Case Report

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A novel *DAX-1* (NR0B1) mutation in a boy with X-linked adrenal hypoplasia congenita

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Abstract

Background: X-linked adrenal hypoplasia congenita (AHC) is caused by mutations in *DAX-1* (NR0B1) playing a key role in adrenal and reproductive development.
Case presentation: Herein we report a 2.5-year-old boy who presented with acute adrenal failure. Family history revealed unexplained death in three brothers of the patient's mother during infancy. Molecular analysis of the *DAX-1* gene revealed the presence of a novel hemizygous mutation, c.870C>A in exon 1, leading to the formation of a premature stop codon. The same mutation was identified in the patient's mother. The truncated mutant protein is most likely misfolded, sequestered in the endoplasmic reticulum and therefore cannot bind to and activate its target DNA sequences in the nucleus.
Conclusions: *DAX-1* mutation must be considered when diagnosis of primary adrenocortical insufficiency is made, especially if there is a history of unexplained death of maternal male relatives.

Keywords: adrenal insufficiency; *DAX-1*; X-linked adrenal hypoplasia congenital.

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Introduction

Hypoplasia/agenesis of adrenals leading to acute adrenal insufficiency with salt-losing crisis as a fatal condition was first reported by Sikl [1] in 1948. In the 1990s specific mutations of *DAX-1* (dosage-sensitive sex reversal – adrenal hypoplasia congenital critical region on the X chromosome 1, known also as NR0B1 [OMIM 300473]), situated in Xp21, were described for the first time to cause X-linked adrenal hypoplasia congenita (AHC) (OMIM 300200). At that time, *DAX-1* was recognized to play a key role not only in the development of the adrenal gland, but also in the development of the testis, ovary, pituitary gland and hypothalamus. These first reports linked X-linked AHC to glycerol kinase deficiency (GKD) and Duchenne muscular dystrophy (DMD), which helped to discover the localization of the gene and its mutations causing X-linked AHC. Since then a wide phenotypic spectrum has been described, patients being mostly affected by frameshift or nonsense mutations that cause premature truncation of the protein.

DAX-1 encodes an orphan nuclear hormone receptor and interacts as a dominant negative regulator with other nuclear receptors, such as SF-1 to regulate gene expression [2]. However, its exact biological role remains mainly unclear. In mice, deletion of exon 2 in *DAX-1* leads to impaired spermatogenesis but normal adrenal function [3]. Interestingly, duplications of *DAX-1* have been described in 46, XY disorder of sexual development (DSD). Possibly, this could mean that over-expression of *DAX-1* suppresses male sexual development [4].

Clinically, most males with X-linked AHC present with acute adrenal failure. Adrenal insufficiency with salt-losing crisis, which is lethal if untreated, can present either in the first 2 months of life or more gradually during childhood [5]. Pubertal development is usually absent. However, some affected boys might develop pubertal sign up to Tanner stage 3, when puberty stops due to disrupted hypothalamic and pituitary release of gonadotropin-releasing hormone and gonadotropins, respectively

[6]. Similarly, a few patients with *DAX-1* mutation were reported to develop ACTH-dependent transient precocious pseudopuberty during infancy/childhood [7, 8].

Herein, we present a novel *DAX-1* mutation leading to X-linked AHC with acute adrenal failure in a 2.5-year-old boy.

