Hypogonadotropic Hypogonadism as a Presenting Feature of Late-Onset X-Linked Adrenal Hypoplasia Congenita

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Mutations in the orphan nuclear receptor DAX-1 cause X-linked adrenal hypoplasia congenita. Affected boys usually present with primary adrenal failure in early infancy or childhood. Impaired sexual development because of hypogonadotropic hypogonadism becomes apparent at the time of puberty. We report adult-onset adrenal hypoplasia congenita in a patient who presented with hypogonadism at 28 yr of age. Although he had no clinical evidence of adrenal dysfunction, compensated primary adrenal failure was diagnosed by biochemical testing. Semen analysis showed azoospermia, and he did not achieve fertility after 8 months of treatment with gonadotropins. A novel Y380D DAX-1 missense mutation, which causes partial loss of function in transient gene expression assays, was found in this patient. This case demonstrates that partial loss-of-function mutations in DAX1 can present with hypogonadotropic hypogonadism and covert adrenal failure in adulthood. Further, an important role for DAX-1 in spermatogenesis in humans is confirmed, supporting findings in the Dax1 (Ahch) knockout mouse.

THE ORPHAN NUCLEAR receptor DAX-1 plays a crucial role in the development and function of the adrenal gland and hypothalamic-pituitary gonadal axis (1–4). Mutations in the gene encoding DAX-1 (AHC) cause X-linked adrenal hypoplasia congenita (AHC) (OMIM: 300200) (1, 2). Boys with this condition usually present with primary adrenal failure in early infancy or childhood (5). Hypogonadotropic hypogonadism (HHG), an associated feature of this disorder, usually becomes apparent during adolescence with impaired or arrested pubertal development. Evidence from Ahch (Dax1) knockout mice (6), and a limited number of patients with AHC (7, 8), suggests that mutations in DAX-1 may cause abnormalities in spermatogenesis, too.

Following the description of isolated HHG in a woman who is homozygous for a DAX1 gene mutation (9) and a report of adult-onset AHC in a patient who presented with mild adrenal failure and partial HHG (8), we considered DAX1 as a candidate gene in patients with HHG or delayed puberty alone. Analysis of more than 100 patients with these conditions failed to reveal any DAX1 gene mutations, and we concluded that abnormalities in DAX-1 are uncommon in patients with hypogonadism in the absence of clinical signs or a family history of adrenal failure (10).

We now report an adult male with partial HHG and covert compensated adrenal failure because of a novel missense mutation in DAX1. This case highlights that hypogonadism may be the presenting feature of X-linked AHC in adulthood. Although rare, this diagnosis should still be considered in young men presenting with hypogonadism, and tests of adrenal function and analysis of DAX1 should be performed when considered appropriate.

Materials and Methods

DNA sequence analysis

After obtaining approval of the local ethical committee, DNA was extracted from the patient’s blood leukocytes using standard methods. The DAX1 gene was PCR amplified using primer pairs and conditions described previously (10). Sequencing reactions were performed in forward and reverse directions using the Taq Big Dye Terminator sequencing kit and an ABI-PRISM 310 automated DNA sequencer (PE Applied Biosystems, Foster City, CA).

Construction of DAX1 expression vectors

DAX1 expression vectors containing the Y380D mutation were created by overlapping PCR, using methods described previously (8, 11). Expression vectors containing wild-type DAX1, the L381H missense mutant (12), a naturally occurring I439S missense mutant found in a patient with adult-onset X-linked (8), and a deletion of the carboxy-terminal region of DAX-1 (del 448–470) were used as positive and negative controls for DAX-1 function, as reported previously (8, 11).
Basal transcriptional activity

The effect of the Y380D mutation on basal transcriptional activity by DAX-1 was investigated using a modified mammalian two-hybrid system (8, 13). The carboxy-terminal region of DAX-1 (codons 207–470) was linked to a GAL4 DNA-binding domain (DBD) in a pBIND expression vector (Promega Corp., Madison, WI) to allow expression of wild-type and mutant GAL4-DAX-1 fusion proteins. These expression vectors (50 ng) were cotransfected with a UAS-TK109 reporter (500 ng) (13).

SF-1-mediated transactivation

The effect of DAX-1 and its mutants on SF-1-mediated transactivation was studied using full-length wild-type or mutant DAX1 cDNA in a pCMX expression vector. The ligand-like binding domain (LBD) of human SF-1 (FTZF1) (codons 133–461) was linked to GAL4DBD in pBIND. These expression vectors (20 ng SF-1, 50 ng DAX-1) were cotransfected with a UAS-TK109 reporter (500 ng) (13).

