

Published in final edited form as:

Sex Dev. 2008 ; 2(4-5): 200–209. doi:10.1159/000152036.

Steroidogenic Factor-1 (*SF-1*, *Ad4BP*, *NR5A1*) and Disorders of Testis Development

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Abstract

Steroidogenic factor-1 (SF-1) (Ad4BP, NR5A1) is a nuclear receptor that regulates many aspects of adrenal and reproductive development and function. Consequently, deletion of the gene (*Nr5a1*) encoding Sf-1 in XY mice results in impaired adrenal development, complete testicular dysgenesis with Müllerian structures, and female external genitalia. Initial efforts to identify *NR5A1* changes in humans focused on 46,XY individuals with combined adrenogonadal failure and Müllerian structures. Although this combination of clinical features is rare, 2 such patients harboring *NR5A1* mutations have been described within the past decade. More recently, however, it has emerged that heterozygous loss of function mutations in *NR5A1* can be found relatively frequently in children and adults with 46,XY disorders of sex development (DSD) but with apparently normal adrenal function. The phenotypic spectrum associated with these changes ranges from complete testicular dysgenesis with Müllerian structures, through individuals with mild clitoromegaly or genital ambiguity, to severe penoscrotal hypospadias or even anorchia. Furthermore, a non-synonymous polymorphism in *NR5A1* may be associated with micropenis or undescended testes within the population. Taken together, these reports suggest that variable loss of SF-1 function can be associated with a wide range of reproductive phenotypes in humans.

Keywords

Gonad; Gonadal dysgenesis; *NR5A1*; Nuclear receptor; Pituitary; Steroidogenesis; Steroidogenic Factor-1; 46,XY DSD

The Identification and Cloning of *NR5A1*

The concept of a common ‘steroidogenic factor’ that could activate multiple different steps in steroidogenesis was first proposed in the early 1990s following the identification of a number of similar regulatory elements in the proximal promoter regions of the cytochrome P450 steroid hydroxylase genes [Rice et al., 1991; Morohashi et al., 1992]. These elements contained variations on an AGGTCA DNA sequence motif, leading to the hypothesis that a common protein – termed ‘steroidogenic factor-1’ (SF-1) – could regulate their transcription.

The gene encoding Sf-1 in the mouse (now termed *Nr5a1*) was first cloned in 1992 from an adrenal cDNA library using a probe corresponding to the DNA-binding domain (DBD) of a related orphan receptor, retinoid X receptor [Lala et al., 1992]. The bovine homologue of this factor (termed adrenal 4-binding protein, Ad4BP) was identified shortly afterwards from

an adrenal cDNA library using the partial sequence of a protein purified from bovine adrenal extracts [Honda et al., 1993]. Both these murine and bovine cDNAs were shown to encode proteins that could activate the promoters of steroid hydroxylase enzymes. Thus, it was concluded that a common steroidogenic factor had been identified.

The mouse gene encoding steroidogenic factor-1 was initially termed *FtzF1*, as it resembles the *Drosophila* orphan nuclear receptor, fushi tarazu factor homolog 1 (*FTZ-F1*) [Ueda et al., 1990; Swift and Ashworth, 1995; Taketo et al., 1995]. This gene was mapped to chromosome 2. The corresponding human gene encoding SF-1 was initially called *FTZFI* but is now more correctly referred to as *NR5A1* (nuclear receptor subfamily 5 group A member 1). This gene was mapped to the long arm of chromosome 9 in humans (9q33) and consists of 7 exons spanning approximately 30 kb of genomic DNA (fig. 1A) [Taketo et al., 1995; Oba et al., 1996; Wong et al., 1996]. Exon 1 is untranslated.

Structure of SF-1

Human SF-1 (NR5A1) is a 461 amino acid protein that shares structural homology with other members of the nuclear receptor superfamily (www.nursa.org). Critical functional domains of this protein include: an amino-terminal 2 zinc finger DNA-binding domain (DBD), an accessory DNA-binding region, a hinge region, and a ligand-binding domain (LBD) that forms an AF-2 structure (fig. 1B). Unlike some nuclear receptors (e.g., androgen receptor), SF-1 does not have a large AF1 domain.

