



Phenotypic spectrum of mutations in DAX-1 and SF-1

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Abstract

SF-1 (steroidogenic factor-1) (NR5A1) and DAX-1 (dosage-sensitive sex-reversal, adrenal hypoplasia congenita, X chromosome) (NR0B1) are orphan nuclear receptors that are expressed in the adrenal gland, gonads, ventromedial hypothalamus (VMH), and pituitary gonadotrope cells. The function of these genes has been clarified by examining the consequences of naturally occurring mutations in humans, as well as targeted disruption of the genes in mice.

Mutations in *DAX1* cause adrenal hypoplasia congenita (AHC), an X-linked disorder characterized by adrenal insufficiency and failure to undergo puberty because of hypogonadotropic hypogonadism. Most *DAX1* mutations introduce frameshifts and/or cause premature termination of the protein. Relatively few missense mutations have been described and all are located within the carboxy-terminal half of the protein. Transfection assays demonstrate that AHC-associated *DAX1* mutations abrogate its ability to act as a transcriptional repressor of SF-1. Most boys affected with AHC present with adrenal insufficiency in early infancy, although a significant fraction present in later childhood or even as young adults. The degree of gonadotropin deficiency is also variable. With the exception of one mild missense *DAX1* mutation, genotype–phenotype correlations have been elusive, suggesting an important role for modifier genes. Targeted mutagenesis of *Dax1* (*Ahch*) in mice reveals an additional role in testis development and spermatogenesis. Similar abnormalities appear to be present in humans.

Targeted mutagenesis of *Sf1* (*FtzF1*) prevents gonadal and adrenal development, and causes male-to-female sex-reversal. A human XY individual with a heterozygous *SF1* mutation presented with adrenal insufficiency and complete sex-reversal; this DNA-binding domain mutation prevents SF-1 stimulation of its target genes.

In addition to their clinical relevance, studies of *SF1* and *DAX1* are proving useful for unraveling the genetic pathways that govern adrenal and gonadal development. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: DAX-1; SF-1; Adrenal; Gonadotropins; Testis; Sex determination

1. Introduction

DAX-1 and steroidogenic factor-1 (SF-1) are orphan nuclear receptors that play a key role in the development and function of the adrenal gland and hypothalamic-pituitary gonadal axis. Their expression co-localizes in the adrenal glands, gonads, VMH, and pituitary gonadotrope cells, and mutations in *DAX1* and *SF1* cause adrenal and reproductive dysfunction in humans (Fig. 1; Table 1).

To date, *DAX1* (*AHC*, NR0B1) mutations have been described in more than 70 individuals or families with X-linked adrenal hypoplasia congenita (AHC) (OMIM:

300200). In this condition, primary adrenal failure is associated with hypogonadotropic hypogonadism (HHG). By contrast, only one mutation in *SF1* (*FTZFI*, NR5A1) has been reported in a child with XY sex-reversal, persistent Müllerian structures and primary adrenal failure (OMIM: *184757). In this review, we will describe the phenotypic spectrum of disorders associated with mutations in *DAX1* and *SF1*, and reveal how the discovery of naturally occurring mutations is helping to unravel the role of these orphan nuclear receptors in human development and disease.

2. X-linked AHC and DAX-1

X-linked AHC is a rare, potentially life-threatening disorder of adrenal gland development, first described

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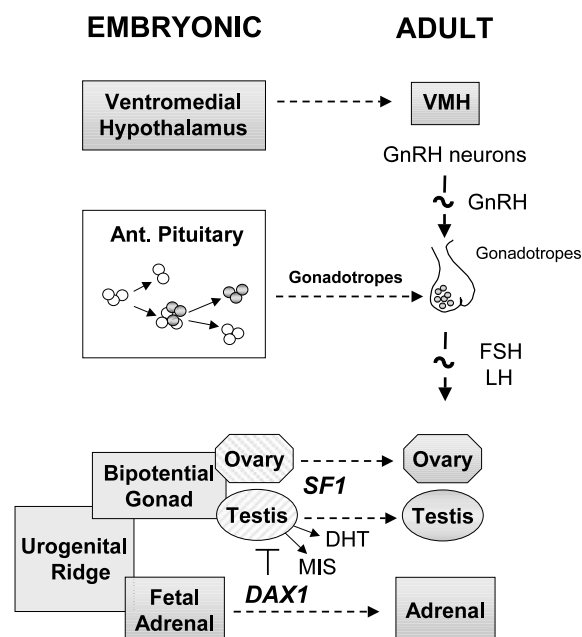


