better accomplish their follow-up. Written informed consent was obtained from parents for the study and publication. Genomic DNA was isolated from peripheral blood leukocytes of the two siblings and their mother. Both exons of DAX1 gene and the intronic flanking sequences were amplified by polymerase chain reaction (PCR) using specific primers and conditions, as described previously (26). The PCR products were pretreated with an enzymatic combination of shrimp alkaline phosphatase and exonuclease I (United States Biochemical Corp, Cleveland, OH) and directly sequenced using the BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA) in an ABI PRISM 310 automatic sequencer.

DAX1/NR0B1A sequence was compared to ENSG00000169297 sequence.

RESULTS

A novel frameshift mutation in exon 2 of DAX1 was identified in the two siblings. An adenine insertion at position 1382 (c1382-1383insA) causes the substitution of a metionin to an asparagine at codon 461 (ATG → AAT), determining a loss of the stop codon at position 470. The study of mother’s DNA confirmed the presence of the same mutation in heterozygous state (Figure 1).
DISCUSSION

Primary adrenal failure is a life-threatening condition that can be caused by a range of etiologies, including autoimmune, metabolic and developmental disorders. DAX1 gene plays an important role in adrenal development and function. Mutations in this transcription factor may cause AHC, a rare cause of primary adrenal insufficiency in childhood (3). Other clinical manifestations may also include isolated mineralocorticoid deficiency (19,27), prolonged testosterone secretion in infancy (28), testicular enlargement (29), genital ambiguity and sex reversal (16,21).

Clinical diagnosis of AHC is not always clearly recognizable and can be overlooked if it is not considered in the differential diagnosis in a context of adrenal crisis. Most of the patients present with failure to thrive, salt-wasting syndrome (hyponatremia, hyperkalemia and metabolic acidosis), hypoglycemia, and skin hyperpigmentation in the first months of life. In some patients, however, adrenal insufficiency may not be evident until they reach adulthood (12).

In the neonatal period, boys generally present signs and symptoms that are indistinguishable from those observed in the salt-losing form of 21-OHD and are frequently misdiagnosed as having this more common disorder (19,29). Moreover, in patients with AHC, plasma steroid determinations may often lead to confusing results in the first weeks of life, probably caused by steroids production by neonates’ persisting adrenal fetocortex (29). Distinguishing these two disorders is important because they differ in their clinical course, steroid management and genetic counseling. In the present report, a diagnostic hypothesis of congenital adrenal hyperplasia was firstly made in the elder brother. In the newborn period, the patient presented salt-losing crisis, but very inexpressive elevation of plasma 17-hydroxyprogesterone levels, not similar to the ones currently seen in neonates with the salt-losing form of 21-OHD. Glucocorticoid and mineralocorticoid replacement was initiated with improvement of their affected male offspring (1).

In our patients, the molecular analysis confirmed a mutation in exon 2 of DAX1 that generated an abnormal protein. The novel frameshift mutation consisting in adenine insertion at 1382 nucleotide, changing the sequence of amino acids forward at 461 codon and determining a loss of the stop codon at position 470. Probably, this new amino acid sequence in this important region of DAX1 protein prevented its accomplishment in adrenal embryogenesis. The majority of mutations in DAX1 causing AHC/HH reported to date are nonsense or frameshift mutations in the C-terminal region of the gene and have been shown to either impair protein folding and nuclear localization or impair transcriptional repression (5,10,21,30,31).

DAX1 has a unique role as a homologous NR superfamily member, acting as a coregulatory protein that inhibits the transcriptional activity of other NRs (32). Lalli and cols. showed that two domains are necessary for this activity: the N-terminal domain (minimally H3 region) and a C-terminal domain (final portion of H11, H12 regions and the intermediary short loop, including the activation function 2 [AF2] domain) (33). These amino acids of C-terminal domain, which mediate ligand binding, dimerization, and nuclear localization, undergoes allosteric conformational changes.
in response to ligand binding, through the hormone-dependent transactivation of its AF2 domain (34), located at codons 461-466. Thus, a mutation in this region, which includes the AF2 domain, could affect the domain structure that involved the ligand binding-dependent functions, as dimerization (35).

This novel mutation described here is associated with an early and severe clinical pattern of presentation. As more and more monogenetic disorders are explained on a molecular level, an important question raised is about the correlation of genotype to phenotype. Recent studies highlight the complexities of DAX1 regulation and function (36,37). There is considerable phenotypic variability associated with DAX1 mutations, probably reflecting a combination of genetic (modifier genes, variability in expressivity and penetrance) and environmental (intercurrent illnesses and other stressors) influences (36). To date, genotype-phenotype correlation cannot be easily predicted (38).

In conclusion, we presented a frameshift mutation in the DAX1 gene in two siblings being initially treated as if they had the diagnosis of 21-OHD salt-losing form of CAH, despite the very inexpressive elevation of 17-OHP levels. An insertion of an adenine base (c1382-1383 A ins), which lead to a pMet461Asp substitution, was found. This mutation had not been previously described in the literature. We believe that obtaining a precise genetic diagnosis of this adrenal disorder, which sometimes cannot be confirmed only by clinical aspects, may have important implications for the long-term management of the disease, for predicting prognosis, investigating possible associated features and for appropriate family counseling.

Acknowledgments: the authors thank Sorahia Domenice and Berenice B. Mendonça, from the Unidade de Endocrinologia do Desenvolvimento, Laboratório de Hormônios e Genética Molecular/LIM 42, of the Hospital das Clínicas of the Faculdade de Medicina of Universidade de São Paulo, Brazil, for their technical assistance.

Disclosure: no potential conflict of interest relevant to this article was reported.

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