

## CLINICAL CASE SEMINAR

# An Amino-Terminal DAX1 (*NROB1*) Missense Mutation Associated with Isolated Mineralocorticoid Deficiency

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**Context:** Mutations in *DAX1* (dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome gene 1; *NROB1*) cause X-linked adrenal hypoplasia congenita, a disease characterized by primary adrenal failure, testicular dysgenesis, and gonadotropin deficiency. Most *DAX1* mutations are deletions, nonsense, or frameshift mutations that markedly impair its transcriptional activity. Missense mutations have been restricted to the carboxy-terminal domain and are associated with more variable clinical phenotypes.

**Objective:** The objective was to identify novel clinical phenotypes associated with *DAX1* missense mutations.

**Patients and Design:** We investigated the genetic basis of isolated mineralocorticoid deficiency in a patient who carries a unique missense mutation (W105C) in the amino-terminal region of *DAX1*.

**D**AX1 (ALSO KNOWN as *AHC*, *NROB1*) encodes an orphan nuclear receptor that regulates the development and function of the adrenal gland, testis, and the hypothalamic-pituitary-gonadal axis (1, 2). *DAX1* acts predominantly as a transcriptional repressor, inhibiting the activity of another nuclear receptor, steroidogenic factor-1 (SF1, NR5A1) (3). The carboxyterminus of *DAX1* is structurally related to the ligand-binding domain (LBD) of other nuclear receptors, whereas the aminoterminalus consists of a unique repeat structure that contains several LXXLL motifs implicated in protein-protein interactions (4). *DAX1* has also been proposed to act as a nucleocytoplasmic shuttling protein, raising the possibility that it exerts additional post-transcriptional regulatory functions (5).

Mutations or deletions of *DAX1* cause X-linked adrenal hypoplasia congenita (AHC) (OMIM, 300200) (1). AHC is an inherited disorder of adrenal gland development, characterized

**Results:** The W105C *DAX1* mutation in the proband was present in three asymptomatic hemizygous males, but it was not detected in the general population. Using *in vitro* studies of *DAX1* expression and function in transfected cells, we demonstrate that the mutant *DAX1* protein exhibits mild loss of function, whether studied for genes it represses or for genes it activates. Structure-function studies suggest that the W105C and other mutations in the aminoterminalus are compensated by the presence of repeated LXXLL motifs that mediate *DAX1* interactions with other proteins.

**Conclusions:** We describe the first missense mutation in the aminoterminalus of *DAX1* and conclude that mutations in this region may be partially compensated by redundant functional domains. Mild *DAX1* mutations may be a cause of isolated mineralocorticoid deficiency. (*J Clin Endocrinol Metab* 92: 755–761, 2007)

by lack of the permanent zone of the adrenal cortex. Boys with this condition usually present with severe primary adrenal failure in infancy or early childhood. Hypogonadotropic hypogonadism becomes apparent at puberty, and infertility results from gonadotropin deficiency in combination with a primary defect in spermatogenesis (6, 7). Almost 100 different mutations in *DAX1* have been described (8, 9), most of which are nonsense or frameshift mutations that cause premature truncation of the protein. Remarkably, missense mutations have been restricted to the carboxy-terminal domain, and have been shown to either impair protein folding and nuclear localization or impair transcriptional repression (10). No missense mutations have been reported in the amino-terminal domain, which contains the repeated LXXLL protein interaction motifs.

In this study, we report a boy who presented with a variant form of AHC (isolated mineralocorticoid deficiency) associated with a W105C missense mutation in the aminoterminalus of *DAX1*. *In vitro* studies were performed to elucidate the functional effects of this mutation and its interactions with the LXXLL repeats.

### Subject and Methods

#### DNA sequencing and mutational analysis

After obtaining institutional approval and written informed consent, genomic DNA was extracted from peripheral blood leukocytes using

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Abbreviations: AF2, Activation function-2 domain; AHC, adrenal hypoplasia congenita; DBD, DNA-binding domain; ER, estrogen receptor; LBD, ligand-binding domain; LXXLL motif, leucine-rich receptor binding motif; SF1, steroidogenic factor-1; WT, wild type.

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standard procedures. The proximal promoter and both exons of *DAX1*, *SF1*, and *CYP11B2* were amplified by PCR using specific oligonucleotide primer pairs and conditions described previously (11). Direct sequencing of PCR products was performed using a Taq big dye terminator sequencing kit and ABI 310 automated sequencer (PE Applied Biosystems, Foster City, CA).

