



Minireview

The role of SF1 in adrenal and reproductive function: insight from naturally occurring mutations in humans

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Abstract

Steroidogenic factor 1 is a monomeric orphan nuclear receptor and one of several hundreds of transcription factors encoded in the human genome. It regulates the transcription of many genes involved in gonadal development, sexual differentiation, steroidogenesis and reproduction. Recently, mutations in the gene encoding SF1 have been identified in several patients with primary adrenal failure and 46,XY sex-reversal. Interpreting the consequences of these mutations provides further understanding of transcription factor haploinsufficiency in human genetic disease as well as the exquisite sensitivity of humans to gene-dosage effects during adrenal and gonadal development. © 2002 Elsevier Science (USA). All rights reserved.

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1. Introduction

In addition to the use of transgenic and knockout animal models to broaden our understanding of developmental and regulatory networks, the identification of naturally occurring mutations in patients with endocrine disorders is providing important insight into the human genes involved in these complex systems. One such gene is *SFI* (steroidogenic factor 1, also known as *NR5A1*), which encodes an orphan nuclear receptor that is pivotal for the development of the entire reproductive network as well as maintenance of endocrine homeostasis in postnatal life. SF1 is required for adrenal and gonadal development and regulates a large group of adrenal and gonadal target genes. Consistent with its temporal and spatial expression pattern, disruption of *Sfi* (the homologue of *SFI*) in mice leads to a phenotype that includes adrenal and gonadal agenesis, impaired synthesis of pituitary gonadotropins, absence of the ventromedial

hypothalamic nucleus (VMH), and abnormal vascularization of the spleen.

Recently, naturally occurring SF1 mutations have been identified in two different 46,XY individuals who manifest complete phenotypic sex-reversal including persistent Müllerian structures and primary adrenal failure. In addition, an *SFI* mutation has been described in a 46,XX female with primary adrenal insufficiency. These reports provide additional evidence that mutations in nuclear receptors and other transcription factors play an important role in human genetic disease. In this review, we will discuss the diversity of these recently identified *SFI* mutations, and we propose that the phenotypic consequences of these changes reflect the mutation site and gene dosage effects.

2. Structure, expression, and function of SF1

The gene encoding SF1 (*SFI*, *NR5A1*) is located on chromosome 9q33 in humans and encodes a 461 amino acid protein that is structurally similar to other members of the nuclear receptor superfamily (Fig. 1) [1,2]. Critical regions of SF1 include a zinc finger DNA-binding

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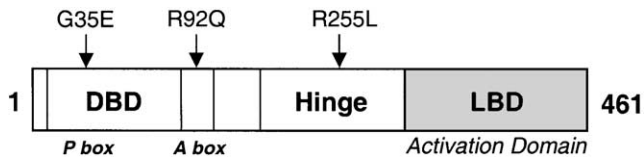


Fig. 1. Schematic representation of SF1. DBD, LBD (which consists of an activation domain), and hinge region are shown. The positions of the mutations G35E, R92Q, and R255L within the P-box, A-box, and hinge region, respectively, are shown by arrows.

domain (DBD), an A-box (or FTZF1 box), a hinge region, and an activation function 2 (AF2) domain. The first zinc finger of SF1 contains a proximal box (P-box), which confers specificity in the recognition of DNA-binding sites [3,4]. The A-box is thought to stabilize monomeric DNA-binding [5,6], whereas the hinge region and the AF2 domain of SF1 are involved in transcriptional activation.

