Importance of Genetic Diagnosis of DAX-1 Deficiency: Example from a Large, Multigenerational Family

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Established Facts

- Inactivating mutations of DAX-1 represent the genetic basis of congenital adrenal hypoplasia.

Novel Insights

- We describe a novel, relatively large deletion within DAX-1, associated with AHC in a large family.
- Identification of this deletion made prenatal genetic diagnosis possible in a female family member at risk for carrying the trait.
- The history of this family illustrates the value of genetic diagnosis in AHC.

Key Words

Adrenal hypoplasia congenital · DAX-1 · Mutation analysis · Adrenal insufficiency · Genetic diseases

Abstract

Background: Inactivating mutations of DAX-1 give rise to the X-linked form of adrenal hypoplasia congenita (AHC). Affected fetuses are at risk of early postnatal Ad-

disonian crisis, but the variable phenotypic expression of DAX-1 insufficiency renders this diagnosis challenging. **Methods:** We describe the familial transmission of AHC over several generations. The proband was diagnosed with adrenal insufficiency at age 3.5 years: molecular analysis revealed a novel, 373-bp deletion including the second exon of DAX-1. Given the familial history of several unexplained deaths in male infants related to the proband via his maternal great-grandmother, we hypothesized that all these boys had been affected with AHC. Another female member of the family being preg-

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nant with a male fetus at the time, we performed DAX-1 analysis on the mother and the newborn. The mother was heterozygous for the deletion, and the newborn hemizygous: he presented an adrenal crisis at 10 days of life, and is now doing well on hormone replacement therapy. **Conclusion:** The unfortunate deaths of male infants at each generation of this family underlie the importance of early and precise diagnosis of this rare condition, stressing the value of genetic diagnosis in six potential female carriers of this family entering their reproductive years.

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Introduction

Adrenal hypoplasia congenita (AHC) is a rare disorder of adrenal gland development initially described in 1948 by Sikl et al. [1]. AHC has an estimated incidence of 1 in 12,500 live births [2, 3], and can be inherited in an X-linked manner or as an autosomal-recessive disease, the two forms exhibiting different adrenal morphologies. The X-linked form, also called cytomegalic, is characterized by the appearance of a disorganized adrenal cortex composed by large cytomegalic vacuolated eosinophilic cells [3–5]. Affected boys present with primary adrenal insufficiency, followed by hypogonadotropic hypogonadism (HH) later in life [6]. The occurrence of adrenal insufficiency typically follows a bimodal age distribution, the adrenal crisis developing either within 2 months of birth or between 2 and 9 years, with a median age at presentation of 3 months [7, 8].

In 1994, DAX-1 (for *D*osage-sensitive sex reversal, *A*drenal Hypoplasia Congenita critical region on the *X* chromosome, gene 1), the human gene responsible for the X-linked form of AHC, was cloned [9–11]. It consists of two exons of 1,168 and 245 bp, respectively, separated by a 3,385-kb intron [12], and codes for an atypical, 470-amino acid member of the orphan nuclear receptor superfamily. DAX-1 is expressed in the prostate, skin, breast, bone and early mouse embryonic stem cells [13], as well as at all levels of the hypothalamic-pituitary-adrenal/gonadal axes [11, 14–16]. Together with another orphan nuclear receptor, steroidogenic factor (SF-1), it participates in the development and functional activation of these two neuro-endocrine systems [4, 8, 17, 18]. DAX-1 has the structural and functional characteristics of a transcriptional regulator [11], with its carboxy-terminal region sharing sequence homology and structural similarities with the ligand-binding domains of several members of the nucle-

ar receptor superfamily. However, DAX-1 also presents a unique 3.5 repeat sequence, consisting of 65–67 amino-terminal acids each, lacking the typical zinc-finger DNA-binding domain of other nuclear receptors but containing cysteines positioned to form a potential novel zinc finger [8, 9, 19]. To date, no candidate ligand has been identified [4, 6].

