Multifunctional role of steroidogenic factor 1 and disorders of sex development

Papel multifuncional do fator esteroidogênico 1 e as doenças do desenvolvimento sexual

Maricilda Palandi de Mello[1], Emerson Salvador de Souza França[1],
Helena Campos Fabbri[1], Andréa Trevas Maciel-Guerra[2], Gil Guerra-Júnior[3]

SUMMARY

Disorders of sex development (DSD) involve several conditions that result from abnormalities during gonadal determination and differentiation. Some of these disorders may manifest at birth by ambiguous genitalia; others are diagnosed only at puberty, by the delayed onset of secondary sexual characteristics. Sex determination and differentiation in humans are processes that involve the interaction of several genes such as *WT1*, *NR5A1*, *NR0B1*, *SOX9*, among others, in the testicular pathway, and *WNT4*, *DAX1*, *FOXL2* and *RSPO1*, in the ovarian pathway. One of the major proteins in mammalian gonadal differentiation is the steroidogenic nuclear receptor factor 1 (SF1). This review will cover some of the most recent data on SF1 functional roles and findings related to mutations in its coding gene, *NR5A1*. Arq Bras Endocrinol Metab. 2011;55(8):607-12

[1] Centro de Biologia Molecular e Engenharia Genética (CBMEG), Universidade Estadual de Campinas (Unicamp), Campinas, SP, Brazil
[2] Departamento de Genética Médica, Faculdade de Ciências Médicas (FCM), Unicamp, Campinas, SP, Brazil
[3] Departamento de Pediatria, FCM-Unicamp, Campinas, SP, Brazil

SUMÁRIO

Os distúrbios do desenvolvimento sexual (DDS) envolvem várias condições que resultam de anormalidades que podem acontecer tanto na determinação como durante a diferenciação gonadal. Algumas dessas doenças podem se manifestar ao nascimento principalmente por genitália ambígua, outras são diagnosticadas apenas na puberdade por atraso no aparecimento de características sexuais secundárias. A determinação e a diferenciação do sexo em seres humanos são processos que envolvem interações entre vários genes nas vias testicular, tais como *NR5A1*, *NR0B1*, *WT1*, *SOX9*, entre outros, e ovariana, tais como *WNT4*, *DAX1*, *FOXL2* e *RSPO1*. Uma das principais proteínas na diferenciação gonadal de mamíferos é o fator esteroidogênico e receptor nuclear 1 (SF1). Esta revisão cobrirá alguns dos dados mais recentes sobre os papéis funcionais de SF1 e as últimas descobertas relacionadas a mutações em seu gene, *NR5A1*. Arq Bras Endocrinol Metab. 2011;55(8):607-12

Correspondence to:
Maricilda Palandi de Mello
Universidade Estadual de Campinas, CBMEG
Av. Cândido Rondon, 400
13083-875 – Campinas, SP, Brazil
mmello@unicamp.br

The presence or absence of the Y chromosome in the karyotype of most mammals, including humans, is linked, respectively, to the processes of male and female sex determination and differentiation (1,2). Sex development is classically divided into three stages: determination, which is chromosomally established at fertilization; differentiation of the gonads from undifferentiated embryonic structures into testes or ovaries; and secondary sexual differentiation, which is the response of innumerous tissues to hormones produced by either gonads to complete sexual maturation during puberty (3). Disorders of sex development (DSD) involve several conditions that result from abnormalities in one of the three stages. Some of these disorders may

manifest at birth by ambiguous genitalia; others are diagnosed only at puberty by delayed onset of secondary sexual characteristics (4,5). In cases of newborns with ambiguous genitalia, the definition of appropriate sex is urgent, because it generally requires a series of surgical procedures, hormone therapy and counseling (5). The understanding of the intricate processes that involve sex determination and differentiation has been a challenge for physicians and scientists in general.

DSD are characterized by incomplete or disordered gonadal or genital development, leading to divergences between genetic sex, gonadal sex and phenotypic sex, specially in individuals with 46,XY karyotype (6,7). These cases present female or ambiguous genitalia that may vary from mild to severe hypospadias, with or without penoscrotal chordee, dysgenetic testes, reduced or null sperm production, and Müllerian structures that may be absent or present as fully developed uterus and fallopian tubes (8). In cases of 46,XY DSD, gonadal dysgenesis refers to a set of abnormalities characterized by dysgenetic gonads as a result of failures in the expression or function of genes involved in testicular development (4,9). Dysgenetic gonads are mainly formed by fibrous tissue without hormonal function that is not able to produce gametes; whereas dysgenetic testes are associated with abnormalities in the differentiation of the Wolffian ducts, in external genitalia virilization, and in the regression of Müllerian ducts (10).