### Construction of human *DAX1* expression vectors

*DAX1* expression vectors (pCMX) containing W105C and a series of artificial N-terminal missense mutations (W105A, W105P) were created by overlapping PCR using methods described previously (12–14). Expression vectors containing cDNA for wild-type (WT) *DAX1* and the naturally occurring L381H missense mutant (13) were used as positive and negative controls for *DAX1* repressor activity. Similarly, triple mutant of the third consensus LXXLL motif were created by introducing alanine substitutions at codons 149 and 150 into the L146A background (AXXAA).

The internally deleted expression vector, which lacks 39 codons flanking codon 105 ( $\Delta 92$ –132), was constructed by ligation of two PCR-generated fragments (after digestion with *SacII* and *XbaI/NheI*, and *EcoRI* and *SplI*, respectively) and a spacer double-stranded oligo with sticky ends (*i.e.* *SplI* and *ScaII* recognition sites) into an empty pCMX vector. The expression vector pBKCMV carrying the WT *DAX1* cDNA was used as a template for generating these two fragments.

To allow antibody-mediated detection of recombinant *DAX1* proteins containing the V5 epitope and a polyhistidine tag, *DAX1* cDNAs for WT, W105C, and Q37X (a naturally occurring severe truncation mutation rescued by in-frame alternate translation) (14) were cloned into pcDNA 6/V5-HisA expression vector (Invitrogen, Carlsbad, CA).

The presence of desired mutations/deletion and the integrity of the constructs were confirmed by direct sequencing before studies of protein expression and function.

### Western blotting

Human embryonic kidney tsa201 cells were transfected with 10  $\mu$ g of pcDNA6/V5-HisA *DAX1* WT or W105C mutant. Equivalent amounts of protein lysates from transfections were resolved with SDS-PAGE and transferred to polyvinylidene difluoride membranes using standard methods. Recombinant *DAX1* was probed with a 1:5,000 dilution of the primary antibody toward the V5 epitope and a 1:10,000 dilution of the secondary antimouse antibody (14).

### Immunocytochemistry

H295R cells were grown on poly-L-lysine coated coverslips, washed three times with PBS, and fixed with 4% neutral-buffered formalin on ice for 30 min. Cells were permeabilized with 0.5% Triton X-100 for 20 min on ice and washed with PBS three times. Blocking solution consisting of either 10% normal goat serum or 10% normal horse serum was

added to the cells for 1 h at room temperature. For immunodetection of endogenous *DAX1*, cells were incubated with anti-*DAX1* rabbit polyclonal antibody (sc-841; Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:50 in blocking solution. For detection of exogenously transfected pcDNA6/V5HisA-WT h*DAX1* or pcDNA6/V5HisA-h*DAX1* W105C, anti-V5 mouse monoclonal antibody (46–0705; Invitrogen, Carlsbad, CA) was diluted 1:100 in blocking solution. V5 and Dax1 were detected using fluorescein-conjugated antimouse IgG (Vector Laboratories, Burlingame, CA) and Texas Red-conjugated antirabbit IgG (Vector Laboratories), respectively (1:100 in blocking solution).

### Functional analysis of WT and mutant *DAX1*

Transient gene expression studies using human embryonic kidney tsa201 were performed in DMEM supplemented with 10% fetal bovine serum and 1% streptomycin/penicillin in a 5% CO<sub>2</sub> atmosphere at 37 C. *DAX1* repression of SF1-mediated transactivation was examined using several different assays. A cDNA encoding the DNA-binding domain of the yeast GAL4 protein fused in frame with the ligand-binding domain of SF1 (codons 133–461) was cloned into the pBIND expression vector. Reporter assays were conducted using 20 ng GAL4-SF1, 50 ng *DAX1* (cloned into pCMX), and 500 ng UAS-TK109luc (15). In a second set of assays, a luciferase reporter construct (500 ng) containing the native rat *LH $\beta$*  promoter (–154 to +5) was cotransfected with expression vectors containing full-length human *SF1* (NR5A1) (20 ng), full-length rat early growth response-1 (*Egr1*) (20 ng), and full-length human WT or mutant *DAX1* (50 ng), as described elsewhere (14–16). Luciferase assays were performed 48 h later.

