

A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans

Steroidogenic factor-1 (SF-1, also known as Ad4BP) is an orphan nuclear receptor that regulates the transcription of an array of genes involved in reproduction, steroidogenesis and male sexual differentiation (for example, *Amh*, *Ahch*, *Cyp11a*, *Star* and those encoding steroid hydroxylases, gonadotropins and aromatase¹). Disruption in mice of the gene encoding Sf-1, *Ftzf1*, causes failure of adrenal and gonadal development, XY sex reversal, persistence of Müllerian structures in males and abnormalities of the hypothalamus and pituitary gonadotropes^{1,2}.

Here we report a human *FTZFI* mutation in a phenotypically female patient who presented with primary adrenal failure in the first two weeks of life (cortisol, 1.2 µg/dl (normal range 5–25 µg/dl); aldosterone, 5.0 ng/dl (6–105 ng/dl); adrenocorticotropin, 1,665 pg/ml (10–80 pg/ml)). Her karyotype was XY, and a presumptive diagnosis of congenital lipid adrenal hyperplasia was made. At ten years, her hormonal status was exam-

ined further before the induction of puberty. Pituitary gonadotropins responded to gonadotropin-releasing hormone stimulation (luteinizing hormone 1.2→8.6 mIU/ml; follicle-stimulating hormone 17.8→38.0 mIU/ml), but there was no testosterone response after stimulation with human chorionic gonadotropin. Notably, normal Müllerian structures were found at laparotomy and streak-like gonads (1.5 cm×0.4 cm×0.2 cm) containing poorly differentiated tubules and connective tissue were removed (Fig. 1a). Transdermal 17β-estradiol gel induced normal breast development. Her uterus grew and regular menstruation occurred after the introduction of cyclical progestogen.

Direct DNA sequencing of *FTZFI* (ref. 3) revealed a heterozygous 2-bp G35E (GGC→GAA) mutation in exon 3, which encodes part of the DNA-binding domain of SF-1 (Fig. 1b). The mutated glycine is the last amino acid in the proximal box (P-box) of the first zinc finger of SF-1 (Fig. 1c). This

region is critical for the recognition of DNA binding sites⁴ and confers specificity to nuclear receptors in the regulation of target genes. Paternity was confirmed using multiple microsatellite markers (data not shown). The mutation creates a novel *EcoRI* site (GAATTC) that is not present in the parents of the patient (who have normal phenotype and sequence) or in 100 controls (200 alleles; Fig. 1d). Although we cannot exclude the possibility of an additional mutation elsewhere in the *FTZFI* locus, direct sequence analysis of the remainder of the coding region, splice sites and promoter region (−873 to +143) did not reveal additional mutations. The coding sequences of other candidate genes, including *SRY*, *STAR* and *AHC* (formerly *DAX1*), are normal.

We created the G35E mutant form of SF-1 by site-directed mutagenesis for use in functional studies. This mutation does not interfere with protein translation, stability or nuclear localization (Fig. 2a,b), but it eliminates the binding of SF-1 to a canonical binding site⁵ (Fig. 2c). Consistent with its impaired DNA binding, the G35E SF-1 mutant does not transactivate a known SF-1-responsive reporter gene (mouse *Cyp11a* promoter; Fig. 2d). Mutant SF-1 does not exhibit dominant-negative activity when co-expressed with wild-type SF-1 (unpublished data).