Fig. 1. *DAX1* and *SF1* expression during embryonic development and in the adult. Expression of these nuclear receptors co-localizes in the shaded structures. *SF1* expression precedes *DAX1* expression slightly in the urogenital ridge. Following gonadal determination, *DAX1* expression in the testis declines, whereas *SF1* is necessary for regulating steroidogenesis (DHT, dihydrotestosterone) and for the production of MIS (Müllerian inhibiting substance). Conversely, *SF1* expression declines in the ovary, although this effect may be more pronounced in the mouse than human.

in 1948 (Sikl, 1948). In this condition, the mature adult zone of the adrenal cortex fails to develop. Rather, large vacuolated 'cytomegalic' cells resembling fetal adrenocortical cells are present (Uttley, 1968). Affected boys develop primary adrenal failure in early infancy or childhood, and require glucocorticoid and mineralocorticoid replacement. Prolonged survival of these children into adulthood revealed that HHG is a condition commonly associated with AHC that manifests as a failure of sexual maturation at the expected time of puberty.

The gene responsible for X-linked AHC (*AHC*) was localized to the short arm of the X-chromosome following the discovery of the rare association of X-linked

AHC with glycerol kinase deficiency (GKD), Duchenne muscular dystrophy (DMD) and ornithine transcarbamylase deficiency as part of a contiguous gene deletion syndrome (Worley et al., 1993). In 1994, the gene *DAX1* (dosage-sensitive sex-reversal, AHC, on the X-chromosome, gene 1) was cloned, and *loss-of-function* mutations in it were shown to cause X-linked AHC and HHG (Muscatelli et al., 1994; Zanaria et al., 1994). Previously, duplication of this region of Xp was found to be associated with dosage-sensitive XY sex-reversal (DSS) in humans (Bardoni et al., 1994). *DAX1* now appears to be the gene responsible for DSS, as *overexpression* of *Dax1* can induce sex-reversal in male mice (Swain et al., 1998).

3. Structure, expression and function of DAX-1

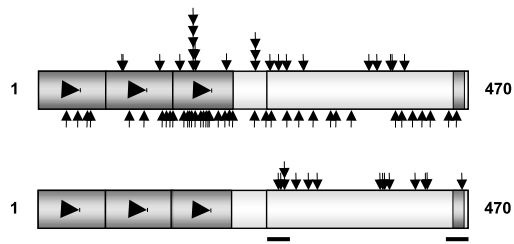
DAX-1 is a 470 amino acid protein encoded by a 5 kb gene containing two exons (Fig. 2a). The amino-terminal domain of *DAX-1* has a unique structure consisting of a 66–67 amino acid repeat motif that does not resemble the DNA-binding domain (DBD) classically found in this region of nuclear receptors. The carboxy-terminal region of *DAX-1* has sequence homology with the putative ligand binding domains (LBD) of several orphan nuclear receptors, including small heterodimer partner (SHP). However, as the name orphan implies, no ligand for *DAX-1* has been identified.

Dax1/DAX1 is expressed in the developing urogenital ridge from E10.5 in mice and 33 dpo in humans. Subsequently, *Dax1/DAX1* is expressed in the primordial adrenal gland, the fetal adrenal gland, and in all layers of the adult adrenal cortex (Guo et al., 1995; Ikeda et al., 1996; Hanley et al., 2000). *Dax1/DAX1* is also expressed in the developing diencephalon (E11.5) and pituitary gonadotropes (E14.5) in mice, and in the hypothalamus and pituitary in humans, consistent with a role in gonadotropin production. Finally, the differentiating mouse gonad also expresses *Dax-1* until E12, after which there is a rapid decline in expression in the testis, but continued expression in the ovary (Ikeda et