In the mouse, *Sf1* is first expressed in the urogenital ridge at embryonic day 9 (E9) [7], and subsequently in the adrenal primordium (E11) and adrenal cortical cells (E13) [8]. A similar expression pattern is seen in humans [9,10]. In the developing gonad, *Sf1* interacts with several transcription factors involved in male sex determination and testis formation (WT1, DAX1, SRY, and SOX9). In Sertoli cells, *Sf1* regulates the expression of *Amh*, which leads to regression of Müllerian structures in males [11]. In Leydig cells, *Sf1* regulates transcription of many enzymes involved in steroidogenesis and testosterone biosynthesis, resulting in virilization of the male fetus. In the developing ovary, *Sf1* transcript levels fall during embryogenesis in the rodent but may persist in humans [9]. Nevertheless, *Sf1* is expressed in the granulosa and theca cells of the adult ovary at the onset of folliculogenesis [12]. Finally, *Sf1* also plays an important role in the development of the VMH and pituitary gonadotropes [13].

SF1/*Sf1* regulates the transcription of a vast array of genes involved in sex determination and differentiation (*WT1*, *DAX1*, *AMH*, *AMHR*), reproduction (*GNRHR*, *GSUA*, *LHB*, *FSHR*, *Oxytocin*, *PRLR*, *INSL3*, *Inhibin a*, *Oct3/4*) steroidogenesis (*ACTHR*, *STAR*, *CYP11*, *CYP19*, *Akr1b7*, etc.), and metabolism (*HDLR*, *SHP*, *SRB1*, *SCP2*) by binding to its cognate sites in their promoters. Putative SF1 response elements have also been identified in the *FATE* (fetal and adult testis expressed transcript) promoter [14]. SF1 is believed to bind to DNA as a monomer, and recognizes DNA-binding sites containing variations on a PyCA AGGTCA DNA sequence motif. Recent evidence suggests that unique geometric features of such DNA sites (i.e., DNA distortion) may be involved in recognition by monomers [15]. The P-box sequence of SF1 is important in determining specificity for these response elements. The A-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 or when the target gene promoter contains a partially conserved or "imperfect" half-site [16]. Once bound, transactivation of target genes by SF1 involves the recruitment of coactivators such as steroid receptor coactivator 1 (SRC1) [17], glucocorticoid receptor interacting protein (GRIP1) [18], CREB-binding protein (CBP)/p300 [19], proline-rich nuclear receptor coregulatory protein (PNRC) [20], or GCN5 [21]. SF1 may also be regulated through interactions with repressors such as DAX1, DP103, and RIP140 [22–25]. SF1-mediated transcription of target genes is repressed in vitro by DAX1 [26] and there is evidence that Dax1 inhibits *Sf1*-mediated steroidogenesis in vivo as well [27].

While SF1 is required for basal expression of steroidogenic enzymes, its role in hormone-dependent regulation of target genes in vivo remains uncertain. The fact that SF1 mediated transactivation of hormone-responsive genes does not involve brisk upregulation of SF1 expression (i.e., an increase in transcript and protein levels) following stimulation of the adrenals, gonads, or pituitary by peptide hormones [28,29] suggests that SF1 can be activated by ligand-dependent or ligand-independent mechanisms [30,31]. Of note, one study has shown that the increase in PKA-mediated SF1 levels is due to decreased SF1 degradation rather than an increase at the transcriptional level [32]. However, SF1 appears to be active in the absence of exogenous ligand and there is evidence that SF1 mediated transcription can be activated by phosphorylation of a serine residue (Ser203) by the mitogen-activated protein kinase (MAPK) signaling pathway [18,33]. On the other hand, phosphorylation of SF1 may reduce or increase gene expression by the recruitment of either a corepressor or a coactivator depending upon cellular and promoter contexts.