The first zinc finger of the DBD of SF-1 contains a proximal (P) box that is involved in the specific recognition of DNA target sequences by nuclear receptors. This P box amino acid sequence is one of the main factors determining nuclear receptor DNA-binding specificity and interfaces with the major groove of DNA by recognizing variations on an AGGTCA motif [Evans, 1988]. The accessory DNA-binding region of SF-1 contains an A box (FTZ box) motif. This region is believed to stabilize DNA binding by interacting with the minor groove of DNA (e.g., an extended 3' flanking sequence of T/CCA) [Ueda et al., 1992; Wilson et al., 1992; Ito et al., 2000; Little et al., 2006]. This accessory DNA-binding region is important as SF-1 is one of the few nuclear receptors that is thought to bind to target genes monomerically rather than as a homo- or heterodimer. However, other transcription factors (e.g., Pitx1, Egr-1, GATA-4, SOX proteins, Foxl2, Lhx3, Isl-1, Sp1, GR) may also play a role in stabilizing SF-1 binding within promoter complexes and conferring synergistic activity [Tremblay and Viger, 2003; Wang et al., 2007].

The hinge region of SF-1 lies between the amino-terminal DBD and carboxy-terminal LBD. This region is important for post-transcription/translation modification (e.g., phosphorylation, SUMOylation) [Hammer et al., 1999; Komatsu et al., 2004; Lee et al., 2005; Lewis et al., 2008]. These modifications up- or down-regulate SF-1 function directly or in response to intracellular signaling pathways. Indeed, SUMOylation may be critical for repressing SF-1 activity in the basal state, and may involve interaction with DP103 and an E3-SUMO ligase complex [Lee et al., 2005].

The LBD region of SF-1 has structural similarity to other nuclear receptors as it contains 12 helices (H1–H12) that can form an AF2 domain for co-factor recruitment. Several nuclear receptor co-activators have been identified that can interact with SF-1 to modulate its activity (e.g., SRC1, GRIP1, CBP/p300, RIP140, PNR, TReP-132, EID-1) [Ito et al., 1998; Park et al., 2007]. The related nuclear receptor DAX1 (NR0B1) can also regulate SF-1 activity through direct and indirect mechanisms [Ito et al., 1997]. SF-1 was thought to be an 'orphan' nuclear receptor for many years as no high-affinity naturally occurring ligand was identified, and it was proposed that the LBD of SF-1 could adopt a stable conformation in the absence of such a molecule [Desclozeaux et al., 2002]. However, several groups have

now been able to crystallize the LBD of SF-1 and have shown that small phospholipid ligands (e.g., phosphatidylinositols) can bind to this structure (fig. 1C) [Krylova et al., 2005; Li et al., 2005; Wang et al., 2005]. These potential ligands can also modulate SF-1 function in vitro and may form an important interface with upstream signaling pathways (e.g., cAMP/diacylglycerol kinase) [Krylova et al., 2005; Li et al., 2007]. The exact in vivo biological role of ligand-mediated SF-1 activation remains to be determined, although a point mutation in the LBD of SF-1 in one patient involves an amino acid (L437) that is likely to be important in receptor-ligand interactions [Lin et al., 2007] (see below). Furthermore, the recent description of putative agonists and antagonists of SF-1 offer a potential opportunity to modulate receptor function in certain situations [Whitby et al., 2006; Del Tredici et al., 2008].

SF-1 Expression and Target Genes

The expression pattern of SF-1 is consistent with its central role in regulating adrenal development, gonad determination and differentiation, and in the hypothalamic-pituitary control of reproduction and metabolism.

In the mouse, Sf-1 is expressed in the early adrenogonadal primordium from 9 dpc and, thereafter, in the developing adrenal gland and gonad [Ikeda et al., 1994; Nef et al., 2005; Val et al., 2007]. In humans, SF-1 expression has been shown in the developing adrenal gland and bi-potential gonad at 32–33 days post conception [Ramayya et al., 1997; Hanley et al., 1999]. Following testis determination (from around 42 days onwards in humans), SF-1 expression is consistently maintained in the somatic cells of the early testis, where it may play a crucial role together with SRY in supporting *SOX9* expression [Sekido and Lovell-Badge, 2008]. In Sertoli cells, SF-1 activates expression of Müllerian inhibiting substance (MIS, anti-Müllerian hormone (AMH)) from around 7 weeks gestation, which leads to the regression of Müllerian structures in the developing male fetus. In Leydig cells, SF-1 activates the expression of steroidogenic enzyme systems from 8 weeks gestation, which results in androgenization of the external genitalia. In females, persistent expression of SF-1 has been reported in early ovarian development in humans [Hanley et al., 1999], whereas Sf-1 expression may decline in the mouse. However, SF-1 is detectable in somatic cells (granulosa and theca cells) of the adult ovary [Murayama et al., 2008]. The exact role SF-1 plays in the ovary is unclear, as the related nuclear receptor LRH-1 (NR5A2) may also have a role in regulating aromatization and oestrogen synthesis.