Case presentation

A 2.5-year-old boy who presented with vomiting and severe slackness was admitted to the emergency department. Physical examination revealed moderate dehydration, underweight (weight: 11 kg [Z-score –1.92], length: 92 cm [Z-score –0.57]), normal developed male genitalia and hyperpigmented lips and nipples. Laboratory evaluation revealed hypoglycemia (2.5 mmol/L), hyponatremia (121 mmol/L), hypochloremia (97 mmol/L), hyperkalemia (4.8 mmol/L), metabolic acidosis (pH 7.33, BE –11.9), low cortisol (1 nmol/L) and elevated ACTH (6040 pg/mL) concentration. Insulin was suppressed and growth hormone appropriately elevated ruling out other endocrine aetiologies for hypoglycemia. A diagnosis of adrenal insufficiency was made and the boy was transferred to the intensive care unit for rehydration and substitution with hydrocortisone and sodium. After stabilization of vital parameters, the patient was switched to oral supplementation with hydrocortisone (14 mg/m^2/day) and fludrocortisone (0.1 mg/day). The boy was discharged from hospital after a couple of days. Growth and psychomotor development have been normal since then. At 4 years of age, his weight is 17 kg (Z-score 0.44) and height is 102.5 cm (Z-score 0.03).

The boy was born after a normal pregnancy. The neonatal period was uneventful. He had a history of recurrent vomiting without diarrhea and increasing hyperpigmentation of the skin over the last 6 months before admission. Psychomotor development was reported to be normal. Family history revealed unexplained deaths in three brothers of the mother during infancy.

On additional laboratory evaluation, congenital adrenal hyperplasia (low 17-hydroxyprogesterone) and adrenoleukodystrophy (normal plasma concentration of very long-chain fatty acids) could be excluded. Negative anti-21-hydroxylase and anti-adrenal autoantibodies made an autoimmune Addison disease very unlikely. Given the family history *DAX-1* mutation was assumed and a molecular analysis initiated.

To this end, genomic DNA was extracted from peripheral blood leukocytes of the patient and the two exons of *DAX-1* (RefSeq NG_009814.1) were amplified and sequenced (conditions available upon request). Direct sequencing of the PCR fragments revealed the presence of a novel hemizygous mutation, c.870C>A in exon 1, leading to the substitution of a cystein at position 290 with a premature stop codon (p.Cys290Ter; Figure 1). The parents were also screened and his mother was shown to be carrier of the same mutation, as to be expected for a X-linked condition.

To further analyze the molecular mechanism of the complete loss of function of the nonsense mutation, we explored the possibility of nonsense-mediated decay (NMD) by stabilizing mRNA through the translation inhibitor cycloheximide. We demonstrated the loss-of-function of *DAX-1* is not due to NMD, as shown by the lack of differences in mRNA stability between WT and mutant *DAX-1* under cycloheximide treatment (data not shown). Furthermore, Western blot analysis showed no decrease in mutant protein quantity or stability, although the detected protein was, as expected, shorter (data not shown).

Molecular models of the wild type and mutant protein were created with the online tool I-TASSER, based on homology modeling. Furthermore, the model was refined with a 5ns refinement molecular dynamics run using YASARA (yasara.org) and finally depicted using PyMol (pymol.org). Thep.Cys290 stop mutation lead to a C-terminal truncation of 180 amino acids from DAX-1 (Figure 2). The homology modeling predicts that the folding of the mutant *DAX-1* is disrupted and, hence, significantly different compared to the predicted protein folding of the WT *DAX-1*. Accordingly, immunofluorescence experiments performed in transfected COS-1 cells showed that whereas WT *DAX-1* correctly concentrated in the nuclei, the mutant protein was detectable only in the cytoplasm of transfected COS-1 cells, and is therefore prevented to exert its function as a transcription factor thus leading to loss-of-function and disease (data not shown).

Statement of ethics

The patient's parents gave their full consent for genetic testing.

Discussion

X-linked adrenal hypoplasia congenita is caused by mutations in the *DAX-1* gene. To date even though over 100 individuals and relatives with X-linked AHC have been reported in the literature, the exact prevalence of NR0B1 mutations causing adrenal insufficiency is not

Figure 1: Mutation analysis.
Electropherograms of directly sequenced PCR-amplified fragments from genomic DNA of the patients, his parents (mother and father) and a control showing a C to A exchange in exon 1 (position c.870), leading to the substitution of a cystein at position 290 with a premature stop codon (p.Cys290Ter). The mutation is hemizygous in the patient and heterozygous in the mother, as to be expected for a X-linked mode of inheritance.

clear. Studies suggest an occurrence of X-linked AHC of around 1:140,000–1:1,200,000 children (or between 1:70,000 and 1:600,000 males) [5].