SF-1/early growth response-1 (Egr-1) synergistic activation of LHβ

The effect of the Y380D mutant DAX-1 on SF-1/Egr-1 synergistic activation of the LHβ promoter was studied by cotransferring full-length human wild-type or mutant pCMXDAX-1 (50 ng), full-length human pCMXSF-1 (20 ng), and full-length rat Egr-1 (20 ng) with a pA3 luciferase reporter (500 ng) containing nucleotides −154 to +5 of the native rat LHβ promoter (14).

Transient gene expression assays

Transient gene expression studies were performed by using human embryonic kidney tsa201 cells grown in DMEM supplemented with 10% FBS and 1% streptomycin/penicillin in a 5% CO2 atmosphere at 37 C. Embryonic kidney tsa201 cells grown in DMEM supplemented with 10% FBS and 1% streptomycin/penicillin in a 5% CO2 atmosphere at 37 C. All transfections were performed in triplicate using calcium phosphate precipitation as described previously (8, 11). Results are expressed as mean ± SEM.

Results

Case report

The proband was referred at 28 yr of age with suspected hypogonadism. Although he reported no concerns about his libido, physical examination revealed underdeveloped secondary sexual characteristics, small testes (5 ml bilaterally), and a eunuchoidal habitus. There was no positive family history of endocrine disorders.

Endocrine evaluation showed low T (between 1.8 and 2.2 nmol/liter) in the presence of detectable levels of gonadotropins (LH 4.4 IU/liter; FSH 5.9 IU/liter) and an impaired gonadotropin response to repeated GnRH stimulation (100 μg gonadorelin, iv) (Fig. 1 and Table 1). Serum PRL (170 ng/ml per hour). Raised PRA (6 ng/ml per hour, normal range 0.2–6 ng/ml per hour). The values on the graph indicate peaks from multiple samples.

TABLE 1. Biochemical features of the patient with adult-onset X-linked AHC due to a Y380D missense mutation in DAX-1

<table>
<thead>
<tr>
<th>DAX1 mutation</th>
<th>Cortisol (nmol/liter)</th>
<th>ACTH (pg/mliter)</th>
<th>T (nmol/liter)</th>
<th>LH/FSH (IU/liter)</th>
<th>Peak LH/FSH (IU/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y380D</td>
<td>150</td>
<td>1800</td>
<td>2.2</td>
<td>4.4/5.9</td>
<td>4.3/6.2</td>
</tr>
<tr>
<td>Normal</td>
<td>140–700</td>
<td>10–60</td>
<td>10–35</td>
<td>0.2–6/0.5–8</td>
<td>5–25</td>
</tr>
</tbody>
</table>

Mutational analysis

DNA sequencing of the AHC (DAX1) gene revealed a hemizygous Y380D missense mutation in DAX-1 in the proband. His mother was heterozygous for this substitution. This Y380D change is unlikely to be a polymorphism because it was not detected in more than 200 alleles screened. The affected tyrosine is highly conserved in DAX-1, the related nuclear receptor, and short heterodimer partner and lies within a cluster of mutations within the carboxy-terminal region of the protein (Fig. 2).

Transient gene expression assays

Introduction of the Y380D mutation into DAX-1 resulted in partial loss of DAX-1-mediated repression in all the transient gene transcription assays tested (Figs. 3 and 4).

In studies of basal transcription, the Y380D and I439S range (serum cortisol, 150 nmol/liter; normal range, 140–700; urinary cortisol, 58 nmol/24 h, normal range 35–270). No serum cortisol response was obtained following cosynthropin stimulation (250 μg iv) (142 nmol/liter at 30 min, 136 nmol/liter at 60 min). Serum electrolytes, aldosterone, and plasma renin activity (PRA) were within the normal range at the time of diagnosis. Physical examination showed no evidence of hyperpigmentation and blood pressure was normal. An abdominal computed tomography scan revealed small adrenal glands. Antiadrenocortical antibodies were negative and very long chain fatty acids were normal. Treatment with cortisone acetate was started (25 mg total daily). Reevaluation 2 yr later (following withdrawal of glucocorticoid replacement for 1 wk) revealed evidence of mild mineralocorticoid deficiency with low-normal serum aldosterone and increased PRA (6 ng/ml per hour, normal range 0.2–2.7 ng/ml per hour).
DAX-1 mutants had 86% and 91% of wild-type repressor activity, respectively, compared with the more profound loss of repression seen with the early-onset L381H mutant (43%) and carboxy-terminal deletion (del448–470) mutant (52%) (Fig. 3).