Table 1
Phenotypic features of *DAX1* and *SF1* mutation in humans and mice (XY)

	DAX-1		SF-1	
	Mouse knockout hemizygous	Human hemizygous	Mouse knockout homozygous	Human (G35E) heterozygous
Adrenal	Fetal zone retained	Failure	Agensis	Failure
Testis	Hypogonadal	Hypogonadal	Agensis	Dysgenetic
Male sexual differentiation	Normal	Normal	XY sex-reversal	XY sex-reversal
Müllerian structures	Absent	Absent	Present	Present
GnRH/FSH/LH	Intact	Deficient	Deficient	Intact (?)
Spermatogenesis	Impaired	Impaired	Absent	Absent

a) DAX-1



b) SF-1

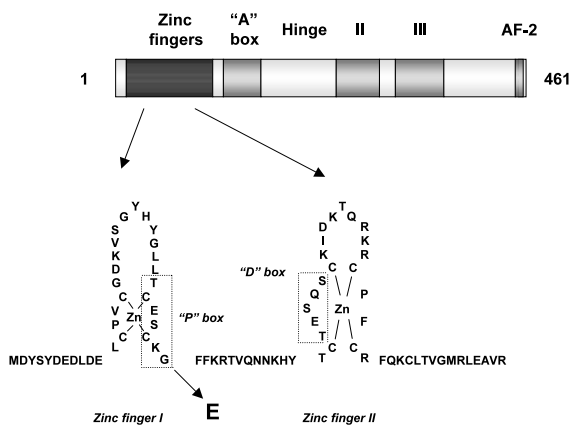


Fig. 2. (a) Schematic representation of DAX-1 to show the range of naturally occurring mutations. Frameshift and nonsense mutations are shown above and below the upper diagram, respectively. Missense mutations are shown above the lower diagram. Putative transcriptional silencing domains are shown as black bars. Reproduced with permission from Reutens et al., 1999. (b) Schematic representation of SF-1. The position of the G35E mutation within the 'P' box of the first zinc finger of SF-1 is shown below. Reproduced with permission from Achermann et al., 1999b.

al., 1996). This sexually dimorphic pattern of expression suggests that *Dax-1* may play a role in sex determination, either as an ovarian determining gene or as a repressor of testicular development. Current evidence suggests that DAX-1 is more likely to be a repressor of testis development, as *DAX1* duplication in humans (Bardoni et al., 1994) and *Dax1* overexpression in mice (Swain et al., 1998) lead to XY sex-reversal. In addition, ovarian development still occurs in female *Dax1* (*Ahch*) knockout mice (Yu et al., 1998) and in a woman homozygous for a *DAX1* gene mutation (Merke et al., 1999). Nevertheless, *DAX1* is once again expressed in the mature testis (Guo et al., 1995; Tamai et al., 1996), where it may influence spermatogenesis directly (Yu et al., 1998).

Although loss of DAX-1 function is associated with adrenal failure and HHG in humans, the majority of functional data suggest that DAX-1 is a repressor of gene transcription. This repression may involve direct binding of DAX-1 to hairpin loop structures in the

promoters of certain target genes (Zazopoulos et al., 1997), or an interaction between DAX-1 and SF-1. This protein–protein interaction may involve specific silencing domains within the carboxy-terminus of DAX-1 (Lalli et al., 1997) or the recruitment by DAX-1 of repressors, such as nuclear receptor co-repressor (NCoR) (Crawford et al., 1998) and Alien (Altincicek et al., 2000). Finally, DAX-1 may also act at the level of post-transcriptional processing by binding to and shuttling mRNA from the nucleus (Lalli et al., 2000).