In addition to its expression in the adrenal gland and gonad, SF-1 is also expressed in the developing ventromedial hypothalamus (VMH), pituitary gonadotropes, and spleen [Luo et al., 1994; Shinoda et al., 1995; Kurrasch et al., 2007]. Extensive evidence now exists that SF-1 can regulate an array of target genes involved in the development and function of many of these tissues (table 1), either through a direct effect on the proximal promoter of these genes [Cammis et al., 1997; Caron et al., 1997; Sugawara et al., 1997; Yu et al., 1998; Wang et al., 2001] or through an influence on more distant regulatory regions or enhancer elements [Sekido and Lovell-Badge, 2008]. Thus, SF-1 might be considered a ‘master-regulator’ of many aspects of adrenal, gonadal, and reproductive development and function, and relatively small changes in SF-1 activity could have clinically significant effects on these different endocrine systems.

Furthermore, much less is known about the regulation of SF-1 expression itself. Despite reports of *NR5A1* promoter or enhancer regulation by factors such as WT1 [Wilhelm and Englert, 2002], SOX proteins, M33 [Katoh-Fukui et al., 2005], Pbx-Hox [Zubair et al., 2006], Pitx2 [Shima et al., 2008], and USF2 [Utsunomiya et al., 2008], or by CpG island methylation [Xue et al., 2007], many of the key mechanisms influencing SF-1 expression

remain to be elucidated. Further research in this area could also have important implications for understanding the role of SF-1 in human disease.

***Nr5a1* Knockout Mice**

The role of *NR5A1* in embryogenesis was demonstrated clearly following the generation of *Nr5a1* (*FtzF1*) knockout mice in the mid-1990s [Luo et al., 1994; Sadovsky et al., 1995; Shinoda et al., 1995]. Despite using different targeting strategies, these animals were reported to have a similar phenotype by several different groups. Mice homozygous for deletion of *Nr5a1* (*Nr5a1*^{-/-}) exhibited complete adrenal and gonadal agenesis. Whilst some evidence of early urogenital ridge formation was seen in *Nr5a1* knockout embryos (e10.5), the progenitor cells rapidly regressed through apoptosis (e11.5–12). Consequently, all animals had severe adrenal failure, a female phenotype, and persistent Müllerian structures in males. In addition to these features, *Nr5a1* knockout mice have abnormal development of the ventromedial hypothalamus, reduced gonadotropin secretion (which responds to some degree to GnRH stimulation), and small spleens. Moreover, increased anxiety is seen in CNS-specific *Nr5a1* knockout mice [Zhao et al., 2008], and late-onset obesity is seen in null mice that are rescued by adrenal transplantation [Majdic et al., 2002]. This latter phenotype is likely to result from the effects of VMH dysregulation on metabolic processing and locomotor activity.

Although heterozygous *Nr5a1* animals (*Nr5a1*^{+/-}) were originally thought to be unaffected, more detailed studies have also revealed a subtle phenotype in these animals [Bland et al., 2004]. These features include smaller testes, abnormal adrenal architecture, and impaired adrenal stress response. Notably, differences in adrenal development between heterozygous animals and their wild-type littermates were more striking during embryonic development than postnatally. Up-regulation of related transcription factors (such as nerve growth factor induced B, NGFI-B) might be one mechanism that can compensate for haploinsufficiency of *Nr5a1* in heterozygous animals and partly ‘rescue’ adrenal function by the time the animals are born [Bland et al., 2004]. It is unclear whether similar mechanisms might act in the fetal gonad, although NGFI-B is not highly expressed here. Thus, a disparity between the ability of the adrenal gland and gonad to compensate for partial loss of *Nr5a1* function might be one mechanism explaining why a gonadal phenotype is more marked than an adrenal phenotype in humans with heterozygous *NR5A1* changes (see below).