3. Targeted mutagenesis of *Sf1*

Mice homozygous for deletion of *Sf1* (–/–) have complete adrenal and gonadal agenesis, male-to-female sex-reversal, and persistence of Müllerian structures in males [34–37]. A virtual absence of the VMH occurs and there is decreased production of gonadotropins [37,38]. These animals are able to respond to GnRH stimulation, suggesting that *Sf1* deficiency does not result in an absolute loss of gonadotropin production from the anterior pituitary [38]. However, a conditional knockout of *Sf1* in the pituitary has confirmed that this nuclear receptor plays an important role in gonadotrope development and/or function [39]. Homozygous (–/–) *Sf1* knockout mice die from steroid deficiency shortly after

birth. When rescued by adrenal transplantation these (–/–) animals develop obesity later in life, establishing them as a novel, monogenic model of late-onset obesity [40]. Majdic et al. have also shown that transplanted adrenals did not overproduce glucocorticoids, and the marked obesity in these animals differed from that seen in mice with deficient estrogen biosynthesis and action (i.e., ER α - and aromatase knockout mice). Thus, based on the importance of the VMH in the regulation of appetite and body weight, the VMH abnormality is the likely basis for increased body weight in these animals.

4. Human *SF1* mutations

The first human *SF1* mutation was identified in a patient with primary adrenal failure, 46,XY sex-reversal, and persistent Müllerian structures [41]. This phenotypically female baby exhibited signs of primary adrenal insufficiency during the first two weeks of life (Table 1). Cortisol (11.7 μ g/dl) and aldosterone (18.7 ng/dl) concentrations were inappropriately low for the clinical context and primary adrenal insufficiency was confirmed by re-evaluation three weeks later (cortisol, 1.2 μ g/dl; ACTH 1165 pg/ml). Treatment with glucocorticoid and mineralocorticoid was instituted and continued throughout childhood. Investigations prior to the induction of puberty showed a moderate gonadotropin response to GnRH stimulation and no testosterone response to exogenous hCG suggesting the absence of functional gonadal tissue. Retained Müllerian structures and streak-like gonads containing poorly differentiated seminiferous tubules and connective tissue were revealed by laparotomy. Ethinylestradiol treatment was used to induce feminization and menstruation occurred after the introduction of cyclical progestogen, confirming the presence of a uterus. Of note, this patient appears to be developing obesity in late adolescence.

Mutation analysis revealed a de novo heterozygous G35E mutation within the P-box of the SF1 DNA-binding domain (Fig. 1). Functional studies showed that this mutation did not interfere with protein expression or nuclear localization. However, as predicted from the location of the mutation in the DNA-binding domain, the mutant SF1 protein failed to bind and transactivate

many SF1 target genes (e.g., *Cyp11a* (*P450sc*), *Dax1*, *Lhb*), especially those containing a variant half-site (see below). Consistent with its inability to bind to DNA, the mutant SF1 protein did not exert a strong dominant negative effect, but competed with wild-type SF1 when expressed in high doses. Therefore, it is likely that the phenotypic manifestations in the patient primarily reflect haploinsufficiency, suggesting that SF1 acts in a dose-dependent manner in humans [16]. Since SF1 regulates so many genes involved in steroidogenesis, a reduced dosage of functional SF1 could have a cumulative effect on multiple steps that regulate glandular development and steroid production.

The phenotype of this patient with a heterozygous point mutation in *SF1* is less severe than the complete adrenal and gonadal agenesis seen in homozygous *Sf1* (–/–) knockout mice. It is notable, however, the careful analyses of heterozygous *Sf1* (–/+) knockout mice also reveal altered adrenal architecture [42]. The fact that heterozygous males have small testes, whereas *Sf1* null males and the above patient have complete sex-reversal, provides further evidence for the exquisite sensitivity of humans to gene-dosage effects during gonadal development.

From these observations, one might expect alternative or milder phenotypes to result from mutations in other regions of *SF1*. Screening of patients with adrenal insufficiency has led to the identification of a second de novo heterozygous SF1 mutation (R255L) in a XX female (Table 1) [43]. This mutation affects a conserved residue in the hinge region of SF1 (Fig. 1). Although the mutation renders the molecule transcriptionally inactive, it does not appear to impair ovarian development, raising the possibility that ovarian development may be less dependent on SF1 than adrenal and testis development. Whether this mutant SF1 will interfere with ovarian function at the expected time of puberty is unknown.