As a repressor of SF-1-mediated transcriptional activation, Y380D and I439S had 90% and 85% of wild-type activity, compared with 43% and 23% for the severe L381H and carboxy-terminal mutants (Fig. 4A).

Finally, in the study of SF-1/Egr-1-mediated synergistic activation of the LHβ promoter, Y380D and I439S had 90% and 85% of wild-type activity, compared with 43% and 23% for the severe L381H and carboxy-terminal mutants (Fig. 4A). The Y380D missense mutation shows partial loss of DAX-1 repressor activity in a transient gene expression assay of basal transcriptional activity. For this experiment we used 50 ng of a pBIND vector containing the putative LBD (207–470) of DAX-1 fused to the GAL4 DBD and 500 ng of a UAS-TK109 luc reporter. Transient transfection studies were performed in triplicate using human tsa201 embryonic kidney cells. Data for each mutant are presented as a percentage (±SEM) of empty vector activity (100%).

Thus, in each of these assays of DAX-1 function, the Y380D mutant exhibits partial loss of function, compared with DAX-1 mutants associated with more severe clinical phenotypes.

Discussion

The association of mutations in the orphan nuclear receptor DAX-1 with X-linked AHC and HHG is well established. Affected boys often present with salt-wasting primary adrenal insufficiency in early infancy (5, 15, 16). Children who do not present early in life tend to present more insidiously throughout childhood (5, 15). Although abnormal puberty owing to a combined hypothalamic and pituitary form of HHG is usually evident about the expected time of puberty (17), several reports have described normal hypothalamic-gonadotrope-Leydig cell axis function in infants with this condition (the so-called minimipuberty of infancy) (12, 15, 18, 19). Thus, abnormalities in the hypothalamic-gonadotrope axis may develop only later in life.

Late-onset X-linked AHC has also been described in a patient who presented with mild adrenal failure at 28 yr of age (8). He was found to have an I439S missense mutation in DAX-1. Although he had evidence of partial HHG on presentation (impaired libido, normal penile length, 6 ml-testes, low basal T of 5.8–8.4 nmol/liter), a moderate gonadotropin response to bolus and repeated GnRH stimulation was demonstrated and he reported having sexual intercourse. Symptoms of adrenal failure (fatigue, weight loss, orthostatic dizziness) over a 5-yr period led him to seek medical attention, rather than concerns about his reproductive function.

The patient described here was referred to an endocrinologist with hypogonadism at 28 yr of age. No personal history of hypoadrenalism was reported and there was no family history of note. He was found to have a hemizygous Y380D mutation in DAX-1. Compared with the previous case of late-onset X-linked AHC, this patient (Y380D) has a more severe reproductive phenotype with a complete lack of gonadotropin response to bolus GnRH stimulation (Fig. 1) and gonadotropin-resistant azoospermia. This case confirms that an adult-onset form of X-linked AHC can occur, and that concern about reproductive function may be the presenting feature rather than symptoms of adrenal failure. The partial loss of function of the Y380D mutant in transient gene ex-
Limited data are available about the role of DAX-1 in human fertility. Azoospermia has been reported in several patients with classic X-linked AHC, and attempts to induce fertility using gonadotropins have been unsuccessful to date (7, 8). The patient with the I439S mutation and late-onset X-linked AHC had oligospermia, consistent with his partial reproductive phenotype. Again, little improvement was seen following gonadotropin treatment (8). The case presented here (Y380D) provides further evidence that DAX-1 affects spermatogenesis and that these patients may be relatively resistant to gonadotropin treatment. Additional reports of reproductive function in patients with DAX-1 mutations are important so that appropriate counseling can be provided and alternative approaches to fertility management such as intracytoplasmic sperm injection may be considered.