Transient gene expression studies using H295R cells were performed in a 1:1 mixture of DMEM and Ham's F12 medium supplemented with 2.5 mM L-glutamine, 15 mM HEPES, 2.5%  $\nu$ -Serum (BD Biosciences, San Jose, CA), and ITS+Premix (BD Biosciences). *DAX1* enhancement of SF1-mediated transactivation was examined in reporter assays using 200-ng full-length human *SF1* (NR5A1), 300-ng full-length human WT or mutant *DAX1* and 500-ng reporter constructs (hCYP11B1 or hCYP11B2; kindly provided by Dr. William Rainey, University of Texas Southwestern, Dallas, TX).

### Statistical analysis

Data are presented as a percentage of maximal stimulation (100%). Bars represent the mean  $\pm$  SEM for three to four independent experiments. Statistical analysis was performed using ANOVA followed by the Fisher *post hoc* test. For clarity, only relevant statistical comparisons are indicated in the figures.

## Results

### Clinical presentation of AHC in the patient

The patient described here is an 11-yr-old prepubertal Caucasian boy of Dutch origin who presented with failure to

**TABLE 1.** Investigations of adrenal and gonadal function in males with the W105C *DAX1* mutation

	Patient	Patient	IV.5	IV.5 <sup>c</sup>	V.5	V.5 <sup>b</sup>	V.8	V.8 <sup>b</sup>
Age	1 month	11 yr	38 yr		20 yr		16 yr	
Puberty			Complete		Complete		Complete	
Testosterone (nmol/liter)	1.8 (1.4–14)		18 (9–32)		18 (9–32)		19 (9–32)	
ACTH (ng/liter) (10–70)		33	15		27		23	
Cortisol ( $\mu$ mol/liter) (0.2–0.53)		0.27 <sup>a</sup>	0.46		0.49 <sup>a</sup>		0.37 <sup>a</sup>	
PRA (fmol/liter·sec) (150–800)	30530	2850	630	2110	490	1020	640	1830
Aldosterone (pmol/liter) (150–1250)	310		80	300	70	280	100	900
Sodium (plasma) (136–146 mmol/liter)			140	139	141	142	144	141
Sodium (urine) (50–220/mmol/V) <sup>d</sup>			259	10 <sup>e</sup>	308	29	246	Below detection
FSH (IU/liter)		2 (0–28)	4.2 (2–15)		2.1 (2–15)		5.1 (2–15)	
LH (IU/liter)		<1 (0–1)	5.5 (2–15)		3.8 (2–15)		6.8 (2–15)	

Basal nonstimulated values are shown. Age-specific normal values are given in parentheses. PRA, Plasma renin activity.

<sup>a</sup> Drawn at 1100 h.

<sup>b</sup> After 5 d of low-salt diet.

<sup>c</sup> After 3 d of low-salt diet.

<sup>d</sup> 24-h urine collection.

<sup>e</sup> 8-h instead of 24-h collection period.

thrive and vomiting at the age of 4 wk. He was moderately dehydrated but not hyperpigmented. Clinical laboratory investigations revealed hyponatremia (110 mmol/liter) and hyperkalemia (7.2 mmol/liter). He was started on hydrocortisone and fludrocortisone replacement with a preliminary diagnosis of congenital adrenal hyperplasia (Tables 1 and 2). Serum creatine kinase, glycerol kinase, and very long chain fatty acids were normal. Ornithine transcarbamylase deficiency was excluded by measuring a normal serum ammonium level. He was subsequently withdrawn from hydrocortisone replacement therapy and has not experienced adrenal crisis while on mineralocorticoid treatment only. Although ACTH levels were not elevated, the first ACTH stimulation test showed subnormal cortisol results. A second test showed normal cortisol values, suggesting sufficient adrenal function (Table 2). Family history was unremarkable except for a diagnosis of unilateral Wilms tumor in a 17-yr-old male cousin at the age of 2 yr.

#### Identification of a *DAX1* mutation

After excluding mutations in the genes encoding aldosterone synthase (*CYP11B2*) (Kiel, M. P., personal communication) and SF1, direct DNA sequencing revealed a novel tryptophan to cysteine missense mutation (W105C, TGG→TGC) in the amino-terminal region of *DAX1* (Fig. 1). This mutation was detected in the proband's mother as well as five other females and, unexpectedly, in three unaffected males in the family (Fig. 2). All three unaffected hemizygous males (IV.5, V.5, and V.8) had no signs and symptoms of adrenal insufficiency or hypogonadism. These males were evaluated further after a 5-d salt-restricted diet. Renin and aldosterone responses were normal, indicating adequate mineralocorticoid reserve.

