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 Addisonian 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-
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 Dosage-sensitive sex reversal, Adrenal Hypoplasia Congenita critical region on the X chromosome, gene J), 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 nuclear receptor superfamily. However, DAX-1 also presents a unique 3.5 repeat sequence, consisting of 65–67 amino 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 hypotalamic 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
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 glucocorticoid 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 17α-hydroxyprogesterone, 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 Pyle 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

<table>
<thead>
<tr>
<th></th>
<th>Values</th>
<th>Normal range</th>
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<tbody>
<tr>
<td>Weight, kg</td>
<td>15 (P50)</td>
<td>–</td>
</tr>
<tr>
<td>Height, cm</td>
<td>110 (&gt;P97)</td>
<td>–</td>
</tr>
<tr>
<td>Plasma/serum analysis</td>
<td></td>
<td></td>
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<tr>
<td>Morning cortisol, nmol/l</td>
<td>205</td>
<td>140–700</td>
</tr>
<tr>
<td>Evening cortisol, nmol/l</td>
<td>166</td>
<td></td>
</tr>
<tr>
<td>17α-Hydroxyprogesterone</td>
<td>0.70</td>
<td>&lt;310</td>
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<tr>
<td>Aldosterone, pg/ml</td>
<td>16.0</td>
<td></td>
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<tr>
<td>Renin, mg/ml × h</td>
<td>14.4</td>
<td></td>
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<tr>
<td>ACTH, pg/ml</td>
<td>2,230</td>
<td></td>
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<tr>
<td>ADH, pg/ml</td>
<td>13.9</td>
<td>&lt;6.7</td>
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<tr>
<td>Sodium, mmol/l</td>
<td>120</td>
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<tr>
<td>Potassium, mmol/l</td>
<td>5.5</td>
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<tr>
<td>Glucose, mmol/l</td>
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<tr>
<td>Urinary analysis</td>
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<tr>
<td>17-Ketosteroids</td>
<td>&lt;LLQ</td>
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<tr>
<td>Gazometry</td>
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<tr>
<td>pH</td>
<td>7.35</td>
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<tr>
<td>pCO₂</td>
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<tr>
<td>HCO₃⁻</td>
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<tr>
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<td>–11.1</td>
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<tr>
<td>SBE</td>
<td>–12.2</td>
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LLQ = Lower limit of quantification.
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'-TGGGTCAATGAGCTAAGTG-3'; reverse, 5'-CATGGTGAACGTCACTGTC-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

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**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.
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.

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References