4. Human DAX-1 mutations

More than 60 different mutations in *DAX1* have now been reported, in more than 70 individuals or families with X-linked AHC (Fig. 2a). Most of these mutations are frameshift or nonsense mutations that result in premature truncation of the DAX-1 protein (Reutens et al., 1999). Disruption of the nine most carboxy-terminal amino acids of DAX-1 (including the putative AF-2 domain) impairs DAX-1 function significantly and results in a severe clinical phenotype. Relatively few missense mutations have been reported in *DAX1*. These mutations appear to cluster within certain regions of the carboxy-terminus of DAX-1, and may provide insight into potentially important domains for DAX-1 function (Zhang et al., 1998). In our experience, approximately 70% of boys with a history of primary adrenal failure and HHG have a mutation in *DAX1*, especially if there is a family history suggesting X-linked inheritance. Nevertheless, the true incidence of X-linked AHC due to *DAX1* mutations is probably less than the 1:12 500 sometimes quoted (Laverty et al., 1973).

4.1. Primary adrenal failure

Boys with *DAX1* mutations usually present with signs and symptoms of primary adrenal insufficiency shortly after birth (60%) or during childhood (40%) (Reutens et al., 1999). This apparently bimodal pattern of presentation may be the result of age-related changes in sodium and fluid intake, mineralocorticoid production and sensitivity, and counter-regulatory stress responses (Reutens et al., 1999). Clinical features in the first months of life include failure to thrive, poor weight gain, vomiting, prolonged jaundice, hyperpigmentation and shock. Biochemical testing usually reveals hyponatremia and hyperkalemia, low cortisol and aldosterone, and elevated ACTH and plasma renin activity. Of note, a normal basal cortisol measured during stress or prior to the onset of symptoms does not exclude impaired adrenal reserve (Achermann et al., 2000; Nakae et al., 1997). In addition, an elevation in 11-deoxycortisol has been documented in some children with X-linked AHC,

perhaps reflecting persistent fetal adrenal activity (Achermann et al., 2000; Peter et al., 1998). Following resuscitation, glucocorticoid and mineralocorticoid treatment is required, with sodium supplementation in the first months of life. In contrast, boys who present in childhood often have a more insidious onset of disease. Signs and symptoms may be subtle, such as nausea, weight loss, or hyperpigmentation, and presentation is sometimes triggered by a stressful event.

Current data suggest that there is little correlation between the type or position of *DAX1* mutation and age at presentation or diagnosis (Reutens et al., 1999). 'Early-' or 'late-presentation' can be a feature in some families where two siblings have the same *DAX1* mutations. In many cases the younger brother is diagnosed at an earlier age, probably reflecting increased awareness of potential adrenal failure by the family or their physicians (Achermann et al., 2000). Further, there appears to be little correlation between the activity of DAX-1 mutants in functional assays and age at presentation, other than the case of a patient who first presented in adulthood (see below). Taken together, these data suggest that additional genetic and epigenetic factors are important in determining the clinical course of X-linked AHC due to DAX-1 mutations (Reutens et al., 1999).

4.2. Hypogonadotropic hypogonadism and impaired spermatogenesis

The association of HHG with X-linked AHC is well established. However, the age at which HHG becomes clinically evident is variable. Although more than 10% of boys with *DAX1* mutations have bilaterally undescended testes at birth (Habiby et al., 1996; Muscatelli et al., 1994), others are reported to have normal HPG activity in infancy (Achermann et al., 2000; Kaiserman et al., 1998; Peter et al., 1998; Takahashi et al., 1997). Further, spontaneous onset of puberty has been reported in several patients with *DAX1* mutations, although pubertal development is incomplete (Bassett et al., 1999). Pulsatile gonadotropin-releasing hormone (GnRH) has been used in an attempt to induce puberty or fertility in patients with *DAX1* mutations. Results are often disappointing, probably reflecting the functional importance of DAX-1 at the level of the pituitary as well as the hypothalamus (Caron et al., 1999; Habiby et al., 1996; Hamaguchi et al., 1998; Seminara et al., 1999; Tabarin et al., 2000). In a limited number of cases, gonadotropins have been used in an attempt to stimulate testosterone production and induce spermatogenesis directly (Seminara et al., 1999; Tabarin et al., 2000). Administration of human chorionic gonadotropin (hCG) stimulates testosterone concentrations into the normal range in most patients, although concomitant administration of follicle stimulating hor-

mone (FSH) was required in one individual (Caron et al., 1999). By contrast, the limited data available suggest that it is difficult to induce spermatogenesis using exogenous gonadotropins in patients with *DAX1* mutations (Seminara et al., 1999; Tabarin et al., 2000). This may reflect a direct effect of DAX-1 on Sertoli cell development and spermatogenesis.