Screening of the *DAX1* gene sequence in 100 healthy Dutch Caucasian individuals (150 X chromosomes) did not reveal the W105C change, suggesting that the isolated mineralocorticoid deficiency in the proband is associated with X-linked AHC.

#### Functional studies of the W105C mutation

Functional studies were performed to assess whether the W105C substitution may represent a hypomorphic allele. First, we confirmed that the W105C mutant was expressed

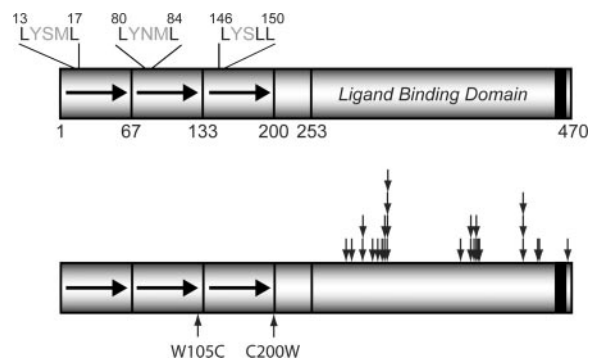


FIG. 1. Schematic representation of *DAX1* mutants. *Upper panel*, The N-terminal repeat region and the locations of three LXXLL motifs (one in each repeat) are shown. *Lower panel*, The sites of amino acid missense mutations identified in *DAX1*. The 27 C-terminal point mutations reported previously (28) are shown above. Those located within the N terminus are shown below.

and efficiently translated in mammalian cells using V5 epitope-tagged WT and W105C *DAX1* mutants (data not shown). Although the *DAX1* W105C mutation was predominantly nuclear localized, the receptor was partially retained in the cytoplasm.

The *DAX1* W105C mutation was tested in several different transfection assays to assess its ability to modulate SF1-mediated transcription. When cotransfected with Gal4-SF1, WT *DAX1* exerted 65% repression, whereas a control AHC mutant (L381H) (13) mediated approximately 10% repression (Fig. 3). The W105C mutant induced approximately 50% repression, indicating that it has diminished repressive activity compared with WT.

Using H295R human adrenocortical cells, *DAX1* enhanced rather than repressed SF1 activity. When cotransfected with a *CYP11B1* reporter, SF1 stimulated activity by 2.1-fold and WT *DAX1* increased this stimulation to 5-fold (Fig. 4). Whereas the control AHC mutant (L381H) (13) exhibited minimal SF1 stimulation (2.3-fold), the W105C mutant conferred partial SF1 stimulation (4.3-fold), indicating that it retains substantial enhancing activity but is less effective than WT.

We next examined *DAX1*-mediated repression using native SF1 and Egr1 stimulation of the LH $\beta$  promoter, because it has been used to characterize a variety of other *DAX1*

TABLE 2. Plasma steroid levels in the proband

	ACTH stimulation				Normal values	
	T = 0	T = 60	T = 0	T = 60	T = 0	T = 60
Age (months)	18		29			
Cortisol ( $\mu$ mol/liter)	0.43	0.49	1.15	1.25	0.20–0.53	>0.50
17-OH progesterone (nmol/liter)	1.20	1.40			0.30–1.30	
Androstenedione (nmol/liter)	0.39	0.41			0.18–0.58	
Aldosterone <sup>a</sup> (nmol/liter)			0.14	0.08	0.11–0.61	0.43–1.43
Corticosterone <sup>a</sup> (B) (nmol/liter)			9.31	46.53	3.50–13.2	32–104
18-OHB <sup>a</sup> (nmol/liter)			2.80	4.10	0.56–1.46	0.83–5.50
Deoxycorticosterone <sup>a</sup> (DOC) nmol/liter)			0.36	0.09	0.15–0.67	0.76–3.12
18-OH DOC <sup>a</sup> (nmol/liter)			1.06	0.95	0.13–0.72	0.58–6.10
Ratio 18-OHB/Aldosterone			20	51		
Ratio B/18-OHB			3.2	11		

T = 60 refers to measurements 60 min after 250  $\mu$ g ACTH (1–24) im.