Human Phenotypes Associated with *NR5A1* Changes

Prompted by the phenotype of *Nr5a1* knockout mice, initial studies to identify *NR5A1* mutations in humans focused on patients with primary adrenal failure, 46,XY gonadal dysgenesis and Müllerian structures. This combination of clinical features is rare, but in 1999 the first human *NR5A1* mutation was identified in an individual with this phenotype (OMIM: 184757) [Achermann et al., 1999]. Whereas the homozygous (*Nr5a1*^{-/-}) mouse has complete agenesis of the adrenal gland and gonads, this patient had decompensating primary adrenal failure presenting after birth and small intra-abdominal gonads removed in early adolescence that contained immature seminiferous tubules (fig. 2A). Thus, the patient's phenotype was milder than that observed in the knockout mouse, where no adrenal glands or gonads are identified. Mutational analysis revealed a de novo heterozygous mutation (p.G35E) in the primary DNA-binding domain (P-box) of *NR5A1* in this patient (fig. 3A–C). Despite extensive efforts, no additional changes were found on the other allele. Functional studies show that the p.G35E change significantly impairs the ability of SF-1 to bind to and activate the promoters of several known SF-1 target genes (fig. 4), although partially preserved activation of some targets with ‘perfect’ response elements is observed [Achermann et al., 1999; Ito et al., 2000; Lin et al., 2007]. Although a significant dominant

negative effect was not seen, co-transfection of increasing amounts of mutant p.G35E SF-1 is able to reduce (but not abolish) wild-type SF-1 function, suggesting that a competitive effect might occur [Achermann et al., 1999; Ito et al., 2000]. Furthermore, more significant disruption of wild-type action by the mutant protein might occur in more complex biological systems (e.g., synergistic activation of target promoters by SF-1 and partner transcription factors such as GATA-4) [Tremblay and Viger, 2003]. On balance, therefore, it is possible that this p.G35E P-box change reduces SF-1 function below haploinsufficiency (50%) but – consistent with the patient's phenotype – does not completely eliminate it.

Further support for a dose-dependent effect of SF-1 on adrenal and testis development came with the report of a patient with a similar phenotype (primary adrenal failure, 46,XY DSD, Müllerian structures) in 2002 [Achermann et al., 2002; Jameson, 2004]. This child was born into a highly consanguineous family and was found to harbor a homozygous p.R92Q mutation in the 'A-box' motif of the accessory DBD of *NR5A1* (fig. 3A–C). As described above, this region is involved in stabilizing binding by monomeric nuclear receptors. Functional studies show that the p.R92Q mutation only partially reduces SF-1 activity and binding to about 30–40%, although variable activation of different promoters is seen (fig. 4) [Achermann et al., 2002; Lin et al., 2007]. Since this region is within the accessory DNA-binding domain, it seems that a homozygous change affecting both alleles is necessary for the full expression of the phenotype observed in this patient. Of note, 3 heterozygous relatives (both parents and a sibling) were clinically normal, with no evidence of adrenal insufficiency or reproductive dysfunction.

The question therefore arose whether milder or variant changes in *NR5A1* could be associated with different adrenal or reproductive phenotypes. Several case reports and cohort studies have now been published that go some way to addressing this issue.

SF-1 and Adrenal Failure

Given the central role that SF-1 plays in adrenal development and steroidogenesis, a number of studies have looked for *NR5A1* changes in children and adults with primary adrenal insufficiency. To date, only one *NR5A1* mutation has been reported (p.R255L) in a 46,XX girl who presented at 2 years of age with primary adrenal failure [Biason-Lauber and Schoenle, 2000]. Ovarian function at this age is difficult to assess but appeared to be intact; it remains to be seen whether this patient will have sufficient ovarian function for normal pubertal development and folliculogenesis. In another study, no *NR5A1* mutations were identified in a small cohort of girls diagnosed with adrenal hypoplasia, nor in women who presented in adulthood with antibody negative Addison's disease [Lin et al., 2006]. Similarly, no significant *NR5A1* changes were found in boys who were diagnosed with adrenal hypoplasia who had been pre-screened and found to be negative for *DAX1* (*NROB1*) mutations [Lin et al., 2006]. Thus, it seems that *NR5A1* mutations are not likely to be a common cause of primary adrenal failure in boys without reproductive dysfunction or 46,XY DSD.

SF-1 and 46,XY DSD

The next major development in human SF-1 biology came in 2004 when Correa et al. [2004] reported a heterozygous frameshift mutation (c.1058_1065delAGCTGGTG) in a 46,XY patient who presented in adulthood with clitoromegaly, primary amenorrhea/absent uterus, impaired breast development, hypertension, and moderate obesity. Adrenal function appeared normal in this case [Correa et al., 2004]. This finding was supported soon afterwards by 2 further case reports of heterozygous *NR5A1* mutations in patients with 46,XY DSD. Mallet et al. [2004] reported a heterozygous nonsense mutation (p.C16X) in a 46,XY newborn with ambiguous genitalia, mildly dysgenetic testes, a uterus, and normal

adrenal function. Similarly, Hasegawa et al. [2004] identified a heterozygous single base pair deletion in exon 2 (c.18delC), resulting in a premature stop codon at position 74, in a 27 year old 46,XY patient with ambiguous genitalia, mildly dysgenetic testes, absent uterus, and normal adrenal function. Taken together these reports suggest that heterozygous disruptive changes in *NR5A1* can present with a phenotype of 46,XY DSD.