5. Variations in phenotype

Several case reports have been published recently that have widened the spectrum of phenotypes associated with *DAX1* mutations. In one report, a homozygous *DAX1* mutation was found in a woman with HHG, but apparently normal ovarian development and normal adrenal function (Merke et al., 1999). This homozygous change was thought to have arisen by the process of gene conversion. Although two hemizygous males in the kindred had a classic phenotype of X-linked AHC with primary adrenal failure, one hemizygous male had preserved fertility and was able to transmit the mutation to his daughter. Since this W172X mutation has been reported in another kindred with classic X-linked AHC, undetected mosaicism or other epigenetic factors may affect the penetrance of the phenotype in this family.

In another report, an I439S missense mutation in DAX-1 was found in a man who first presented with mild adrenal failure and incomplete HHG in his twenties (Tabarin et al., 2000). Functional studies reveal that this mutation causes a partial loss of function in a variety of transient gene expression assays. In the example shown in Fig. 3, wild-type DAX-1 represses synergistic activation of the *LHβ* promoter by SF-1 and early growth response-1 (Egr-1). Approximately 50% repression is seen with DAX-1 mutants associated with 'classic' AHC (R267P, ΔV269, Δ448–470). The I439S mutant exhibits partial loss of repression (20%). This apparent association between functional genotype and phenotype remains unique among patients with *DAX1* mutations. Nevertheless, *DAX1* should now be considered a candidate gene in patients presenting with adult-onset adrenal failure and partial HHG.

Finally, extreme delayed puberty was reported in several heterozygous female carriers of *DAX1* mutations in one family where hemizygous males had classic features of X-linked AHC (Seminara et al., 1999). This phenotype may have been due to skewed X-inactivation. Taken together, these reports led us to hypothesize that *DAX1* could be a candidate gene for mutation in patients with familial and sporadic forms of HHG and delayed puberty in the absence of adrenal failure (Achermann et al., 1999a). However, direct sequencing of over 100 patients failed to reveal any mutations in *DAX1*.

6. Mutational analysis of family members

Following the identification of a *DAX1* mutation, genetic screening and counseling should be offered to boys at risk of developing adrenal failure, and potential female carriers who could transmit a mutation to their sons. To illustrate this, we recently found a *DAX1* mutation in the eight months old asymptomatic younger brother of a boy with adrenal failure (Achermann et al., 2000). Investigations revealed compensated primary adrenal failure and impaired adrenal reserve despite normal basal cortisol concentrations, confirming that he was at risk of developing adrenal failure.

7. Targeted mutagenesis of *Dax1* (*Ahch*)

We performed targeted mutagenesis of *Dax1* (*Ahch*) to produce a murine model of X-linked AHC and to study the role of *Dax1* in development (Yu et al., 1998). A 'Cre-loxP' targeting strategy was necessary as *Dax1* seems to be essential for embryonic stem (ES) cell survival and because mutations in *Dax1* cause infertility in males (ES cells are XY-derived). No gross abnormalities in adrenal function were detected in knockout mice, although there is a delay in adrenal X-zone regression that may mimic the human adrenal pheno-

type to some extent. The most striking effect of the mutation was seen in the testis. Male *Dax1* knockout mice are hypogonadal and infertile, despite having sufficient testosterone production for the formation of male internal and external genitalia, and apparently adequate gonadotropin production. Progressive seminiferous tubule degeneration, loss of germ cells, impaired spermatogenesis and Leydig cell hyperplasia were seen. *DAX-1* may therefore be involved in Sertoli cell function and spermatogenesis directly, and the *Dax1* (*Ahch*) knockout may represent a good model for examining the role of *Dax1* in these processes (Table 1). The normal ovarian development and fertility seen in female *Dax1* knockout mice indicate that *Dax1* is not an ovarian determination gene.