<sup>a</sup> Measured by M. Peter by RIA after extraction and chromatography [reference values according to M. Peter *et al.* (29)].

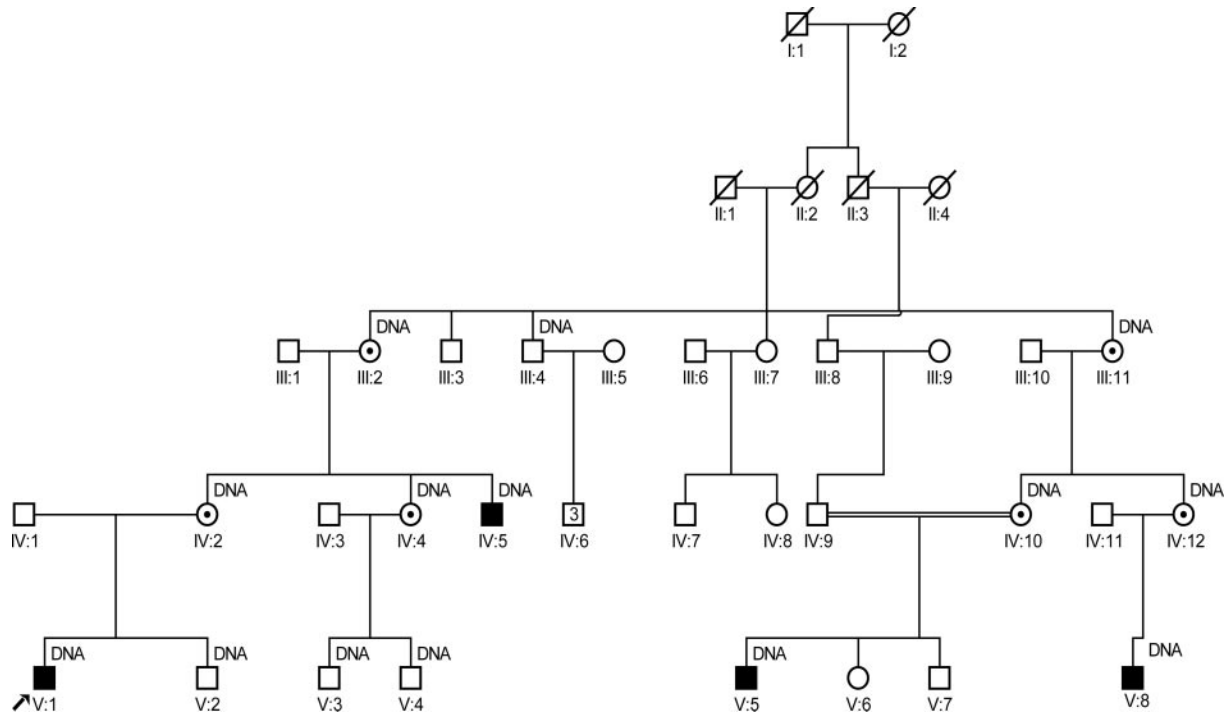


FIG. 2. Kindred analysis of the proband and identification of nucleotide changes in *DAX1*. ■, Hemizygous males; ⊙, heterozygous carrier females. Generations are indicated in Roman numerals. Arrow indicates proband. Genotyping was performed in all individuals labeled with “DNA.” IV:5, Asymptomatic, normal semen analysis; V:5, asymptomatic male, unilateral Wilms tumor at age 2 yr.

mutants (13) (Fig. 5A). WT DAX effectively suppressed SF1-mediated transcription (86% repression), whereas transcriptional repression was largely eliminated in the carboxy-terminal L381H mutant (18% repression). Using this reporter system, the W105C mutant retained transcriptional repression (84% repression), although it was consistently lower than WT DAX1 in multiple experiments. The substitution of a Cys residue at codon 105 creates the possibility of forming nonproductive disulfide bonds. Thus, to assess further the

functional importance of the Trp105, this residue was also substituted with alanine and proline. Each of these substitutions caused greater loss of repression (64% repression) than the Cys mutant. Thus, mutations at Trp105 consistently impair transcriptional repression but the Trp to Cys substitution appears to be better tolerated than certain other residues.