Over 80 mutations of DAX-1 have been described in patients and families affected with the X-linked form of AHC [4]. Most of them are frameshift or nonsense, but some missense mutations have also been reported [19–22]. They result in various truncations of the critical carboxy-terminal region of the protein, which invariably abrogate its transcriptional repressor activity [23, 24]. There is a wide range of phenotypic expression of DAX-1 insufficiency, going from the classical acute adrenal crisis occurring in early infancy to a more insidious and progressive onset of adrenal insufficiency appearing later in childhood [7, 25], or even in adulthood [26, 27]. Similarly, the associated HH, with absent or delayed puberty, can be either of hypothalamic or pituitary origin [4, 28]. Finally, rare variant phenotypes include isolated HH in a woman homozygous for a DAX-1 nonsense mutation [29] and extreme pubertal delay in heterozygous female carriers [6].

This variable phenotypic expression of DAX-1 insufficiency underlies the considerable importance that genetic counseling may have for known carrier mothers. Indeed, prenatal or early postnatal genetic testing can identify male fetuses at risk of developing AHC before the onset of an Addisonian crisis, allowing better and faster hormone replacement therapy of affected patients [30].

This study presents an extended kindred with X-linked AHC transmitted over five generations, and reports the clinical features of a young boy who presented with adrenal insufficiency at the age of three years. He harbors a novel 373-bp deletion inducing a complete deletion of exon 2 of DAX-1. This large family, comprising many potential female carriers of reproductive age, illustrates the complexity of the issues related to genetic diagnosis of heritable diseases.

Case

Patient

The proband (fig. 1, V:7) is a Caucasian male infant, born at term after a normal pregnancy (weight at birth: 3.7 kg; length at birth: 54 cm). He is the first male of three infants, having two older sisters. He had a completely uneventful infancy until the age of

Fig. 1. Family tree. Legends: grey circle = probable female carrier. Black square with cross = possible hemizygous male. Black square = hemizygous male. Black circles = heterozygous female. Arrow indicates the proband.

3 5/12 years when he presented to the emergency room with fever, vomiting and signs of dehydration. Biochemical work-up disclosed the presence of hypoglycemia, hyponatremia, hyperkalemia and metabolic acidosis, suggesting combined gluco- and mineralocorticoid deficiency. Further work-up revealed a weight of 15 kg (P50) for a height of 110 cm (>P97), low serum cortisol levels, low serum 17 α -hydroxy-progesterone, low serum aldosterone with high serum renin activity levels, elevated ACTH and ADH levels as well as undetectable urinary 17-ketosteroids (table 1), all compatible with combined glucocorticoid and mineralocorticoid insufficiency. At this time, he was discharged with an initial diagnosis of adrenal insufficiency of unknown etiology. He was therefore put on steroid supplementation with hydrocortisone and fludrocortisone (15 mg and 0.1 mg/day, respectively). At age 3 and 7/12, a new physical examination revealed no hypospadias, stage I pubertal development according to Tanner, and scrotal testes of 1 ml each with the presence of a hydrocele on the right testicle. Bone age according to Greulich and Pile was estimated at 3.5 years.

The boy is now 9 9/12 years, and is still receiving steroid replacement therapy (hydrocortisone, 22.5 mg/day and fludrocortisone, 0.1 mg/day) with reportedly good adhesion to treatment. He has remained on the 95th percentile of his growth curve (142 cm) and on the 97th percentile of his weight curve (48 kg), with an estimated bone age of 7 years.