The report of another *SF1* mutation in a patient with 46,XY sex-reversal is of particular interest, as the mode of inheritance appears to be autosomal recessive (i.e., members of the family who are heterozygous for the mutation do not have a significant phenotype) (Fig. 2) [44]. The phenotype of this patient is remarkably similar to that of the previous XY individual; namely, early-onset primary adrenal failure due to glucocorticoid and

Table 1
Phenotypic characteristics of SF1 mutations in humans

| Mutation | Inheritance | Karyotype-sex | Gonad | Uterus | Adrenal function | Gonadotrope function | Ref. |
|----------|------------------------|---------------|------------|---------|------------------|----------------------|---------|
| G35E | Dominant ^a | XY-female | Dysgenetic | Present | Failure | ? Partial defect | [16,41] |
| R92Q | Recessive ^b | XY-female | Dysgenetic | Present | Failure | ? | [44] |
| R255L | Dominant ^a | XX-female | Ovary | Present | Failure | ? | [43] |

^a De novo mutation.

^b Heterozygous family members tested have normal HPA and HPG activity [44].

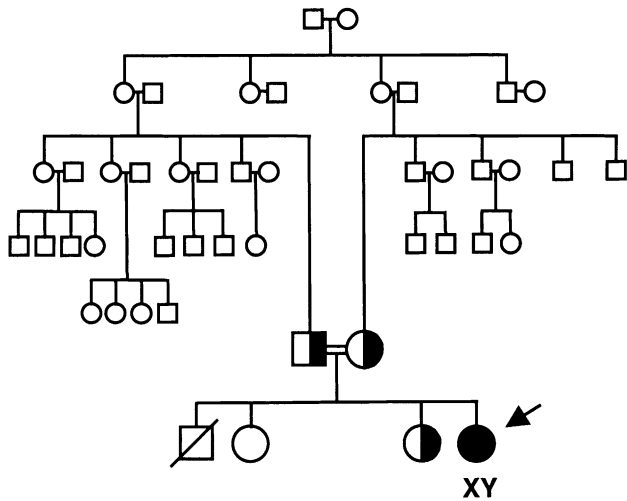


Fig. 2. Segregation of the R92Q substitution in a kindred with a *SF1* mutation. The parents are first cousins. The index case, shown by the arrow, had a hypoglycemic convulsion shortly after birth and evidence of progressive primary adrenal failure in the neonatal period. Heterozygous individuals (denoted by half-shaded symbols) apparently have a normal phenotype (reproduced with permission from Achermann et al. [44]).

mineralocorticoid insufficiency, and 46,XY sex-reversal due to gonadal dysgenesis (Table 1). This mutation affects the A-box region of SF1 that modulates DNA-binding by monomers (Figs. 1 and 3) [16,45]. Heterozygous family members are phenotypically normal (confirmed by biochemical and dynamic endocrine testing), despite having one mutant allele. In contrast, inheritance of two copies of the partially active SF1 mutant causes a phenotype that is similar to that of haploinsufficiency of SF1. These observations underscore the exquisite sensitivity of developmental pathways to gene dosage and residual function of SF1 in humans.

5. From clinical observations to molecular basis of endocrine disease

The data derived from functional studies of these naturally occurring mutations provide important insight into our understanding of the molecular basis of the transcription factor-DNA interactions central to the