Within the past year, 2 larger cohort studies and 2 case reports have identified a further 11 individuals or families with heterozygous *NR5A1* changes and a range of 46,XY DSD phenotypes but normal adrenal function [Coutant et al., 2007; Lin et al., 2007; Reuter et al., 2007; Köhler et al., 2008]. The prevalence of *NR5A1* changes in these cohorts has been approximately 15% (4/30) [Lin et al., 2007; Köhler et al., 2008]. The range of mutations and spectrum of phenotypic features described to date are shown in table 2 and figure 2B, C. In most cases, the degree of underandrogenization was greater than the degree of testicular dysgenesis, suggesting that impaired androgen synthesis contributes a significant element to the phenotype. Müllerian structures may be present or absent, and Wolffian structures are often seen. In 2 cases, double heterozygous inheritance of a disruptive mutation with a potentially hypomorphic polymorphism (p.G146A) may have resulted in a more severe clinical phenotype [Reuter et al., 2007; Köhler et al., 2008].

Several of these *NR5A1* changes are heterozygous nonsense or frameshift mutations which might affect RNA stability through nonsense-mediated decay or produce a truncated non-functional protein (p.Y138X, c.424_427dupCCCA, c.536delC, c.1277dupT). However, several novel heterozygous missense mutations in *NR5A1* have also been reported, which are providing insight into critical domains for SF-1 function (fig. 3A). Most of these missense mutations lie within the primary or accessory DNA-binding domains of *NR5A1* and affect highly-conserved amino acid residues (p.V15M, p.C33S, p.M78I, p.R84H, p.R84C, p.G91S) (fig. 3B, C). As expected, these changes interfere with DNA binding in electromobility shift assays and impair transcriptional activation in transient gene expression assays using known *NR5A1* target promoter constructs (fig. 4). No dominant negative effects were seen for these point mutants when wild-type and mutant *NR5A1* were co-transfected into neutral cells, or when mutant *NR5A1* was transfected into *NR5A1* expressing cells, suggesting that haploinsufficiency of *NR5A1* may be sufficient to cause the phenotype. Furthermore, several point mutants (e.g., p.V15M, p.C33S, p.M78I) showed focal aggregation into sub-nuclear bodies, which has been seen previously with certain naturally occurring mutations in other nuclear receptors (e.g., androgen receptor, glucocorticoid receptor) (fig. 4C, D) [Lin et al., 2007; Köhler et al., 2008]. The exact biological consequence of this phenomenon is unclear. Finally, the p.L437Q change in the LBD of *NR5A1* involves an amino-acid residue that is predicted to contribute to the ligand-binding interface of SF-1. This patient is notable as he has a milder phenotype (penoscrotal hypospadias), is raised male, and may have impaired gonadotropin secretion in adolescence (fig. 2C). This mutation has partial residual function in Leydig and Sertoli cell lines (fig. 4B), which is consistent with the less severe phenotype in this patient [Lin et al., 2007].

Although most *NR5A1* mutations appear to arise de novo, another interesting feature that has emerged from this recent work is that approximately one third of heterozygous *NR5A1* changes can be inherited from the mother in a sex-limited dominant fashion [Coutant et al., 2007; Lin et al., 2007; Reuter et al., 2007; Köhler et al., 2008]. That is, a woman can carry the heterozygous change without having ovarian dysfunction, but pass it on to sons who are affected. This situation is highlighted in the family reported by Coutant et al. [2007] in which a heterozygous c.536delC change was inherited from the mother to two 46,XY children with ambiguous genitalia and who were diagnosed with partial androgen insensitivity syndrome. The fact that sex-limited dominant inheritance can mimic an X-linked disorder suggests that *NR5A1* mutations should be sought in cases of potential partial

androgen insensitivity syndrome when several 46,XY offspring are affected but the androgen receptor sequence is normal. Furthermore, the long-term effects of heterozygous *NR5A1* mutations in females are not known, so ovarian function should be monitored with time in this cohort.

In addition to these specific *NR5A1* changes, genomic disruption of the locus containing *NR5A1* (9q33.3) has also been reported in association with human phenotypes. For example, haploinsufficiency of *NR5A1* and *LMX1B* has been described in a patient with genitopatellar syndrome, whereas somatic duplication of *NR5A1* is seen in some pediatric adrenal tumors [Figueiredo et al., 2005; Schlaubitz et al., 2007].