Missense mutations in DAX-1 are relatively rare. Approximately 15 missense mutations in DAX-1 have been reported, and these appear to cluster within specific regions of the putative LBD (11). The Y380D mutation affects a tyrosine in an amino acid (L381H) adjacent to this codon has been reported in a patient with classic early-onset adrenal failure (12). This L381H mutation causes severe loss of function in gene transcription studies (Figs. 3 and 4). These amino acids are predicted to lie within the hydrophobic core of the putative LBD (11, 25), although the exact structural significance of mutations in this region will not be revealed until the crystal structure of DAX-1 is solved. However, the partial loss of DAX-1 repression seen in functional studies with the Y380D and I439S mutants is clearly consistent with the late-onset phenotype in these patients and contrasts with the lack of a genotype-phenotype correlation for DAX-1 mutations associated with an earlier-onset classical phenotype (11).

The identification of a Y380D missense mutation in DAX-1 in a man referred with hypogonadism at 28 yr of age confirms that a late-onset form of X-linked AHC exists and that patients can present with a predominant reproductive rather than adrenal phenotype. The diagnosis of covert compensated primary adrenal failure in this patient has important clinical implications, especially given the apparent development of impaired mineralocorticoid function with time. Most diagnoses of X-linked AHC are made in the pediatric endo-

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**Fig. 4.** The effect of the Y380D missense mutation on DAX-1 repression activity in transient gene expression assays of SF-1-mediated transactivation (a) and SF-1/Egr-1-mediated synergistic activation of the native LHβ promoter (b). A. Transfections were performed using 20 ng of a pBINDGAL4 fusion protein containing the activation domain of SF1 (residues 133–461), 50 ng pCMX-DAX1 expression vector containing the full-length wild-type or mutant cDNA, and 500 ng of a UAS-TK109Luc reporter. B. Cotransfection of human SF1 (20 ng) with rat Egr-1 (20 ng) produced synergistic activation of the rat LHβ gene promoter (−154 to +5) (500 ng). The effect of wild-type DAX-1 and its mutants is shown in the figure. Data for each mutant are presented as a percentage (+SEM) of empty vector activity (100%).

expression assays is consistent with the delayed presentation of this patient.

We have addressed the issue of a predominant reproductive phenotype because of DAX1 mutations previously (10). Following the report of isolated HHG in the absence of adrenal failure in a woman who is homozygous for a DAX1 mutation through probable gene conversion (9), we considered DAX1 as a candidate gene in patients with isolated HHG or constitutional delay of puberty. The DAX1 gene was sequenced in more than 100 patients (95 males, 11 females) with these conditions, but no mutations were found (10). The case described here shows that patients with DAX1 mutations can present with a reproductive phenotype, and the measurement of ACTH and PRA might be considered in these cases. However, we know from our previous studies that such a presentation is relatively rare.

Evidence from overexpression of DAX1/Dax1 in humans (20) and mice (21) has shown that this nuclear receptor plays a key dosage-sensitive role in gonadal development. Further, targeted deletion of Dax1 (Afh) in the mouse causes impaired spermatogenesis and infertility (6). Dax1 knockout mice have quite marked abnormalities of testicular structure with dilated seminiferous tubules, blockage of the rete testis, and proximal/middle efferent tubules because of aberrantly located Sertoli cells and ectopic and hyperplastic Leydig cells (22). Sertoli cell “rescue” of Dax1 expression has been performed by crossing Dax1 knockout mice with a transgenic line that express Dax1 from a Müllerian inhibiting substance promoter (23). These rescued animals have restored fertility, but the abnormalities in testicular architecture persist. Further, Dax1 knockout mice have marked up-regulation of testicular aromatase activity, elevated intratesticular E2, and elevated serum E2 and PRL (24). Of note, serum E2 and PRL in this patient were normal.

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[50x283]activation of the rat LH of human tant cDNA, and 500 ng of a UAS-TK109luc reporter. B. Cotransfection DAX1 expression vector containing the full-length wild-type or mu-

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[50x319]sor activity in transient gene expression assays of SF-1 mediated 

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FIG. 4. The effect of the Y380D missense mutation on DAX-1 repres-

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[50x265]effect of wild-type DAX-1 and its mutants is shown in the figure. Data 

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[50x274]SEM) of empty vector 

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[50x310]performed using 20 ng of a pBINDGAL4 fusion protein containing the 

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[50x355]sor activity in transient gene expression assays of SF-1 mediated 

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crine clinic. This case demonstrates that it is important for adult endocrinologists to be aware of this condition, the spectrum of its presentation, and the implications of mutational analysis of DAX1 for genetic counseling, carrier detection, and the appropriate management of fertility.

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