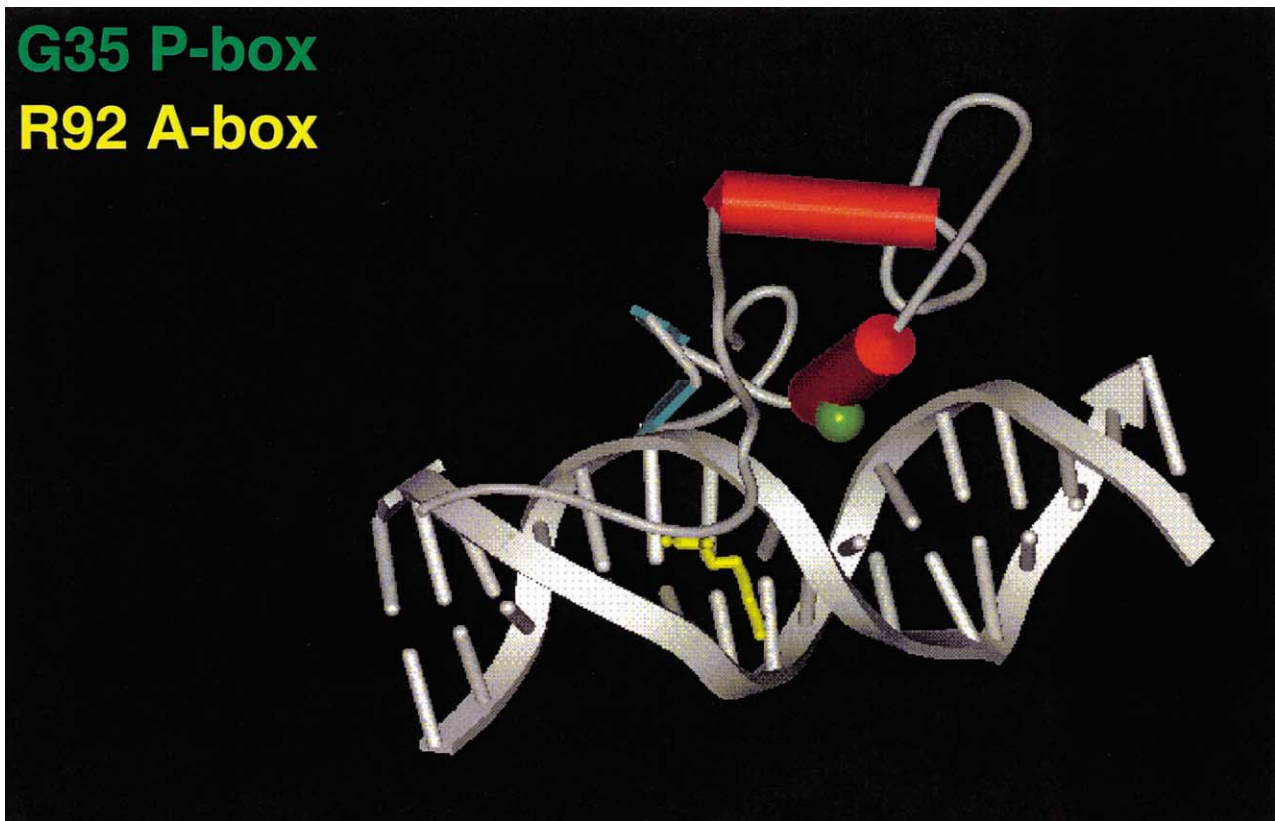


Fig. 3. Model of SF1 binding based upon the crystal structure of nerve growth factor-induced-B (NGFI-B) bound to DNA as a monomer. The position of amino acid 35 within the P-box is shown in green and the amino acid 92 within the A-box is shown in yellow. The P-box amino acids bind to the half-site sequence (variations on AGGTCA) within the major groove of DNA, whereas the A-box is believed to bind to the 5'-flanking sequence (T/CCA) within the minor groove of DNA (reproduced with permission from Achermann et al. [44]).