SF-1 and Other Reproductive Phenotypes

Given the potential dose-dependent effects of SF-1 on reproductive function, it might be predicted that milder functional changes in SF-1 could be associated with less severe reproductive phenotypes.

In a recent study of 24 patients with bilateral anorchia (vanishing testis syndrome) with or without micropenis, a heterozygous p.V355M in *NR5A1* was found in one of a pair of dizygotic twin brothers [Philibert et al., 2007]. This p.V355M did not significantly affect protein expression, nuclear localization or DNA binding, but did reduce SF-1-dependent transcriptional activation by about half, most likely through its effect on the helical conformation of the LBD. Unfortunately, long-term follow up data were not available for the twin brother, who also harbored the p.V355M change. Nevertheless, this study suggests that rare variants in *NR5A1* may disrupt its function significantly enough to be a susceptibility factor for milder reproductive phenotypes.

A common non-synonymous polymorphism in *NR5A1* (p.G146A) has also been shown to have mildly altered functional activity in a number of studies, with approximately 80% of wild-type SF-1-mediated transcriptional activation in some assay systems [WuQiang et al., 2003]. A link between this polymorphism and micropenis or cryptorchidism has been proposed in 2 population-based association studies [Wada et al., 2005, 2006]. Thus, milder changes in *NR5A1* may also be important susceptibility factors for a range of reproductive phenotypes in the population, perhaps together with other oligogenic influences.

Conclusion

The past decade has seen considerable progress in our understanding of the role of SF-1 in adrenal and reproductive function. Identifying patients with naturally occurring *NR5A1* mutations is providing important insight into the role of this nuclear receptor in endocrine development and disease. Despite initial efforts aimed at identifying *NR5A1* changes in individuals with combined adrenal and gonadal phenotypes, it is now emerging that changes in *NR5A1* are a relatively common cause of 46,XY disorders of sex development. Whether this cohort of patients will develop adrenal insufficiency with time remains to be seen.

Acknowledgments

JCA is a Wellcome Trust Senior Research Fellow in Clinical Science (079666).

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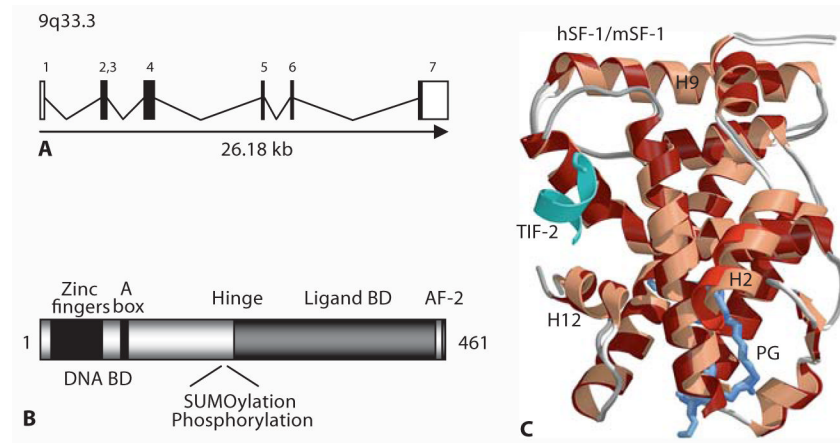


Fig. 1. **A** An overview of the genomic structure of *NR5A1* (encoding SF-1). **B** Cartoon showing key structural domains of SF-1 (Ad4BP, NR5A1). **C** Crystal structure model of the ligand-binding domain of human SF-1 (dark red ribbons) superimposed with that of mouse Sf-1 (light red ribbons) bound to TIF-2. The small phospholipid ligand is shown in blue. Reproduced with permission from Krylova et al. [2005].

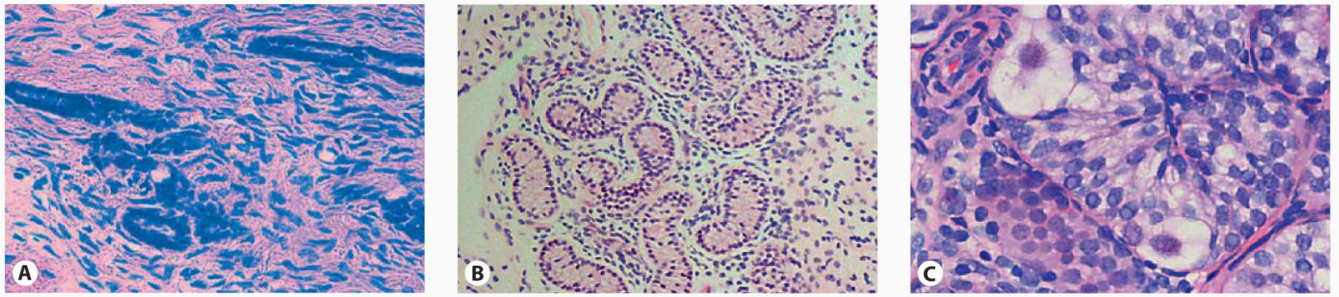


Fig. 2.

