The role of DAX-1 (NR0B1) and steroidogenic factor-1 (SF-1, NR5A1) in human adrenal function

Ranna El-Khairi, Alejandro Martinez-Aguayo, Bruno Ferraz-de-Souza, Lin Lin, John C Achermann

Developmental Endocrinology Research Group, Clinical and Molecular Genetics Unit, UCL Institute of Child Health, University College London, London, UK

Short title: DAX-1 (NR0B1) and SF-1 (NR5A1) in adrenal function

Keywords: DAX-1, SF-1, X-linked adrenal hypoplasia congenita, hypogonadotropic hypogonadism, primary adrenal insufficiency, Addison disease, 46,XY disorders of sex development (DSD), nuclear receptors, infertility, primary ovarian insufficiency.

Correspondence:

Dr John C Achermann
Developmental Endocrinology Research Group,
Clinical & Molecular Genetics Unit,
UCL Institute of Child Health,
University College London,
30 Guilford Street,
London WC1N 1EH, UK
Phone: 44 207 905 2887
Fax: 44 207 404 6191
Email: j.achermann@ich.ucl.ac.uk

Abstract

The nuclear receptor transcription factors, DAX-1 (*NR0B1*) and SF-1 (*NR5A1*), regulate many aspects of adrenal and reproductive development and function. Disruption of the genes encoding these factors can be associated with pediatric adrenal disease. DAX-1 mutations are classically associated with X-linked adrenal hypoplasia congenita (AHC), hypogonadotropic hypogonadism (HH) and impaired spermatogenesis. However, other phenotypes are also being reported, such as isolated mineralocorticoid insufficiency, premature sexual development, primary adrenal insufficiency in a 46,XX patient and late onset X-linked AHC and/or HH. SF-1 mutations have also been associated with primary adrenal insufficiency, together with 46,XY disorders of sex development (DSD). However it is emerging that SF-1 changes are a relatively rare cause of primary adrenal failure in humans, and most individuals with SF-1 mutations have a spectrum of 46,XY DSD phenotypes. These conditions range from 46,XY females with streak gonads and Müllerian structures, through children with ambiguous genitalia and inguinal testes, to severe penoscrotal hypospadias with undescended testes. Therefore, the human gonad appears to be more sensitive than the adrenal gland to loss of SF-1 function. This review will focus on the expanding range of phenotypes associated with DAX-1 and SF-1 mutations.

Introduction

The human adrenal gland first develops from an area of intermediate mesoderm at around four weeks post conception and undergoes rapid growth and differentiation during fetal life into a fully functional organ capable of releasing cortical and medullary hormones. Defects in several different transcriptional, signaling and mitogenic pathways can result in underdevelopment of the adrenal gland, leading to the clinical condition congenital adrenal hypoplasia (or adrenal hypoplasia congenita; AHC) [1]. Many of these processes also regulate adrenal function postnatally and throughout the lifespan, potentially resulting in primary adrenal insufficiency when disruption occurs.

In this chapter we will focus on the role of the nuclear receptor transcription factors DAX-1 (*NR0B1*) and steroidogenic factor-1 (SF-1, *NR5A1*, Ad4BP) in human adrenal development and function. The importance of these factors in human adrenal disease is now well established, but the spectra of phenotypes associated with these conditions continue to expand.

DAX-1 (NR0B1)

DAX-1 (Dosage-sensitive sex reversal - Adrenal hypoplasia congenita critical region on the X chromosome 1) is an atypical nuclear receptor first reported in 1994 to be associated with X-linked adrenal hypoplasia congenita (X-linked AHC, OMIM 300200) [2]. The genetic locus for this transcription factor was mapped to the short arm of the X chromosome (Xp21) as X-linked AHC can occur as part of a contiguous gene deletion syndrome with glycerol kinase deficiency (GKD) and Duchenne Muscular Dystrophy (DMD). *DAX1* (officially called *NR0B1*) encodes an atypical nuclear receptor with a

ligand-like binding domain at the carboxyl-terminus and an unusual amino-terminal region containing 3.5 repeats of an LXXLL-motif containing sequence (fig. 1). DAX-1/*NR0B1* is expressed in key areas of the adrenal gland, gonad, and central reproductive axis during development and post-natal life, consistent with its role in the development and function of these endocrine systems.