Lin et al. [5] reported finding *DAX-1* mutations in all patients with adrenal insufficiency of their cohort (8 cases of a cohort of 117 patients) when family history revealed adrenal failure or unexpected death in males together with a history of arrested or absent puberty. This underlines the importance of taking an exact family history suggesting any insight into possible adrenal disease. In line with such findings and the fact that *DAX-1* mutations follow an X-linked pattern of inheritance, family history of three maternal male relatives dying during neonatal period was suggestive for the *DAX-1* defect being retrospectively the putative cause of their early death.

Genetic analysis revealed a novel hemizygous mutation, c.870C>A, in exon 1. This mutation led to the formation of a premature stop codon resulting in a C-terminal

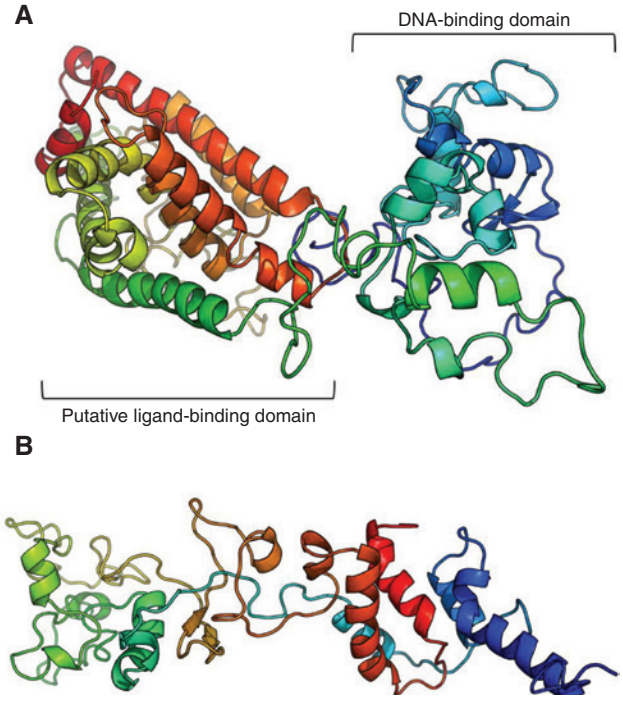

Figure 2: Model of wild type and p.Cys290Stop DAX-1 mutant proteins.
(A) DAX-1 consist of two domains, the N-terminal DNA-binding domain and the C-terminal putative ligand-binding Domain. Predicted wild-type *DAX-1* protein model. (B) The *DAX-1* p.Cys290 stop mutation leads to a loss of 180 amino acids, which in turn results in a predicted change in protein folding according to homology modeling.

truncation of 180 amino acids from *DAX-1*. The *DAX-1* gene encodes a protein that belongs to the nuclear hormone receptor superfamily with an amino-terminal region and a carboxyl-terminal region [2]. Nonsense and frameshift mutations can be located throughout the *NR0B1* gene. Missense mutations tend to be more prevalent in certain regions of the ligand-like binding domain, in highly conserved amino acids [9]. The C terminus shows characteristics of a nuclear hormone receptor ligand-binding domain. In 1997, Lalli et al. [10] discovered that the DAX-1 C terminus contains transcriptional silencing activity, which can be transferred to a heterologous DNA-binding domain. Nakae found that especially mutations in the C-terminus of the DAX-1 protein cause AHC. They postulated that the C-terminal domain of the DAX-1 protein is important for normal adrenal gland development. Even impairment of 11 amino acids in this region can lead to disrupted adrenal cortical differentiation [11].