Gonadal dysgenesis may be classified as pure (complete), partial (incomplete) or mixed dysgenesis (11). Complete gonadal dysgenesis (CGD) is characterized by phenotypically female individuals without genital ambiguity, and presence of dysgenetic gonads, and normal Müllerian derivatives. It may occur in individuals with normal karyotypes, 46,XX or 46,XY (12). Individuals with XY CGD are prone to developing gonadal tumors (13). Conversely, incomplete gonadal dysgenesis (DGI) is characterized by individuals 46,XY without mosaicism, with partial testicular differentiation, derivatives of Müllerian ducts and genital ambiguity. Usually, seminiferous tubules are present and associated with areas similar to ovarian stroma. Internal genitalia consists of derivatives of both Wolffian and Müllerian ducts (12). The presence of a second cell lineage presenting 45,X in the karyotype characterizes mixed gonadal dysgenesis (14).

After two decades of the identification of *SRY* gene as the sex-determining region on the Y chromosome responsible for initiating male development, it is well known that sex determination and differentiation in humans are processes that involve the interaction of several genes, such as *WT1*, *NR5A1*, *NR0B1*, *SOX9*, among others, in the testicular pathway; and *WNT4*, *DAX1*, *FOXL2* and *RSPO1,* in the ovarian pathway (8,15). Figure 1 illustrates the main steps and genes involved in gonadal differentiation that result in male and female characteristics.

One of the major proteins in mammalian gonadal differentiation is the steroidogenic nuclear receptor factor 1 (SF1) now known as nuclear receptor subfamily 5 group A member 1 (NR5A1 [MIM 184757]). The human SF1 protein is formed by 461 amino acids and is a member of the orphan nuclear receptor family, considered the main regulator of enzymes involved in adrenal and gonadal steroidogenesis, and is expressed in undifferentiated gonads before SRY (16). The expression of *NR5A1* gene, which encodes SF1 protein, is necessary at three stages throughout testis determination and differentiation: in the formation of bipotential gonads; in the Sertoli cells, to regulate the expression of anti--Müllerian hormone gene (AMH); and in the Leydig cells, to regulate the expression a number of steroid hormones (17,18). Therefore, it plays an important role in the expression of male specific genes. In female development, it is also actively present and participates in different steps of ovarian development and function (19). The wide range of action of this protein became evident after observations in mice, showing that the gene is expressed very early in the urogenital ridge, and acts later on in the development of the adrenals, gonads, pituitary, and ventromedial hypothalamus (20,21).

SF1 was first isolated in 1992 during the search for elements that regulate the proximal promoter region of the cytochrome P450 21-hydroxylase enzyme (22). SF1 protein is highly expressed in steroidogenic tissues, such as gonads, adrenals, and placenta, and regulates almost all the enzymes related to this process (23). It is essential not only in adrenal and gonadal development and sex differentiation, but also plays important physiological roles in the central nervous system (21). As illustrated in figure 1, after testicular determination, SF1 regulates the expression of anti-Mülllerian hormone in Sertoli cells, which leads to regression of Müllerian structures during fetal development (18). In Leydig cells, SF1 activates the expression of steroidogenic enzymatic system, resulting in virilization of external genitalia and testicular descent (24,25). In females, SF1 is expressed in ovarian theca cells and granulosa
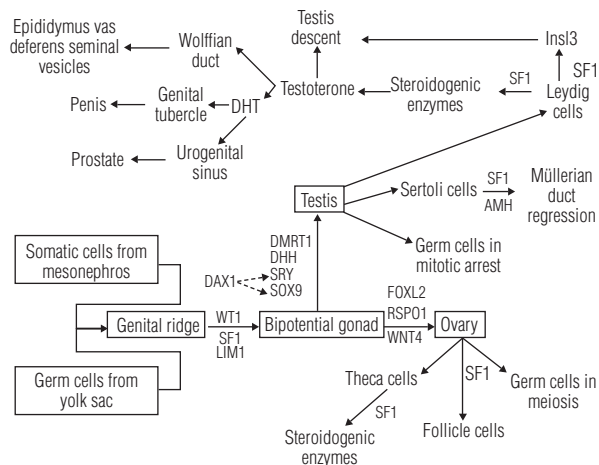
Figure 1. Sexual development cascade [adapted from Swain (1) and Biason-Lauber (2)]. The most important proteins in male and female pathways are shown in the figure. WT1 and SF1 are expressed in the bipotential gonad that develops into an ovary under the action of LIM1, FOXL2, RSPO1 and WNT4, whereas in the presence of SRY, a testis develops with additional action of SOX9, DHH and DMRT1, among others. In the testis, germ cells, Sertoli cells, and Leydig cells differentiate. SF1-regulated production of AMH, Insl3, and testosterone induce the differentiation of the male-specific internal and external genitalia. In the ovaries, beside germ cells, there are follicle cells and theca cells, in which SF1-regulation is also important.

cells, early in folliculogenesis (26). In addition, SF1 is the main regulator of cholesterol metabolism in steroidogenic cells, stimulating the expression of almost all factors involved in the mobilization of cholesterol and steroid hormone biosynthesis (27).