PCR and Direct DNA Sequencing of DAX-1 Gene

The following studies have been approved by the ethical committee of our institution, and formal informed consent was obtained from the patient's mother as well as from the other members of the family that were studied. Peripheral blood leukocytes from the proband (V:7), his stepsister (V:5), his mother (IV:3), his second-cousin (V:13) and his maternal aunt (IV:10) were obtained for genomic DNA extraction using commercially available reagents (Nucleon BACC2, Amersham Pharmacia Biotech, Little Chalfont,

Table 1. Clinical and biochemical characteristics of the proband at presentation

	Values	Normal range
Weight, kg	15 (P50)	–
Height, cm	110 (>P97)	–
Plasma/serum analysis		
Morning cortisol, nmol/l	205	140–700
Evening cortisol, nmol/l	166	
17 α -Hydroxyprogesterone	0.70	
Aldosterone, pg/ml	16.0	<310
Renin, mg/ml $\times$ h	14.4	lying: 0.2–2.8 standing: 1.5–5.7
ACTH, pg/ml	2,230	20–60
ADH, pg/ml	13.9	<6.7
Sodium, mmol/l	120	
Potassium, mmol/l	5.5	
Glucose, mmol/l	3.8	
Urinary analysis		
17-Ketosteroids	<LLQ	
Gazometry		
pH	7.35	
pCO ₂	3.04	
HCO ₃	12.4	
ABE	–11.1	
SBE	–12.2	

LLQ = Lower limit of quantification.

Fig. 2. a Schematic representation of the 373-bp deletion encompassing the entire exon 2 of DAX-1. The arrows indicate the primers used for the amplification (S = sense, AS = anti-sense). **b** Photograph of a gel illustrating the shorter mutated fragment amplified by this set of primers. N = Negative control; MT = mutant; Het = heterozygote; WT = wild type.

England). Control DNA was obtained from 2 normal volunteers. Overlapping genomic DNA fragments encompassing the two exons of the DAX-1 gene were amplified by PCR, using the sets of forward and reverse primers and the reaction conditions previously described by Salvi et al. [25] plus an additional set of primers specifically used to amplify the region bearing the deletion (forward, 5'-TGGGTGCAATGAGCTAAGTG-3'; reverse, 5'-CATGGTGAAGTGCCTACTGC-3'). Direct DNA sequencing of purified PCR products was performed by Microsynth (Balgach, Switzerland), using an automated sequencer. For improved accuracy and precision, sequencing of PCR fragments was performed in both directions. Alignment and comparisons between the wild-type and mutated DAX-1 gene were achieved using the programs BestFit and PileUp from GCG sequence analysis package (Genetic Computer Group, Madison, Wisc., USA).

Results

Mutation Analysis of the DAX-1 Gene

Figure 2a illustrates schematically the novel deletion of DAX-1 identified in this family. PCR amplification of a fragment theoretically comprising the entire exon 2 in the proband resulted in a slightly smaller amplicon than in controls (fig. 2b), suggesting the presence of a deletion in this region. This hypothesis was confirmed by direct sequencing of the amplicon encompassing this region.

The deletion extends from position 6021 throughout position 6394, completely eliminating exon 2 of the gene. Following this finding, both the proband's mother (IV:3) and stepsister (V:5, same mother, different father), the latter being 27 years old, were found heterozygous for the novel deletion.

As illustrated by the family tree (fig. 1), the proband is the only male offspring of 4 children from 2 different sisters (IV:1 and IV:3) on that side of the family. We then heard of the recent unexplained death of a male infant at age 1 4/12 years, in another side of the family related to the proband via his maternal great-grandmother (II:1). Since this unfortunate child (V:12), the only male offspring of 5 different children from 2 sisters (IV:5 and IV:7) was related to the proband via their common maternal great-grandmother (II:1), we hypothesized that the same DAX-1 deletion could have been transmitted to that side of the family. Upon further questioning of various members of this large family, we discovered the existence of three other unexplained deaths of male infants in their first months of life (I:1, III:2, IV:6).

As the third sister in the fourth generation on that side of the family (IV:10) was pregnant with a male fetus at the time (V:13), DAX-1 analysis in the newborn was performed. This infant presented an adrenal crisis at the

age of 10 days of life, which could be managed promptly with proper electrolyte and fluid support followed by adequate hormone replacement therapy.