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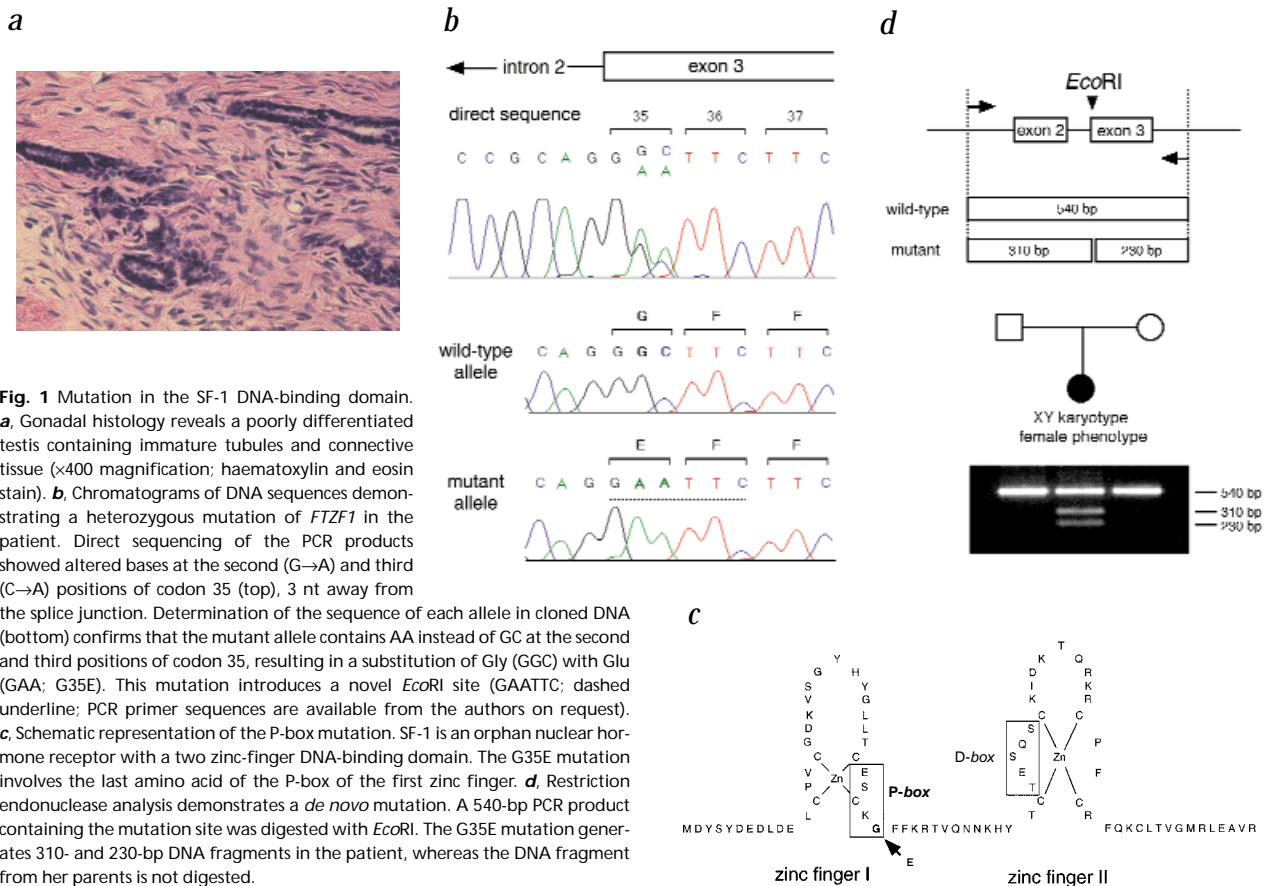
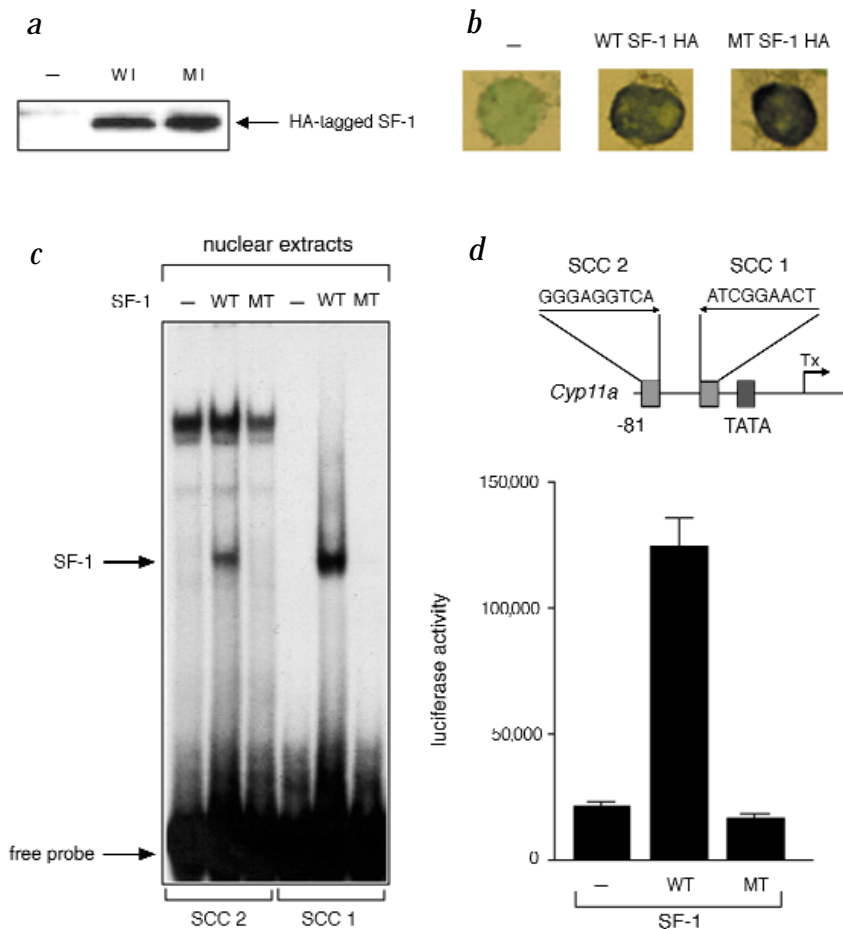