Gonadal histology in several patients with *NR5A1* (*SF-1*) mutations (haematoxylin & eosin). **A** Gonad removed in early adolescence from an individual with an adrenal and gonadal phenotype (G35E) showing scattered seminiferous tubules and fibrous tissue (original magnification $\times 400$). **B** Gonadal biopsy taken during orchidopexy at 6 years of age in a boy with a milder phenotype (L437Q) showing hyalinized seminiferous tubules and a predominant Sertoli-Cell-Only phenotype (original magnification $\times 100$). **C** Histology following gonadectomy at 4 months of age in a patient with clitoromegaly (G91S) showing relatively intact seminiferous tubules containing germ cells (original magnification $\times 400$). Reproduced with permission from Achermann et al. [1999] and Lin et al. [2007].

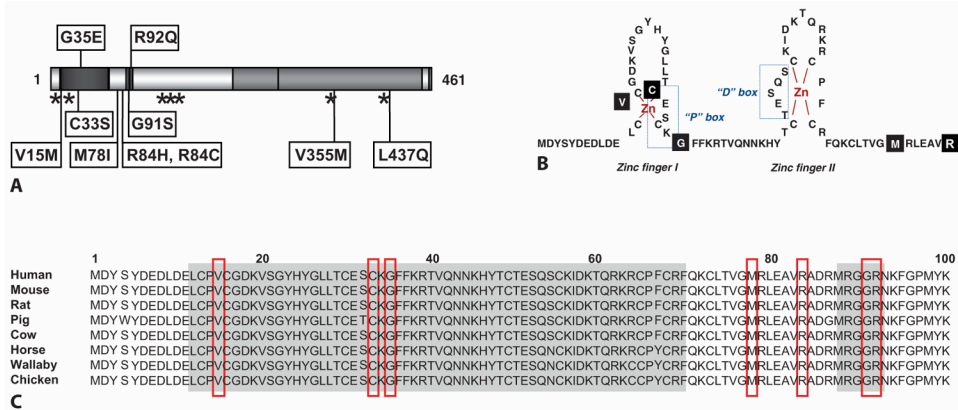


Fig. 3. **A** Cartoon of *NR5A1* showing the positions of missense (labeled) and nonsense/frameshift (asterisks) mutations in *NR5A1*. Those changes associated with a combined adrenal and gonadal phenotype are shown above; those associated with variants of 46,XY DSD but normal adrenal function are shown below. **B** Overview of the zinc-finger region of the DNA-binding domain (DBD) of SF-1 showing the positions of missense mutations in black boxes. **C** These missense changes affect highly conserved amino acids in this region. Modified with permission from Lin et al. [2007].