Discussion

More than 50 years after the first description of AHC by Sikl et al. [1], the etiology and molecular mechanisms of this rare disorder appear more precisely defined, with over 80 DAX-1 mutations described [4]. Despite these advances, the well recognized clinical heterogeneity of the adrenal syndrome associated with DAX-1 insufficiency remains a diagnostic challenge to the clinician: indeed, some patients present an adrenal crisis within days after birth while others do not develop adrenal insufficiency until they reach adulthood [5, 7, 27, 30].

In the present study, we report a novel 373-bp deletion of DAX-1 encompassing the entire second exon, and examine the transmission of the disorder in male and female members of an extended kindred. This mutation, by deleting the C-terminal region of the DAX-1 protein, is probably responsible for a loss of function of this protein. Indeed, the C-terminal portion of DAX-1 is functionally important for its transcription repressor activity, and truncations of this portion of the protein invariably impair its function [23, 24].

The history of this particular family exemplifies some of the difficulties inherent to the clinical heterogeneity of the syndrome. The proband developed adrenal insufficiency only 2.5 years after birth, whereas his distant cousin, although carrying the exact same deletion of DAX-1, experienced an adrenal crisis at the age of 10 days. Such heterogeneity in the phenotypic expression of a given mutation of DAX-1 has already been described in several kindreds [7, 10, 28, 31–34]. It is probably one of the reasons why all the attempts at drawing genotype-phenotype correlations in DAX-1 insufficiency have remained largely elusive. Various hypotheses have been proposed to explain this lack of correlation: differences in activity threshold, existence of potential salvage mechanisms for persistence of fetal adrenal cortex [35], presence of modifying genes and/or environmental factors are among the mechanisms potentially affecting clinical presentation [36]. However, all these hypotheses remain purely speculative at this point.

For all these reasons, the diagnosis of DAX-1 insufficiency remains challenging to the clinician, and can very easily be overlooked if one does not consider it in the differential diagnosis of an Addison crisis in a neonate. The

reality of this diagnostic challenge is dramatically illustrated by member V:12 of the family described here. Despite the history, in all the preceding generations of his family, of unexplained deaths of male offspring in early infancy, this unfortunate boy died very recently at the age of 1.5 years, with a clinical presentation compatible with an unrecognized adrenal crisis. The family that we present here also highlights both the importance and the difficulties of molecular diagnosis and genetic counseling. Indeed, there are six potential female carriers of the deletion in the last generation (V:1; V:6; V:8; V:9; V:10; V:11), with an a priori risk of between 25 and 50% [34]. These women were all born between 1977 and 1990, and are therefore either reaching or are well into their reproductive years. Genetic diagnosis and counseling according to their carrier status should be proposed to all women within such kindreds [9], to allow earlier instauration of life-saving steroid therapy in their affected male offspring.

In conclusion, we present here a large family carrying a novel deletion in DAX-1 that illustrates the X-linked transmission of the defect over several generations. The unfortunate deaths of male infants at each generation underlies the importance of early and precise diagnostic of this rare condition. Moreover, this latter finding stresses the value of genetic diagnosis and counseling for six potential female carriers of the deletion entering their reproductive years.

Acknowledgment

This work was supported by grant 32-00B0-100 858/1 (to F.P.P.) from the Swiss National Science Foundation.