The “classical” clinical features of X-linked AHC are: 1) primary adrenal failure presenting in early infancy or childhood; 2) hypogonadotropic hypogonadism presenting as absent or arrested puberty; and 3) impaired spermatogenesis [2,3]. Boys with this condition usually need glucocorticoid and mineralocorticoid replacement throughout life and induction of secondary sex characteristics with testosterone. In general, GnRH pumps or recombinant gonadotropins are not effective at reversing azoospermia [4,5].

Approximately one-third of boys with X-linked AHC have a deletion of *DAX1* (*NR0B1*) [6]. A contiguous gene deletion syndrome affecting another Xp locus gene occurs in about half of these cases. Approximately two-thirds of individuals have point mutations in the *DAX1* (*NR0B1*) gene. These changes can be insertions, deletions or nonsense changes scattered throughout the two exons. The most common point mutations are frameshift changes (approximately 50%) and nonsense mutations (approximately 30%) (fig.1, upper panel). Missense mutations have been reported in 20% of patients with point mutations and are mostly located within the putative ligand-binding region of DAX-1 (fig.1, lower panel) [6]. These changes can cause several different defects such as altered protein structure, reduced protein-co-factor interactions, or disrupted nuclear localization [7,8]. Missense changes in the amino-terminus of DAX-1 are very rare.

In addition to the classic phenotype of X-linked AHC described above, several variant or partial phenotypes have now been reported (table 1). These include predominant mineralocorticoid insufficiency [9]; hyperandrogenism in early childhood [10]; early puberty with later pubertal arrest [11]; and adrenal insufficiency in a girl with an Xp deletion and extreme skewed X-inactivation [12]. Furthermore, late-onset X-linked AHC has been described in several men who presented with primary adrenal failure or hypogonadotropic hypogonadism in late adolescence or early adulthood [13,14]. In some cases, these milder conditions may be due to missense mutations with partial loss-of-function, or to premature stop codons at the aminoterminus of the protein with subsequent translation of an alternative isoform of the DAX-1 protein that retains partial function due to preserved LXXLL domains [13,14].

The precise functional role of DAX-1 in adrenal development and function remains unclear. Many studies have shown that DAX-1 can act as a repressor of gene transcription through its effect on the related nuclear receptor steroidogenic factor-1 (SF-1, NR5A1). Indeed, crystallography of DAX-1 bound to the related receptor LRH-1 (NR5A2) predicts that two DAX-1 proteins bind to the ligand-like binding domain of the partner, thereby potentially altering its activation capacity [15]. However, such a repressor role for DAX-1 is paradoxical given the fact that loss-of-function changes in DAX-1 result in adrenal hypoplasia. Two alternative hypotheses have been suggested. Firstly, DAX-1 may be needed to prevent adrenal stem cell differentiation, so that expansion of a pool of progenitor stem cells can occur before these cells mature into a mature steroidogenic lineage [7]. Loss of DAX-1 repression would result in premature differentiation without prior expansion, so that the total number of cells is reduced and adrenal hypoplasia results. Alternatively, DAX-1 may actually function as an activator of gene transcription in certain cases, such as with the steroid receptor RNA activator

(SRA) or on specific promoters (e.g. pre-B-cell leukaemia transcription factor 1; PBX1). These activator effects may be cell- or time-specific [16,17].

Although the exact mechanisms underlying X-linked AHC and its associated reproductive consequences remain to be fully elucidated, understanding the underlying pathogenic mechanism is important to gain new insight into this condition and if new treatment approaches are to be developed.