Phenotypes in patients harboring a *DAX-1* mutation show wide heterogeneity. As there is no genotype-phenotype correlation, *DAX-1* mutation does not predict which clinical characteristics can be expected. Moreover, age of onset of AHC and the position of the mutation do not correlate [11]. Within the same family harboring the same mutation onset of clinical manifestation can vary. This may also be suggested in our family where manifestation of adrenal insufficiency in our patient occurred only in the 3rd year compared to his uncles with suggested *DAX-1* mutation who died in the 1st year. Such findings suggest various degrees of penetrance due to co-modulating and/or epigenetic or nongenetic factors influencing *DAX-1* activity and integrity and thereby the clinical course of AHC [12].

In conclusion, we identified a novel hemizygous mutation in a patient with X-linked AHC. This mutation led to the formation of a premature stop codon, resulting in a C-terminal truncation of 180 amino acids from DAX-1, which is probably misfolded, sequestered in the endoplasmic reticulum and most likely degraded. Differentiating adrenal insufficiency due to the *DAX-1* mutation from other causes is of great importance since it has implications for future follow-up and treatment as well as family planning and counseling.

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References

1. Sikl H. Addison's disease due to congenital hypoplasia of the adrenals in an infant aged 33 days. J Pathol Bacteriol 1948;60:323.
2. Iyer AK, McCabe ER. Molecular mechanisms of DAX1 action. Mol Genet Metab 2004;83:60–73.
3. Yu RN, Ito M, Saunders TL, Camper SA, Jameson JL. Role of Ahch in gonadal development and gametogenesis. Nat Genet 1998;20:353–7.
4. Bardoni B, Zanaria E, Guioli S, Floridia G, Worley KC, et al. A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. Nat Genet 1994;7:497–501.
5. Lin L, Gu WX, Ozisik G, To WS, Owen CJ, et al. Analysis of DAX1 (NR0B1) and steroidogenic factor-1 (NR5A1) in children and adults with primary adrenal failure: ten years' experience. J Clin Endocrinol Metab 2006;91:3048–54.

6. Seminara SB, Achermann JC, Genel M, Jameson JL, Crowley WF Jr. X-linked adrenal hypoplasia congenita: a mutation in DAX1 expands the phenotypic spectrum in males and females. J Clin Endocrinol Metab 1999;84:4501–9.
7. Domenice S, Latronico AC, Brito VN, Arnhold IJ, Kok F, et al. Adrenocorticotropin-dependent precocious puberty of testicular origin in a boy with X-linked adrenal hypoplasia congenita due to a novel mutation in the DAX1 gene. J Clin Endocrinol Metab 2001;86:4068–71.
8. Yeste D, Gonzalez-Nino C, Perez de Nanclares G, Perez-Nanclares G, Audi L, et al. ACTH-dependent precocious pseudopuberty in an infant with DAX1 gene mutation. Eur J Pediatr 2009;168:65–9.
9. Achermann JC, Ito M, Silverman BL, Habiby RL, Pang S, et al. Missense mutations cluster within the carboxyl-terminal region of DAX-1 and impair transcriptional repression. J Clin Endocrinol Metab 2001;86:3171–5.
10. Lalli E, Bardoni B, Zazopoulos E, Wurtz JM, Strom TM, et al. A transcriptional silencing domain in DAX-1 whose mutation causes adrenal hypoplasia congenita. Mol Endocrinol 1997;11:1950–60.
11. Nakae J, Tajima T, Kusuda S, Kohda N, Okabe T, et al. Truncation at the C-terminus of the DAX-1 protein impairs its biological actions in patients with X-linked adrenal hypoplasia congenita. J Clin Endocrinol Metab 1996;81:3680–5.
12. Peter M, Viemann M, Partsch CJ, Sippell WG. Congenital adrenal hypoplasia: clinical spectrum, experience with hormonal diagnosis, and report on new point mutations of the DAX-1 gene. J Clin Endocrinol Metab 1998;83:2666–74.