References

- Sikl H: Addison's disease due to congenital adrenal hypoplasia. *J Pathol Bacteriol* 1948;60: 323–326.
- Bacon GE, Kelch RP: Congenital adrenal hyperplasia due to 21-hydroxylase deficiency: a review of current knowledge. *J Endocrinol Invest* 1979;2:93–100.
- Lavery CR, Fortune DW, Beischer NA: Congenital idiopathic adrenal hypoplasia. *Obstet Gynecol* 1973;41:655–664.
- Phelan JK, McCabe ER: Mutations in NR0B1 (DAX1) and NR5A1 (SF1) responsible for adrenal hypoplasia congenita. *Hum Mutat* 2001; 18:472–487.
- Peter M, Viemann M, Parts CJ, Sippel 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; 83:2666–2674.
- 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: 4501–4509.
- Reutens AT, Achermann JC, Ito M, Gu WX, Habiby RL, Donohoue PA, Pang S, et al: Clinical and functional effects of mutations in the DAX-1 gene in patients with adrenal hypoplasia congenita. *J Clin Endocrinol Metab* 1999; 84:504–511.
- Clipsham R, McCabe ER: DAX1 and its network partners: exploring complexity in development. *Mol Genet Metab* 2003;80:81–120.
- Guo W, Mason JS, Stone CG Jr, Morgan SA, Madu SI, Baldini A, Lindsay EA, et al: Diagnosis of X-linked adrenal hypoplasia congenita by mutation analysis of the DAX1 gene. *Jama* 1995;274:324–330.
- Muscattelli F, Strom TM, Walker AP, Zanaria E, Recan D, Meindl A, Bardoni B, et al: Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Nature* 1994; 372:672–676.
- Zanaria E, Muscattelli F, Bardoni B, Strom TM, Guioli S, Guo W, Lalli E, et al: An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. *Nature* 1994;372:635–641.
- Guo W, Burris TP, Zhang YH, Huang BL, Mason J, Copeland KC, Kupfer SR, et al: Genomic sequence of the DAX1 gene: an orphan nuclear receptor responsible for X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 1996; 81:2481–2486.
- Ho J, Zhang YH, Huang BL, McCabe ER: NR0B1A: an alternatively spliced form of NR0B1. *Mol Genet Metab* 2004;83:330–336.
- Guo W, Burris TP, McCabe ER: Expression of DAX-1, the gene responsible for X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism, in the hypothalamic-pituitary-adrenal/gonadal axis. *Biochem Mol Med* 1995;56:8–13.
- Ikeda Y, Swain A, Weber TJ, Hentges KE, Zanaria E, Lalli E, Tamai KT, et al: Steroidogenic factor 1 and Dax-1 colocalize in multiple cell lineages: potential links in endocrine development. *Mol Endocrinol* 1996;10:1261–1272.
- Swain A, Zanaria E, Hacker A, Lovell-Badge R, Camerino G: Mouse Dax1 expression is consistent with a role in sex determination as well as in adrenal and hypothalamus function. *Nat Genet* 1996;12:404–409.
- Swain A, Narvaez V, Burgoyne P, Camerino G, Lovell-Badge R: Dax1 antagonizes Sry action in mammalian sex determination. *Nature* 1998;391:761–767.
- Bardoni B, Zanaria E, Guioli S, Floridia G, Worley KC, Tonini G, Ferrante E, et al: A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. *Nat Genet* 1994;7:497–501.
- Zhang Z, Burch PE, Cooney AJ, Lanz RB, Pereira FA, Wu J, Gibbs RA, et al: Genomic analysis of the nuclear receptor family: new insights into structure, regulation, and evolution from the rat genome. *Genome Res* 2004;14: 580–590.
- Abe S, Nakae J, Yasoshima K, Tajima T, Shinohara N, Murashima M, Satoh K, et al: Novel missense mutation (Leu466Arg) of the DAX1 gene in a patient with X-linked congenital adrenal hypoplasia. *Am J Med Genet* 1999;84: 87–89.