Steroidogenic factor-1 (SF-1, NR5A1)

SF-1 is a related nuclear receptor that is expressed in the adrenal gland, gonads, ventromedial hypothalamus, gonadotropes and spleen during development and into postnatal life. Like DAX-1, SF-1 has a carboxyl-terminal ligand-like binding domain, but its amino-terminal DNA-binding domain is more typical of the nuclear receptor superfamily (fig.2). This region contains a two zinc finger DNA-binding domain (with a "P-box" motif), an accessory region ("A-box") that supports monomeric binding, and a hinge region that can undergo post-translational modification (e.g. phosphorylation, SUMOylation). SF-1 was originally thought to be an "orphan" nuclear receptor as no naturally-occurring ligand was known. Recent data following crystalization of the ligand-binding domain suggest that this region may contain a small phospholipid molecule [18]. Although it is still not know if a high-specificity natural ligand exists for SF-1, this finding raises the possibility that SF-1 function could be modulated by signaling pathways, environmental chemicals or pharmacological agents.

SF-1 regulates an array of target genes involved in adrenal and reproductive development and in steroidogenic and metabolic function. Complete deletion of the gene

encoding Sf1 in the mouse causes adrenal and gonadal agenesis, a female phenotype and Müllerian structures in XY mice, partial gonadotropin deficiency, and late-onset obesity in animals rescued by adrenal transplantation [19].

Initial attempts to identify SF-1 changes in humans focused on individuals with a similar collection of features (i.e., primary salt-losing adrenal insufficiency, 46,XY disorders of sex development (DSD) and Müllerian structures). This is a very rare phenotype in humans and in more than a decade only two such patients have been reported who harbor SF-1 changes. The first individual described has a *de novo* heterozygous p.G35E change in the “P-box” of SF-1 [20]. This motif forms the principle DNA-binding structure of SF-1 by interfacing with the major groove of DNA. The p.G35E may have a mild competitive or dominant negative effect in certain assay systems but the effect on different promoters can be variable and the phenotype may be the result of reduced SF-1 transactivation on multiple targets throughout the genome and at different stages of development; therefore, a potentially complex and non-linear system. The importance of SF-1 in regulating human adrenal and gonad development and function was confirmed following the report of an infant with a similar phenotype (primary salt-losing adrenal failure, 46,XY DSD, Müllerian structures) who had inherited a homozygous p.R92Q alteration in SF-1 in a recessive fashion [21]. This change lies within the “A-box” of SF-1 and interferes with monomeric DNA binding stability. Again, the effects of this homozygous change are complex and variable, but in a range of promoter assays mean functional activity was in the order of 30-40% of wild-type. Although a 46,XX girl with primary adrenal failure was reported to have a heterozygous point mutation in SF-1 (p.R255L) [22], a systematic study of children and adults with a range of potential adrenal hypoplasia phenotypes did not reveal any additional mutations in SF-1 in a cohort of more than 70 DAX-1 negative individuals tested [6]. Therefore, SF-1 mutations

are a well-established but relatively rare cause of primary adrenal failure in humans. No complete loss of function changes or deletions of SF-1 have been described.

More recently, an association between SF-1 changes and 46,XY DSD has emerged [for example, see 23]. These children typically harbor heterozygous nonsense or frameshift mutations in SF-1 that are predicted to cause haploinsufficiency of the protein, or heterozygous missense changes with severe loss of function but no clear dominant negative activity. Affected individuals have a spectrum of 46,XY DSD phenotypes ranging from 46,XY females with streak gonads and Müllerian structures, through children with ambiguous genitalia and inguinal testes, to severe penoscrotal hypospadias with undescended testes [24]. In these cases there seems to be a predominant androgen biosynthesis defect with variable defects in testis development and/or integrity.

SF-1 changes often occur *de novo,* but can also be transmitted in a recessive pattern or from the mother in a sex-limited dominant manner. Other missense mutations in SF-1 have be found in association with bilateral anorchia (vanishing testis syndrome) with micropenis [25] and in women with primary ovarian insufficiency or premature menopause [26]. Of note, where data are available, adrenal function appears to be normal in all these individuals. Therefore, the human gonad appears to be more sensitive than the adrenal gland to loss of SF-1 function, although this group of patients will need long-term follow up to establish whether there is a risk of developing adrenal failure with time. The potential metabolic effects of SF-1 disruption also need addressing in the long-term, as ventromedial hypothalamic defects and late-onset obesity have been reported in mice [27], and obesity and hypertension have been noted in some patients.