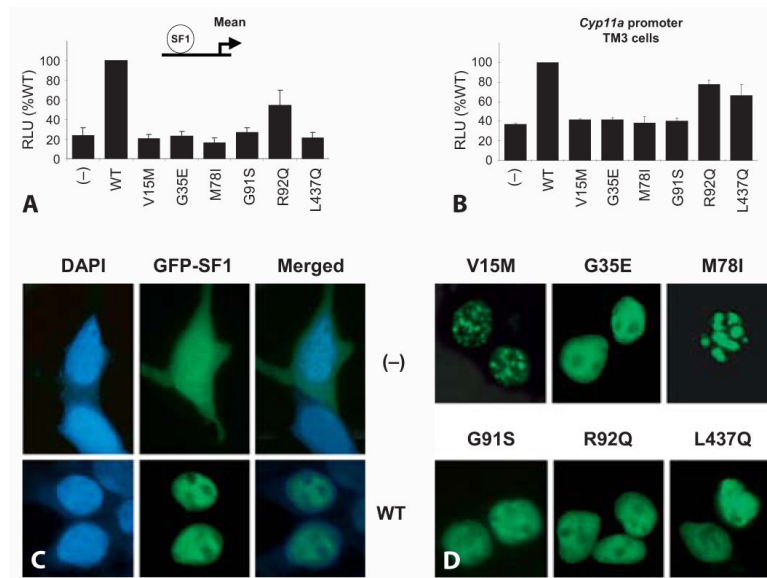


Fig. 4. Functional analysis of wild-type (WT) SF-1 and SF-1 mutants associated with 46,XY DSD. **A** Mean transcriptional activity following studies of 5 different promoters (*Cyp11a*, *MIS*, *Cyp19*, *Ins13*, *LHβ*) in transient transfection assays using *tsa201* (human embryonic kidney) cells. Most mutants found in patients are present in a heterozygous state and show impaired function. The R92Q change is found in a homozygous state and has partial function. **B** The L437Q mutation associated with penoscrotal hypospadias has partial function in a TM3 Leydig cell line. **C–D** GFP-tagged SF-1 shows normal nuclear localization in most cases. However, 2 point mutants (V15M, M78I) show aggregation in subnuclear foci. Modified with permission from Lin et al. [2007].

Table 1

A selection of SF-1 target genes involved in endocrine development and function

Gonad/ reproduction	Steroidogenesis/ adrenal	Metabolism/ CNS
<i>NR0B1 (DAX1)</i>	<i>MC2R (ACTHR)</i>	<i>POU5F1</i>
<i>VNN1</i>	<i>CYP11A1</i>	<i>NR0B2 (SHP)</i>
<i>SOX9</i>	<i>CYP11B2</i>	<i>SCARB1</i>
<i>FATE1</i>	<i>STAR</i>	<i>AKR1B7</i>
<i>ZNF461 (GIOT-1)</i>	<i>HSD3B2</i>	<i>NPC1</i>
<i>AMH (MIS)</i>	<i>SULT2A1</i>	<i>NOS1</i>
<i>AMHR2</i>	<i>ADCY4</i>	<i>CNR1</i>
<i>INSL3</i>	<i>NOV (CCN3)</i>	<i>BDNF</i>
<i>GnRH/GnRHR</i>	<i>CYB5A</i>	<i>UCN3</i>
<i>LHB/LHCGR</i>	<i>CYP19</i>	<i>CRHR2</i>
<i>FSHB/FSHR</i>	Hydroxylases	
<i>INHA</i>		
<i>OXT</i>		
<i>CXorf6 (MAML2)</i>		

Table 2
Molecular and clinical features of 46,XY individuals with mutations in *SF-1 (NR5A1)*

Mutation	Form	Inheritance	Adr	External genitalia	Testis	Uterus	Reference
p.G35E	hetero	de novo	+	female	severe	+	Achermann et al. [1999]
p.R92Q	homo	recessive	+	female	NK	+	Achermann et al. [2002]
c.424_427dup + p.G146A	hetero + SNP	NK	-	female	severe	+	Köhler et al. [2008]
p.M78I	hetero	SLD	-	female	mild	(+)	Lin et al. [2007]
p.V15M	hetero	de novo	-	female	mild	-	Lin et al. [2007]
p.R84C+ p.G.146A	hetero + SNP	SLD+ SNP	-	ambiguous	mild	-	Reuter et al. [2007]
p.C91S	hetero	SLD	-	ambiguous	mild	(+)	Lin et al. [2007]
p.C16X	hetero	de novo	-	ambiguous	mild	+	Mallet et al. [2004]
c.184delC	hetero	de novo	-	ambiguous	mild	-	Hasegawa et al. [2004]
c.1058_1065del	hetero	de novo	-	ambiguous	regress ^a	-	Correa et al. [2004]
p.C33S	hetero	de novo	-	ambiguous	mild	-	Köhler et al. [2008]
p.R84H	hetero	de novo	-	ambiguous	normal	-	Köhler et al. [2008]
p.Y138X	hetero	de novo	-	ambiguous	mild	-	Köhler et al. [2008]
c.536delC	hetero	SLD	-	ambiguous	normal	-	Coutant et al. [2007]
c.1277dupT	hetero	SLD	-	ambiguous	normal	-	Köhler et al. [2008]
p.L437Q	hetero	de novo	-	hypospadias	mild	-	Lin et al. [2007]
p.V355M	hetero	SLD	-	micropenis	regress	-	Philibert et al. [2007]

Modified with permission from Köhler et al. [2008]. hetero = Heterozygous; homo = homozygous; SNP = single nucleotide polymorphism; NK = not known; SLD = sex-limited dominant; Adr = adrenal failure; + = present; - = absent; (+) = remnant present; ambiguous = clitoromegaly or ambiguous genitalia.

^a Gonadal tissue not found at laparoscopy at age 31 years.