Because the functional effect of mutations at W105 were relatively mild and missense mutations have not been reported previously in the amino-terminal region of DAX1, we

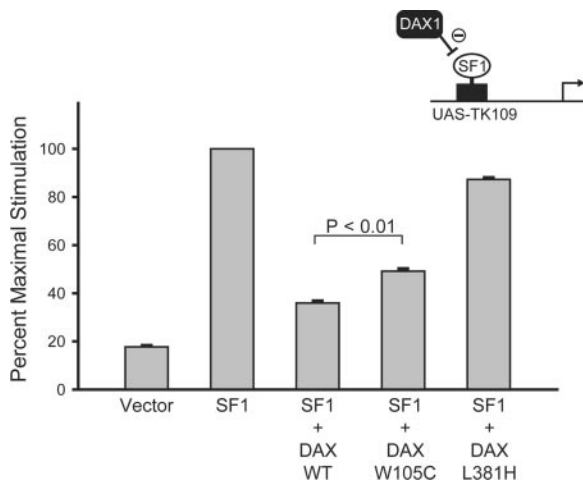


FIG. 3. Loss of transcriptional repression by the W105C *DAX1* mutant using a GAL4-SF1 reporter. Assays were conducted using UAS-TK109luc as a reporter gene in TSA201 cells. Reporter activity was measured in response to GAL4-SF1 in combination with wild-type or various DAX1 mutants. W105C showed partial loss of DAX1 repressor function compared with the more profound loss of repression seen with the C-terminal missense mutant (L381H).

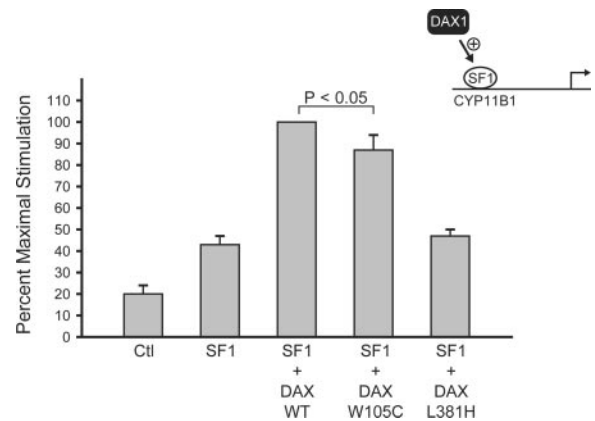
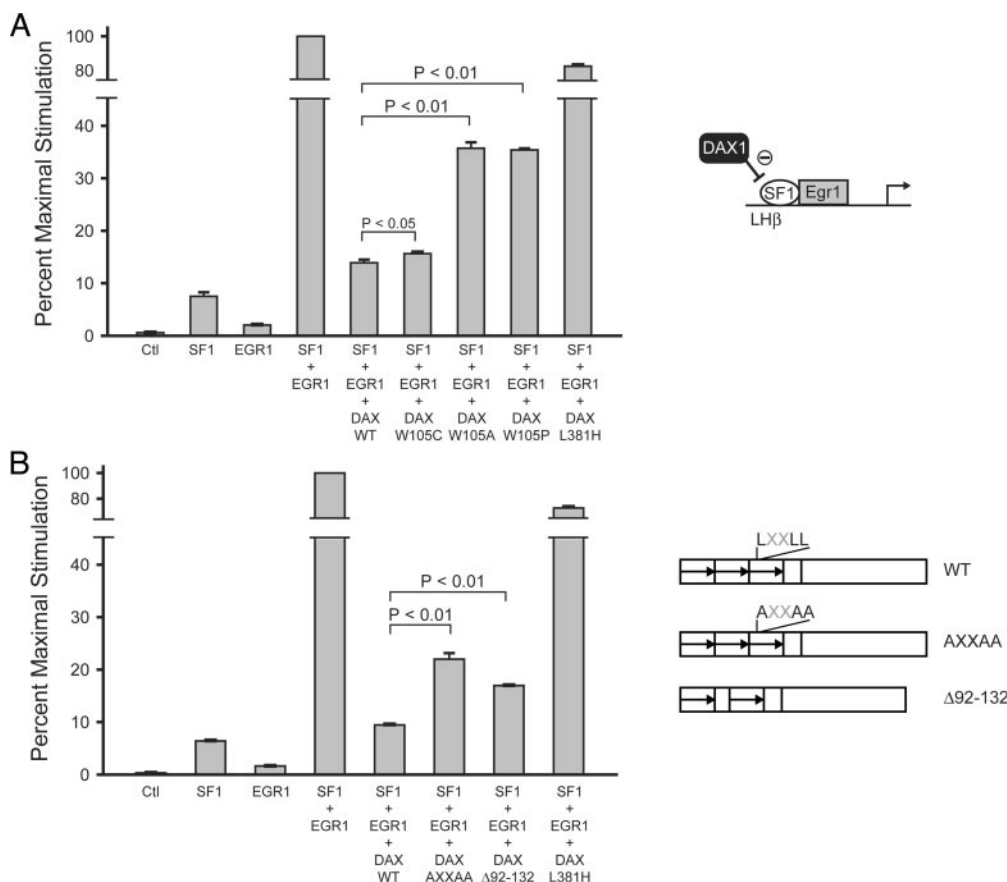