A polymorphic variability in SF-1 may influence some of these processes in the population.

Finally, overexpression of SF-1 may act as a drive to pediatric adrenal tumorigenesis, as somatic duplication or multiple copy numbers of the locus containing SF-1 have been found in some children with adrenal tumors on a background of loss of heterozygosity for *TP53* [28]. In such cases, SF-1 could be a future therapeutic target for treatment of this rare but important group of conditions.

Acknowledgements

JCA is a Wellcome Trust Senior Fellow in Clinical Science (079666). AMA holds a scholarship from Becas-Chile (www.becaschile.cl). BFdS holds a scholarship from Capes / Brazil (4798066). We thank physicians and families who have been involved in our studies over the years and recognize the invaluable contribution Keith L. Parker has made in this field.

References

1 Else T, Hammer GD. Genetic analysis of adrenal absence: agenesis and aplasia. Trends Endocrinol Metab 2005;16:458-68.

2 Muscatelli F, Strom TM, Walker AP, Zanaria E, Recan D, Meindl A, Bardoni B, Guioli S, Zehetner G, Rabl W, Schwarz HP, Kaplan J, Camerino G, Meitinger T, Monaco AP. Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. Nature 1994;372:672-6.

3 Reutens AT, Achermann JC, Ito M, Ito M, Gu WX, Habiby RL, Donohoue PA, Pang S, Hindmarsh PC, Jameson JL. Clinical and functional effects of mutations in the DAX-1 gene in patients with adrenal hypoplasia congenita. J Clin Endocrinol Metab 1999;84:504-11.

4 Mantovani G, Mancini M, Gazzano G, Spada A, Colpi GM, Beck-Peccoz P, Persani L. Somatic mutational analysis of DAX1 in testes from men with idiopathic azoospermia. Fertil Steril 2005;84:1542-4.

5 Seminara SB, Achermann JC, Genel M, Jameson JL, Crowley WF, Jr. X-linked adrenal hypoplasia congenita: a mutation in DAX1 expands the phenotypic spectrum in males and females. J Clin Endocrinol Metab 1999;84:4501-9.

6 Lin L, Gu WX, Ozisik G, To WS, Owen CJ, Jameson JL, Achermann JC. Analysis of DAX1 (NR0B1) and steroidogenic factor-1 (NR5A1) in children and adults with primary adrenal failure: ten years' experience. J Clin Endocrinol Metab 2006;91:3048-54.

7 Lalli E, Sassone-Corsi P. DAX-1, an unusual orphan receptor at the crossroads of steroidogenic function and sexual differentiation. Mol Endocrinol 2003;17:1445-53.

8 Lehmann SG, Wurtz JM, Renaud JP, Sassone-Corsi P, Lalli E. Structure-function analysis reveals the molecular determinants of the impaired biological function of DAX-1 mutants in AHC patients. Hum Mol Genet 2003;12:1063-72.

9 Wiltshire E, Couper J, Rodda C, Jameson JL, Achermann JC. Variable presentation of X-linked adrenal hypoplasia congenita. J Pediatr Endocrinol Metab 2001;14:1093-6.

10 Landau Z, Hanukoglu A, Sack J, Goldstein N, Weintrob N, Eliakim A, Gillis D, Sagi M, Shomrat R, Kosinovsky EB, Anikster Y. Clinical and genetic heterogeneity of congenital adrenal hypoplasia due to NR0B1 gene mutations. Clin Endocrinol (Oxf) 2010;72:448-54.

11 Domenice S, Latronico AC, Brito VN, Arnhold IJ, Kok F, Mendonca BB. Adrenocorticotropin-dependent precocious puberty of testicular origin in a boy with X-linked adrenal hypoplasia congenita due to a novel mutation in the DAX1 gene. J Clin Endocrinol Metab 2001;86:4068-71.