8. Identification of steroidogenic factor-1

SF-1, and the bovine homologue Ad4BP, were cloned from adrenal cDNA libraries in 1992 and 1993, respectively (Honda et al., 1993; Lala et al., 1992). The existence of a common 'steroidogenic factor' had been proposed following the identification of similar regulatory elements in the proximal promoter regions of the cytochrome P450 steroid hydroxylase gene family (Morohashi et al., 1992; Rice et al., 1991). Both SF-1 and Ad4BP were shown to stimulate the promoter activity of these target genes. The mouse gene encoding this protein was mapped to chromosome 2 and named *FtzF1*, as it resembles the *Drosophila* orphan nuclear receptor, *fushi tarazu* factor 1 (FTZ-F1) (Swift and Ashworth, 1995; Ueda et al., 1990). The human homologue, *FTZF1/NR5A1* contains seven exons and has been mapped to chromosome 9q33 (Taketo et al., 1995; Wong et al., 1996).

9. Structure, expression and function of steroidogenic factor-1

SF1 (*FTZF1/NR5A1*) encodes a 461 amino acid protein that is structurally similar to other members of the nuclear receptor superfamily (Fig. 2b). Critical regions of SF-1 include a two zinc finger DBD, an 'A' box (or FTZF1 box), a hinge region, and an activation function-2 (AF-2) domain. The first zinc finger of SF-1 contains a proximal ('P') box, which confers specificity in the recognition of DNA-binding sites (Mader et al., 1989; Umesono and Evans, 1989). The 'A' box may stabilize DNA binding (Ueda et al., 1992; Wilson et al., 1992), whereas the hinge region and the AF-2 domain of SF-1 are involved in transcriptional activation.

The timing and expression pattern of *SF1* is consistent with its critical role in adrenal development, steroidogenesis, and gonadal differentiation (Fig. 1). In

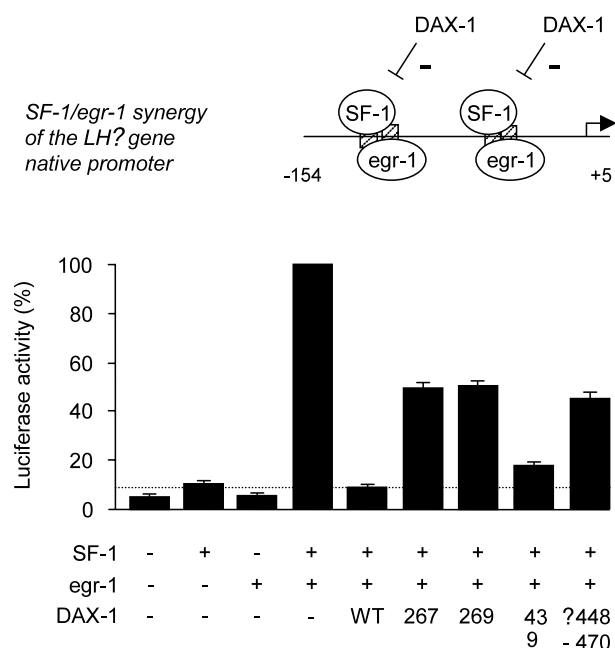


Fig. 3. Synergistic activation of the *LH β* gene promoter by SF-1/Egr-1 is repressed by wild-type DAX-1. The missense mutation R267P, single nucleotide deletion d269V, and carboxy-terminal deletion mutant (amino acids 448–470) show a loss of repression, and represent naturally occurring missense and truncation mutants, respectively. The I439S mutation has partial loss of function. This mutation was found in a patient with delayed onset adrenal failure and partial HHG. Reproduced with permission from Tabarin et al., 2000.