FIG. 4. Loss of transcriptional activation by the W105C *DAX1* mutant using a CYP11B1 reporter. Assays were conducted using CYP11B1-Luc as a reporter gene in H295R adrenal cells. Reporter activity was measured in response to SF1 in combination with wild-type or various DAX1 mutants. WT DAX1 stimulates this reporter gene. W105C showed partial loss of DAX1 stimulation function compared with the more profound loss seen with the C-terminal missense mutant (L381H).



**FIG. 5.** Transcriptional activity of various DAX1 aminoterminal mutants. Assays were conducted in TSA201 cells using the native rat *LHβ* promoter (−154 to +5) cotransfected with expression vectors containing *SF1* (*NR5A1*), early growth response-1 (*Egr1*), and WT or various mutant forms of *DAX1*. **A**, Substitution of various amino acids for W105. Partial loss of repressor function was similarly observed with native rat aromatase (*Cyp19*) promoter (data not shown). Furthermore, exchanging leucines at positions +1 or +4 in each motif with an alanine (L13A, L80A, L84A, L146A, L149A, and L150A) was similar to WT activity; tyrosine instead of a critical serine at position −2 of the third consensus motif (S144Y) was also well-tolerated (data not shown). **B**, Effect of mutations of the aminoterminal repeated sequences in *DAX1*. Assays were conducted using the native rat *Lhβ* promoter (−154 to +5) cotransfected with expression vectors containing *SF1* (*NR5A1*), early growth response-1 (*Egr1*), and WT or various mutant forms of *DAX1*. The locations of the *DAX1* mutants are depicted at the right of the figure. Note that deleting a block of 39 codons flanking codon 105 does not cause a loss of function.

performed additional structure-function analyses to explore the possibility that the functional effects of mutations in this region might be minimized because of the redundant LXXLL motifs that mediate *DAX1* interactions with other proteins (3, 4, 17). Individual Leu to Ala substitutions in each Leu of the 3 LXXLL motifs (residues 13, 17, 80, 84, 146, 149, and 150) did not significantly alter *DAX1* repression (data not shown), consistent with functional redundancy among these motifs (17). However, when all three Leu residues in the third LXXLL motif were substituted with Ala, *DAX1* repression was reduced from 91 to 78%, but the protein still retains substantial repression compared with the carboxy-terminal L381H mutant (27% repression) (Fig. 5B). Because the W105C missense mutation lies between the second and third LXXLL motifs, we considered the possibility that alterations in this region might disrupt interactions between these hydrophobic protein binding sites. However, a deletion (92–132) between these motifs minimally reduced transcriptional repression. Taken together, these findings suggest that the amino terminus of *DAX1* is relatively resistant to mutational effects because of redundant LXXLL motifs. Specifically, mu-

tation of W105 or deletion of the region containing this residue minimally impairs *DAX1* repression of *SF1*-mediated transcription.

### Discussion

Most patients with X-linked AHC present with adrenal crisis in early childhood and have combined glucocorticoid and mineralocorticoid deficiency (18). The disorder is also characterized by failure to enter puberty, reflecting hypogonadotropic hypogonadism (6). Rarely, individuals have been described with milder forms of AHC, characterized by incomplete or delayed onset of adrenal insufficiency, and arrested puberty or mild gonadotropin deficiency (12, 14, 15). These milder clinical presentations have been associated with *DAX1* mutations that do not exhibit complete loss of function. For example, two missense mutations in the *DAX1* carboxy-terminal region (I439S, Y380D) retain partial repression of *SF1*-mediated transcription in transient gene expression studies (12, 15). In another example, alternative translation downstream of a Q37X mutation in *DAX1* allowed

production of reduced amounts of an amino-terminally truncated DAX1 isoform that retained partial transcriptional repression (14). A C200W substitution may also be associated with variable expression of AHC (19).