12 Shaikh MG, Boyes L, Kingston H, Collins R, Besley GT, Padmakumar B, Ismayl O, Hughes I, Hall CM, Hellerud C, Achermann JC, Clayton PE. Skewed X inactivation is associated with phenotype in a female with adrenal hypoplasia congenita. J Med Genet 2008;45:e1.

13 Tabarin A, Achermann JC, Recan D, Bex V, Bertagna X, Christin-Maitre S, Ito M, Jameson JL, Bouchard P. A novel mutation in DAX1 causes delayed-onset adrenal insufficiency and incomplete hypogonadotropic hypogonadism. J Clin Invest 2000;105:321-8.

14 Ozisik G, Mantovani G, Achermann JC, Persani L, Spada A, Weiss J, Beck-Peccoz P, Jameson JL. An alternate translation initiation site circumvents an amino-terminal DAX1 nonsense mutation leading to a mild form of X-linked adrenal hypoplasia congenita. J Clin Endocrinol Metab 2003;88:417-23.

15 Sablin EP, Woods A, Krylova IN, Hwang P, Ingraham HA, Fletterick RJ. The structure of corepressor Dax-1 bound to its target nuclear receptor LRH-1. Proc Natl Acad Sci U S A 2008;105:18390-5.

16 Ferraz-de-Souza B, Martin F, Mallet D, Hudson-Davies RE, Cogram P, Lin L, Gerrelli D, Beuschlein F, Morel Y, Huebner A, Achermann JC. CBP/p300-interacting transactivator, with Glu/Asp-rich C-terminal domain, 2, and pre-B-cell leukemia transcription factor 1 in human adrenal development and disease. J Clin Endocrinol Metab 2009;94:678-83.

17 Xu B, Yang WH, Gerin I, Hu CD, Hammer GD, Koenig RJ. Dax-1 and steroid receptor RNA activator (SRA) function as transcriptional coactivators for steroidogenic factor 1 in steroidogenesis. Mol Cell Biol 2009;29:1719-34.

18 Krylova IN, Sablin EP, Moore J, Xu RX, Waitt GM, MacKay JA, Juzumiene D, Bynum JM, Madauss K, Montana V, Lebedeva L, Suzawa M, Williams JD, Williams SP, Guy RK, Thornton JW, Fletterick RJ, Willson TM, Ingraham HA. Structural analyses reveal phosphatidyl inositols as ligands for the NR5 orphan receptors SF-1 and LRH-1. Cell 2005;120:343-55.

19 Schimmer BP, White PC. Minireview: Steroidogenic factor 1: Its roles in differentiation, development, and disease. Mol Endocrinol 2010. [Epub ahead of print]

20 Achermann JC, Ito M, Ito M, Hindmarsh PC, Jameson JL. A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. Nat Genet 1999;22:125-6.

21 Achermann JC, Ozisik G, Ito M, Orun UA, Harmanci K, Gurakan B, Jameson JL. Gonadal determination and adrenal development are regulated by the orphan nuclear receptor steroidogenic factor-1, in a dose-dependent manner. J Clin Endocrinol Metab 2002;87:1829-33.

22 Biason-Lauber A, Schoenle EJ. Apparently normal ovarian differentiation in a prepubertal girl with transcriptionally inactive steroidogenic factor 1 (NR5A1/SF-1) and adrenocortical insufficiency. Am J Hum Genet 2000;67:1563-8.

23 Lin L, Philibert P, Ferraz-de-Souza B, Kelberman D, Homfray T, Albanese A, Molini V, Sebire NJ, Einaudi S, Conway GS, Hughes IA, Jameson JL, Sultan C, Dattani MT, Achermann JC. Heterozygous missense mutations in steroidogenic factor 1 (SF1/Ad4BP, NR5A1) are associated with 46,XY disorders of sex development with normal adrenal function. J Clin Endocrinol Metab 2007;92:991-9.