Table 2

SF-1 target genes involved in sexual differentiation, reproduction and steroidogenesis

<i>Sexual differentiation</i>	<i>Steroidogenesis</i>
WT-1	ACTH-R
DAX-1	StAR
MIS	P450scc
MIS-R	3 β -HSD
<i>Reproduction</i>	21-Hydroxylase
GnRH-R	11 β -Hydroxylase
α -GSU	17 α -Hydroxylase
LH β	P450aldo
Oxytocin	Aromatase
Prolactin-R	<i>Metabolism</i>
LEY I-L (INSL3)	HDL-R
Inhibin α	SHP
Oct-3/4	SR-B1

the mouse, *Sf 1* is first expressed in the urogenital ridge at embryonic day 9 (E9) (Hatano et al., 1994), and subsequently in the adrenal primordium (E11) and adrenal cortical cells (E13) into adulthood (Morohashi et al., 1994). A similar expression pattern is seen in humans (Hanley et al., 1999a; Ramayya et al., 1997). In the developing gonad, Sf-1 interacts with several transcription factors involved in the process of male sex determination and testis formation (WT-1, DAX-1, SRY, and SOX9). In Sertoli cells, Sf-1 regulates the expression of Müllerian inhibiting substance (MIS), which leads to regression of Müllerian structures in males (Shen et al., 1994). In Leydig cells, Sf-1 regulates steroidogenesis and testosterone biosynthesis, allowing virilization of the male fetus. In the developing ovary, *Sf 1* transcript levels fall during embryogenesis in the rodent but may persist in humans (Hanley et al., 1999a). Nevertheless, *Sf 1* is expressed in the granulosa and theca cells of the adult ovary at the onset of folliculogenesis (Takayama et al., 1995). Finally, Sf-1 also plays an important role in the development of the ventromedial hypothalamus and pituitary gonadotropes (Ingraham et al., 1994).

SF-1 regulates the transcription of many genes involved in sex determination and differentiation, reproduction, and steroidogenesis (Table 2) by binding to specific response elements in their promoters. SF-1 is believed to bind DNA as a monomer, and recognizes DNA-binding sites containing variations on a PyCA AGGTC A DNA sequence motif. The P-box sequence of SF-1 is important in determining specificity for these response elements. The A-box (or FTZ-F1 box) may be involved in stabilizing monomeric binding, through its interaction with the PyCA of the 5'-flanking sequence. This interaction may be particularly important when DNA binding affinity is compromised following mutation of the P-box (see below) or when the target gene promoter contains an 'imperfect' SF-1 half-site (Ito et al., 2000). Once bound, transactivation of target genes

by SF-1 involves the recruitment of co-activators such as steroid receptor co-activator-1 (SRC-1) (Ito et al., 1998), glucocorticoid receptor interacting protein (GRIP1) (Hammer et al., 1999), CREB-binding protein (CBP)/p300 (Monte et al., 1998) or proline-rich nuclear receptor coregulatory protein (PNRC) (Zhou et al., 2000). It remains unclear whether a specific ligand modulates the activation of SF-1. Although oxysterols were proposed to be SF-1 ligands (Lala et al., 1997), subsequent experiments showed their effect to be weak (Mellon and Bair, 1998). It remains possible that SF-1 is a modulator of gene transcription that does not require ligand activation. In fact, Hammer et al., 1999 showed that SF-1 mediated transcription can be regulated by phosphorylation of a single serine residue (Ser203), acting through the mitogen-activated protein kinase (MAPK) signaling pathway. This finding therefore provides an additional mechanism through which SF-1 may exert its actions on target genes.

10. Targeted mutagenesis of Sf-1 (Ftz-F1)

Several groups have reported targeted deletion of *Sf 1* (*FtzF1*) in mice (Luo et al., 1994, 1995; Sadovsky et al., 1995; Shinoda et al., 1995). Mice homozygous for the gene deletion ($-/-$) have complete adrenal and gonadal agenesis, male-to-female sex-reversal, and persistence of Müllerian structures in males. Adrenal failure becomes apparent after birth. A virtual absence of the VMH occurs and decreased gonadotropin levels are found in pituitary gonadotropes (Ikeda et al., 1995; Shinoda et al., 1995). Mice kept alive by steroid replacement therapy are able to respond to GnRH treatment, suggesting that Sf-1 deficiency does not result in an absolute loss of gonadotropin production by these anterior pituitary cells (Ikeda et al., 1995).