Fig. 1 Mutation in the SF-1 DNA-binding domain. **a**, Gonadal histology reveals a poorly differentiated testis containing immature tubules and connective tissue (×400 magnification; haematoxylin and eosin stain). **b**, Chromatograms of DNA sequences demonstrating a heterozygous mutation of *FTZFI* in the patient. Direct sequencing of the PCR products showed altered bases at the second (G→A) and third (C→A) positions of codon 35 (top), 3 nt away from the splice junction. Determination of the sequence of each allele in cloned DNA (bottom) confirms that the mutant allele contains AA instead of GC at the second and third positions of codon 35, resulting in a substitution of Gly (GGC) with Glu (GAA; G35E). This mutation introduces a novel *EcoRI* site (GAATTC; dashed underline; PCR primer sequences are available from the authors on request). **c**, Schematic representation of the P-box mutation. SF-1 is an orphan nuclear hormone receptor with a two zinc-finger DNA-binding domain. The G35E mutation involves the last amino acid of the P-box of the first zinc finger. **d**, Restriction endonuclease analysis demonstrates a *de novo* mutation. A 540-bp PCR product containing the mutation site was digested with *EcoRI*. The G35E mutation generates 310- and 230-bp DNA fragments in the patient, whereas the DNA fragment from her parents is not digested.

Fig. 2 Functional analyses of mutant SF-1. **a**, Expression of mutant SF-1. Western-blot analysis was performed with anti-influenza haemagglutinin (HA) antibody using nuclear extracts collected from human embryonic kidney tsa 201 cells transfected with an empty vector (-), or HA-tagged wild-type (WT) or mutant (MT) SF-1 expression vectors¹⁴. **b**, Nuclear localization of mutant SF-1. Cells transfected with an empty vector or HA-tagged wild-type or mutant SF-1 expression vectors were subjected to immunocytochemical analysis using anti-HA antibody. Nuclei of cells expressing wild-type or mutant SF-1 proteins exhibit dark-brown staining, whereas the nuclei of cells transfected with an empty vector stain light green. **c**, DNA binding of mutant SF-1. Nuclear extracts prepared from cells transfected with an empty vector or wild-type or mutant SF-1 expression vectors were subjected to electrophoretic mobility shift assay using ³²P-radiolabelled probes (20 fmol) corresponding to each of the two SF-1 sites in the mouse *Cyp11a* (P450_{ssc}) promoter¹⁵. **d**, Transcriptional activity of mutant SF-1. A luciferase reporter construct (500 ng) containing the mouse *Cyp11a* (P450_{ssc}) promoter (-81 to +42) was transfected into tsa 201 cells with an empty vector, or with wild-type or mutant SF-1 expression vectors (20 ng). Results are the mean ± s.e.m. of triplicate transfections.



This case demonstrates that SF-1 is essential for normal adrenal and gonadal development in humans, as predicted from targeted disruption of *Ftzh1* in mice^{1,2}. The adrenal and gonadal features in the patient reported here are not as severe as those described in homozygous mice, and gonadotropin release is preserved. It is notable, however, that *Ftzh1*^{+/-} mice are phenotypically normal. The more penetrant phenotype in humans may be a consequence of tissue- or promoter-specific effects of this particular mutation in the P-box. Although no dominant-negative activity was observed in transient expression assays, it is also possible that the mutant protein exerts inhibitory activity in some tissues or with a subset of target genes. Targeting specific missense mutations into the *Ftzh1* locus of transgenic lines may allow direct comparisons of phenotypes in mice and humans.

This SF-1 mutation causes complete XY sex reversal, including normal female external genitalia and retention of the uterus. This contrasts with disorders of steroid biosynthesis, in which no uterus is present. Our findings provide evidence that SF-1 regulates the regression of Müllerian structures in humans, either through direct actions on the *AMH* (formerly *MIS*) promoter⁶ or secondary to an abnormality of Sertoli-cell development or function. The presence of immature tubules in the gonad is consistent with *SRY* being the primary testis-determining gene in mammals. The incomplete gonadal development observed here, however, suggests that SF-1, and the genes that it regulates, are necessary for subsequent male sexual differentiation.

There are now a number of examples of dosage-sensitive mechanisms of sex-determination and differentiation in humans. In addition to mutations in *SRY*, XY sex reversal may result from overexpression of *AHC* as a consequence of Xp duplication⁷, or underexpression of the autosomal genes *SOX9* (ref. 8) and *WT1* (refs 9,10) because of heterozygous mutations or deletions. This report suggests that *FTZFH1* also functions in a dosage-dependent manner to regulate male sex determination. Humans may be more sensitive to these gene-dosage phenomena than mice, as *Ahch* (formerly *Dax1*) overexpression in male mice only causes sex reversal in the genetic background of a weakened *Tdy* (formerly *Sry*) allele¹¹ and XY heterozygous *Wtl* mice are phenotypically male¹². Studies of the interactions of SF-1, WT-1 and DAX-1 *in vitro* are also consistent with a dose-dependent mechanism for their actions as transcription factors¹³.

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