- Achermann JC, Ito M, Silverman BL, Habiby RL, Pang S, Rosler A, Jameson JL: Missense mutations cluster within the carboxyl-terminal region of DAX-1 and impair transcriptional repression. *J Clin Endocrinol Metab* 2001;86: 3171–3175.
- Zhang YH, Huang BL, Anyane-Yeboah K, Carvalho JA, Clemons RD, Cole T, De Figueiredo BC, et al: Nine novel mutations in NR0B1 (DAX1) causing adrenal hypoplasia congenita. *Hum Mutat* 2001;18:547.
- Lalli E, Bardoni B, Zazopoulos E, Wurtz JM, Strom TM, Moras D, Sassone-Corsi P: A transcriptional silencing domain in DAX-1 whose mutation causes adrenal hypoplasia congenita. *Mol Endocrinol* 1997;11:1950–1960.
- Ito M, Yu R, Jameson JL: DAX-1 inhibits SF-1-mediated transactivation via a carboxy-terminal domain that is deleted in adrenal hypoplasia congenita. *Mol Cell Biol* 1997;17: 1476–1483.
- Salvi R, Gomez F, Fiaux M, Schorderet D, Jameson JL, Achermann JC, Gaillard RC, et al: Progressive onset of adrenal insufficiency and hypogonadism of pituitary origin caused by a complex genetic rearrangement within DAX-1. *J Clin Endocrinol Metab* 2002;87: 4094–4100.
- Achermann JC, Gu WX, Kotlar TJ, Meeks JJ, Sabacan LP, Seminara SB, Habiby RL, et al: Mutational analysis of DAX1 in patients with hypogonadotropic hypogonadism or pubertal delay. *J Clin Endocrinol Metab* 1999;84:4497–4500.
- Tabarin A, Achermann JC, Recan D, Bex V, Bertagna X, Christin-Maitre S, Ito M, et al: A novel mutation in DAX1 causes delayed-onset adrenal insufficiency and incomplete hypogonadotropic hypogonadism. *J Clin Invest* 2000; 105:321–328.
- Habiby RL, Boepple P, Nachtigall L, Sluss PM, Crowley WF Jr, Jameson JL: Adrenal hypoplasia congenita with hypogonadotropic hypogonadism: evidence that DAX-1 mutations lead to combined hypothalamic and pituitary defects in gonadotropin production (see comments). *J Clin Invest* 1996;98:1055–1062.
- Merke DP, Tajima T, Baron J, Cutler GB Jr: Hypogonadotropic hypogonadism in a female caused by an X-linked recessive mutation in the DAX1 gene. *N Engl J Med* 1999;340:1248–1252.
- Achermann JC, Silverman BL, Habiby RL, Jameson JL: Presymptomatic diagnosis of X-linked adrenal hypoplasia congenita by analysis of DAX1. *J Pediatr* 2000;137:878–881.
- Hay ID, Smail PJ, Forsyth CC: Familial cytomegalic adrenocortical hypoplasia: an X-linked syndrome of pubertal failure. *Arch Dis Child* 1981;56:715–721.
- Wiltshire E, Couper J, Rodda C, Jameson JL, Achermann JC: Variable presentation of X-linked adrenal hypoplasia congenita. *J Pediatr Endocrinol Metab* 2001;14:1093–1096.
- Yanase T, Takayanagi R, Oba K, Nishi Y, Ohe K, Nawata H: New mutations of DAX-1 genes in two Japanese patients with X-linked congenital adrenal hypoplasia and hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 1996;81:530–535.
- Schwartz M, Blichfeldt S, Muller J: X-linked adrenal hypoplasia in a large Greenlandic family: detection of a missense mutation (N440I) in the DAX-1 gene; implication for genetic counselling and carrier diagnosis. *Hum Genet* 1997;99:83–87.
- Loke KY, Larry KS, Lee YS, Peter M, Drop SL: Prepubertal diagnosis of X-linked congenital adrenal hypoplasia presenting after infancy. *Eur J Pediatr* 2000;159:671–675.
- McCabe ER: Editorial: Vulnerability within a robust complex system-DAX-1 mutations and steroidogenic axis development. *J Clin Endocrinol Metab* 2002;87:41–43.