11. Human SF-1 (FTZF1) mutation

Recently, we reported a human *SF1* mutation in a patient with primary adrenal failure, XY sex-reversal and persistent Müllerian structures (Achermann et al., 1999b). The phenotypically female patient exhibited signs of primary adrenal insufficiency during the first two weeks of life and presented in vascular collapse at 17 days of age. Her cortisol (11.7 μ g/dl) and aldosterone (18.7 ng/dl) were inappropriately low given the clinical condition and primary adrenal insufficiency was confirmed by re-evaluation three weeks later (cortisol, 1.2 μ g/dl; ACTH 1165 pg/ml). She was treated with glucocorticoid and mineralocorticoid replacement throughout childhood. Investigations prior to the induction of puberty showed a moderate gonadotropin response to GnRH stimulation and no testosterone

response to exogenous hCG. Laparotomy revealed normal Müllerian structures and streak-like gonads containing poorly differentiated seminiferous tubules and connective tissue. Ethinylestradiol treatment was used to induce feminization and menstruation occurred after the introduction of cyclical progestogen, confirming the presence of a uterus.

Mutation analysis revealed a heterozygous G35E mutation in the 'P' box of the SF-1 DBD in the patient that was not present in her normal parents or 200 control alleles (Fig. 2b). Functional studies showed that this mutation did not interfere with protein expression or nuclear localization. However, as predicted from the site of the mutation in the P-box, the mutant SF-1 failed to bind and transactivate various SF-1 target genes such as *Cyp11a* (*P450scc*), *Dax1*, or *LHβ*.

The phenotype of this patient with a heterozygous point mutation in SF-1 is much less severe than the complete adrenal and gonadal agenesis seen in homozygous *Sf1* (–/–) knockout mice. In contrast, recent evidence suggests that heterozygous *Sf1* (–/+) knockout mice have a partial adrenal phenotype, not as severe as that seen in this patient. We have been unable to demonstrate dominant negative activity by the human mutant SF-1 protein, even though this mutant can bind to and partially transactivate certain promoters containing classic SF-1 response elements (Ito et al., 2000). It is likely, therefore, that SF-1 acts in a dosage-dependent manner. Since SF-1 regulates so many of the genes involved in steroidogenesis (Table 2), haplo-insufficiency of SF-1 could have a cumulative effect on each step of steroid production. Mice may be more resistant to abnormalities of adrenal development, given the comparative phenotypes of patients with *SF1* or *DAX1* mutations with mice with targeted deletions of these genes (Table 1). Indeed, mice may also be more resistant than humans to dose-dependent sex-reversal, based on studies of *DAX1* overexpression (Swain et al., 1998), *WT1* mutation (Kreidberg et al., 1993), and the case of an SF-1 mutation.

12. Summary

The identification of naturally occurring mutations in DAX-1 and SF-1 has underscored the importance of these orphan nuclear receptors in human endocrine development and disease, and is helping to determine critical domains of these proteins that are involved in transcriptional regulation.

Mutations in *DAX1* should be sought in boys with primary adrenal failure and HHG, especially if a family history suggests X-linked inheritance. Identifying these mutations is important so that appropriate treatment and counseling can be given to the proband, and so that 'at risk' family members can be tested and coun-

seled regarding their risk of developing the disorder or passing it on to their offspring. Recently, the phenotypic spectrum associated with *DAX1* mutations has been extended to include a man with adult-onset adrenal failure, partial HHG and impaired spermatogenesis, and females with HHG or delayed puberty. *DAX1* should also be considered a candidate gene for mutation in patients with these conditions, although our studies suggest that mutations in *DAX1* are unlikely to be common in patients with reproductive disorders in the absence of a personal or family history of adrenal failure.

The single *SF1* mutation identified in a patient with primary adrenal failure, XY sex-reversal and persistent Müllerian structures confirms the important role that *SF1* plays at many levels of sex determination, reproduction, and steroidogenesis. Additional *SF1* mutations are being sought in patients with a similar phenotype. Given the effect of this heterozygous P-box mutation on the regulation of target genes, it remains possible that homozygous mutations in *SF1*, or mutations in a different region of *SF1*, could produce variant phenotypes. Further, the clinical phenotype of a *SF1* mutation in a genotypic female (XX) remains to be described, although primary adrenal failure and impaired ovarian development or steroidogenesis might be predicted to occur.

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