The clinical presentation of AHC in the proband described here is unusual in two respects. First, the patient had prominent hypoaldosteronism without clear evidence of glucocorticoid insufficiency. Although transient neonatal hypoaldosteronism or progressive mineralocorticoid deficiency with elevated plasma renin activity have been described in AHC (W39X and Y380D, respectively) (14, 15), these patients had concomitant glucocorticoid deficiency. Second, several male relatives who carry the W105C mutation are clinically unaffected, without evidence of adrenal or reproductive dysfunction. These features indicate phenotypic heterogeneity, presumably caused by the effects of other genes that modify or compensate for DAX1 function (20, 21). Alternatively, environmental events such as illness or exposure to medications could unmask underlying adrenal dysfunction. The variable expression of the AHC phenotype is reminiscent of other genetic disorders, particularly when the mutation has partial effects on protein function. For example, in the syndrome of resistance to thyroid hormone, the R316H mutation in thyroid hormone receptor  $\beta$  is associated with variable degrees of hormone resistance among family members with the same mutation (22, 23).

An additional unusual feature of the W105C mutation is its location in the amino-terminal region of DAX1, because each of the previously identified missense mutations cluster within the carboxy-terminal region (13). The idea that the W105C mutation is a disease-causing allele, as opposed to a polymorphism, is supported by its absence among healthy individuals. Of note, this tryptophan residue is highly conserved in several other species including mouse, monkey, and pig (24). The functional studies of the W105C mutation indicate that it consistently reduces DAX1 repression of SF1-mediated transcription, although the loss of function is much less than seen with other DAX1 mutations. Of note, we also found loss of DAX1 enhancement of SF1-mediated transcription of the CYP11B1 promoter by the W105C mutant. This is the first report of DAX1 regulation of CYP11B1 and the observed transcriptional stimulation is reminiscent of that seen previously for the CYP11A1 promoter. Although the mechanistic basis remains unknown for how DAX1 can mediate transcriptional activation for some promoters and repression for others, the loss of DAX1 function occurs for both DAX1 mediated repression and activation. These findings are consistent with other studies suggesting a correlation between DAX1 function *in vitro* with the severity of clinical phenotype as assessed by age of onset and severity of adrenal and reproductive abnormalities (12–15). It should also be recognized, however, that these transcriptional assays may not necessarily reflect all of the functions of DAX1, including its important developmental roles (25).

The function of the DAX1 amino terminus is incompletely understood, although there is increasing evidence that this region mediates interactions with other proteins such as SF1 or ER (3, 4, 17). This region has been shown to use three distinct LXXLL-like motifs (4, 17) that were initially identified as binding sites of nuclear receptors coregulators (26).

All three copies of the LXXLL motifs are conserved among different species (human, mouse, and pig) except in chicken and alligator, which only have one copy (24). Structure–function studies of this region suggest partial functional redundancy since mutations in all three repeats are necessary to abrogate protein–protein interactions and transcriptional repression by DAX1 (4, 17). The location of the W105C mutation between the second and third LXXLL motifs (Fig. 1) led us to consider that it might function by disrupting the actions of these motifs. However, consistent with previous studies (4, 17), we find that mutations in either the second or third LXXLL motif, or deletion of the intervening region, has minimal effect on DAX1-mediated repression, suggesting alternative explanations. For example, Trp105 may play some other structural role, such as altering DAX1 nuclear localization (10) or inducing protein misfolding, particularly because a Cys residue is introduced by the mutation. Because mutations to several other residues (Ala, Pro) have even more pronounced effects than the identified Cys mutation, it is possible that Trp105 is directly involved in DAX1 structure and function. A nonsense mutation (W105X) in a boy with classic AHC (27) also raises the possibility that codon 105 may be a hot spot for mutations.

In summary, we describe the first amino-terminal missense mutation in DAX1 in a subject with atypical and mild AHC, suggesting that missense mutations in the DAX1 amino terminus may impair protein function sufficiently to cause clinical presentation of AHC. These findings expand the phenotypic spectrum of AHC and suggest that DAX1 mutations may cause hypoaldosteronism, with normal glucocorticoid and gonadotropin production.

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