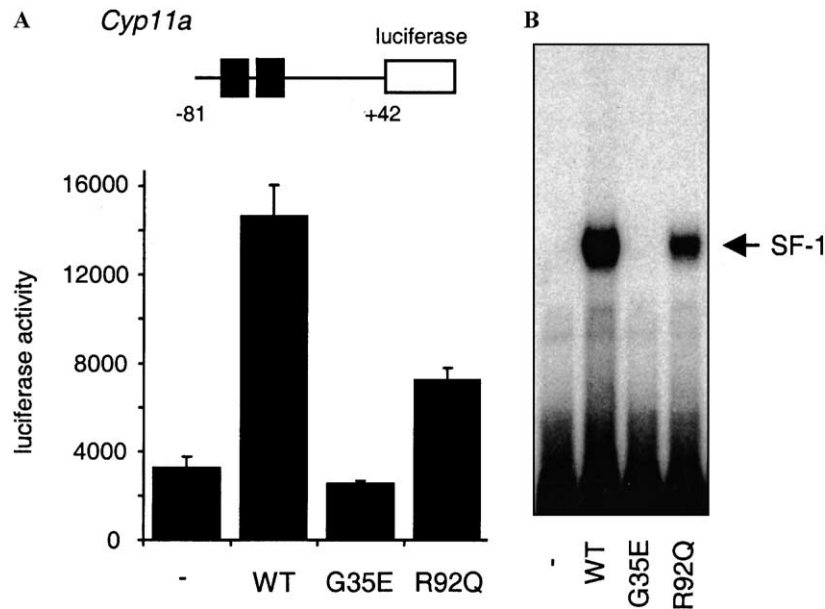


Fig. 4. Functional effects of SF1 mutations. (A) The R92Q A-box mutant shows impaired activation of a critical SF1 target gene, *Cyp11a* (P450sc), and (B) reduced binding to a probe corresponding to an SF1 binding site of this promoter (reproduced with permission from Achermann et al. [44]).

regulation of endocrine genes. It seems that the existence of variant response elements in SF1 target genes is a feature modulating SF1 transcriptional activity (e.g., proximal and distal variant SF1 sites in *Cyp11a* promoter and a composite SF1 site in *Dax1* promoter) [16,44]. Surprisingly, in vitro evidence suggests that wild-type SF1 protein exhibits stronger binding to the variant half-site sequence compared to the “consensus” half-site sequence found in the *Cyp11a* promoter (see [16] and Fig. 1B therein).

Although most of the SF1 mutations described to date alter DNA-binding, each occurs within a different domain of this orphan nuclear receptor. The interaction between P-box codon 35 and the major groove of the DNA helix dictates binding to the AGGTCA motif. As a result, mutations in the P-box sequence (G35E) interfere with SF1 binding, especially to promoters containing variant half-site sequences (e.g., *Cyp11a* 3', TCA AGGCTA) (Fig. 4). There is heterogeneity in the SF1 binding interactions with various target sequences, and mutation of critical P-box residues (e.g., G35E) may not be sufficient to abolish recognition of SF1 response elements and subsequent activation of certain target genes (e.g., *aromatase*, see [16] and Figs. 1B and 2A therein). As mentioned above, the A-box region interacts primarily with the PyCA 5'-flanking sequence and the first part of the half-site, which is located within the minor groove of the DNA helix. This interaction is probably weakened by the loss of charge resulting from the R92Q mutation. However, since this protein–DNA interface is less important than that between the P-box and major groove, only partial loss of transcriptional activity and

binding by the R92Q mutant is seen compared to the G35E mutant, and a homozygous state is necessary for development of the clinical phenotype (Figs. 3 and 4).

In conclusion, we propose that gene dosage and residual function, as well as alterations in the specificity for target genes, can influence the phenotypic consequences of mutations in many developmental transcription factors. Mutations in other regions of the protein or in different amino acids could potentially result in milder or variant clinical presentations in patients who are 46,XY, and subtle forms of adrenal insufficiency could be expected to occur in females heterozygous for hypomorphic alleles of SF1.

Note added in proof. A heterozygous frameshift mutation resulting from the deletion of eight nucleotides at position 2783 and leading to a carboxy-terminal truncated SF1 protein has been described in an individual with 46,XY sex-reversal and clitoromegaly but no uterus, and apparently normal adrenal function. This case provides further evidence for the importance of functional activity of SF1 and raises the possibility that other mutations in this transcription factor might have milder or tissue specific effects in humans [46].

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