24 Köhler B, Lin L, Mazen I, Cetindag C, Biebermann H, Akkurt I, Rossi R, Hiort O, Gruters A, Achermann JC. The spectrum of phenotypes associated with mutations in steroidogenic factor 1 (SF-1, NR5A1, Ad4BP) includes severe penoscrotal hypospadias in 46,XY males without adrenal insufficiency. Eur J Endocrinol 2009;161:237-42.

25 Philibert P, Zenaty D, Lin L, Soskin S, Audran F, Leger J, Achermann JC, Sultan C. Mutational analysis of steroidogenic factor 1 (NR5a1) in 24 boys with bilateral anorchia: a French collaborative study. Hum Reprod 2007;22:3255-61.

26 Lourenco D, Brauner R, Lin L, De PA, Weryha G, Muresan M, Boudjenah R, Guerra-Junior G, iel-Guerra AT, Achermann JC, McElreavey K, Bashamboo A. Mutations in NR5A1 associated with ovarian insufficiency. N Engl J Med 2009;360:1200-10.

27 Majdic G, Young M, Gomez-Sanchez E, Anderson P, Szczepaniak LS, Dobbins RL, McGarry JD, Parker KL. Knockout mice lacking steroidogenic factor 1 are a novel genetic model of hypothalamic obesity. Endocrinology 2002;143:607-14.

28 Almeida MQ, Soares IC, Ribeiro TC, Fragoso MC, Marins LV, Wakamatsu A, Ressio RA, Nishi MY, Jorge AAL, Lerario AM, Alves VAF, Mendonca BB, Latronico AC. Steroidogenic Factor 1 Overexpression and Gene Amplification Are More Frequent in Adrenocortical Tumors from Children than from Adults. J Clin Endocrinol Metab 2010;95:1458-62.

29 Achermann JC, Silverman BL, Habiby RL, Jameson JL. Presymptomatic diagnosis of X-linked adrenal hypoplasia congenita by analysis of DAX1. J Pediatr 2000;137:878-81.

Figures legends

Fig. 1. Cartoon of DAX-1 structure and a selection of the nonsense (closed circles), frameshift (open circles) and missense changes reported. Those changes associated with a milder phenotype (*) or isolated mineralcorticoid deficiency (**) are indicated. The potential LXXLL domains are shown below the protein structure. LBD = ligand binding domain. (Modified with permission from Lin et al., J Clin Endocrinol Metab 2006; 91: 3048-3054. Copyright © 2006 by The Endocrine Society).

Fig. 2. Cartoon of SF-1 structure and a selection of the changes reported in patients with adrenal insufficiency and/or reproductive phenotypes. Nonsense, frameshift and missense changes are shown. BD = binding domain, PAI = primary adrenal insufficiency, DSD = disorder of sex development, POI = primary ovarian insufficiency. (Modified with permission from Lin et al., J Clin Endocrinol Metab 2006; 91: 3048-3054. Copyright © 2006 by The Endocrine Society)

Table 1. Phenotypes associated with changes in DAX-1 (*NR0B1*).

Clinical phenotype	**Example ref**
Primary adrenal insufficiency, HH, infertility	[2,3]
Isolated mineralocorticoid insufficiency	[9]
Premature sexual development	[10,11]
Adrenal insufficiency or delayed puberty in girls with skewed X inactivation or gene conversion	[12]
Late-onset X-linked AHC or HH in early adulthood	[13,14]
Presymptomatic diagnosis	[29]

AHC = adrenal hypoplasia congenita, HH = hypogonadotropic hypogonadism

Table 2. Phenotypes associated with changes in SF-1 (*NR5A1*).

Clinical phenotype	**Example ref**
Primary adrenal insufficiency, 46,XY female, streak gonads, uterus	[20, 21]
Primary adrenal insufficiency in 46, XX girl	[22]
46,XY DSD, streak gonads, uterus	[23]
46,XY DSD, clitoromegaly/ambiguous, inguinal testes, no uterus	[23]
Severe penoscrotal hypospadias, small penis, undescended testes	[24]
Bilateral anorchia, small penis	[25]
Primary ovarian insufficiency	[26]

DSD = disorder of sex development