NR5A1 is an autosomal gene located at 9q33 (OMIM 184757). It expands over 30 kb of genomic DNA divided into one non-coding exon followed by six coding exons (28). The structure of human SF1 protein includes: a DNA-binding domain (DBD) containing two zinc fingers, a ligand-binding domain (LBD), two functional activation domains (AF-1 and AF-2); an accessory region, and a hinge region. The first zinc finger contains a proximal (P box) region, that is involved in nuclear receptor specific recognition of DNA target sequences (29). The region contains an accessory box that stabilizes DNA binding. The hinge region is important for SF1 transcriptional activity (30). Moreover, unlike most nuclear receptors, SF1 binds DNA as a monomer with high affinity for the region 5'YCAA-GGYCR'3 (where Y = T / C, R = G / A) (31). As a member of the NR5A subfamily, it has the DBD extended by a FTZ-F1 box, which is important for DNA anchoring. This box contains a T-box that supports an A-box that, in turn, interacts co-operatively with the P-box in the first Zn-finger of the DBD, and defines the specificity of the monomeric DNA binding (20).

Similar to the great majority of transcription factors, the activation of SF1 transcriptional activity requires the interaction with other proteins through its protein activation domains (20). Furthermore, transcriptional activity of SF-1 is modified by post-translational modifications such as phosphorylation/dephosphorylation, acetylation and SUMOylation (20,21). Its expression is precisely regulated in a time- and tissue-dependent manner by promoters formed through alternative non-coding exons 1 (32,33), upstream stimulatory factors 1 and 2 (USF1, USF2) (34), interactions at the basal promoter region with different regulatory elements (20), and methylation at the basal promoter region, as well as at intronic enhancers (35).

Several studies have shown that the *NR5A1* gene is highly conserved among species, and the overall amino acid similarity between mice and humans is 95%. Homozygous mutations that inactivate SF1 in mice are manifested by the absence of adrenal and gonadal development, the absence of pituitary gonadotropins and structural changes in the ventral and median hipotalamus regions. *Nr5A1* gene deletions in such animals cause complete adrenal and gonadal agenesis, defects in virilization and retention of Müllerian ducts in XY animals (36,37). Abnormalities in the pituitary and in the brain, specifically in the ventromedial hypothalamus, are also attributed to the *Nr5A1* deletion (21).

Sequencing *NR5A1* gene of an XY female revealed the heterozygosity for the missense mutation p.Gly35Glu (38). This was the first mutation described in humans, and the reported patient presented primary adrenal insufficiency, complete gonadal dysgenesis, and Müllerian duct persistence. It was a *de novo* heterozygous mutation, leading to an amino acid substitution in the first zinc finger P-box DNA-binding region, severely affecting SF1 function (38,39). A homozygous patient with similar phenotype had been subsequently described as carrying the p.Arg92Gln mutation located in the A-box region. This inherited mutation caused partial loss of SF1 function *in vitro*, what explained that heterozygous carriers were normal (40). Several studies showed an haploinsufficiency effect caused by inactivating mutations in the *NR5A1* gene in 46,XY heterozygous individuals with gonadal dysgenesis and without adrenal failure (41-43). Actually, mutations in the *NR5A1* gene may be more frequent in patients with gonadal dysgenesis without adrenal insufficiency than in patients with

both gonadal dysgenesis and adrenal insufficiency (43). Considering the type of each mutation already identified in *NR5A1*, it is difficult to establish a direct phenotype--genotype correlation (Figure 2). If we consider that there is a dosage effect, it is difficult to understand why all patients heterozygous for severe nonsense mutations occurring in the first and second coding exons, as well as other frameshift mutations that are considered to undergo mRNA nonsense-mediated decay, do not present the severe phenotype, including adrenal insufficiency. Nevertheless, heterozygous mutations such as p.Glu11Ter, p.Cis16Ter and p.Glu51ArgfsX23 were identified in patients with, respectively, XY DSD and severe penoscrotal hypospadia, and cryptorchidism without adrenal insufficiency. Most mutations were found in heterozygous patients either as *de novo* mutations or as inherited from a non-affected parent, indicating a dominant inheritance with different degree of penetrance. It is interesting to note that from the nine mutations identified in XY DSD and in XX primary ovarian insufficiency, eight are nonsense or frameshift mutations (Figure 2), and most of them had been inherited. Conversely, there are two cases of homozygosity for inherited mutations enabling the conclusion that, in those cases, the mutation was transmitted in a recessive manner. The p.Arg92Gln described above is one, and the other is p.Asp293Asn, which was found segregating in a family, and resulted in three different phenotypes: XX primary ovarian insufficiency, XY incomplete gonadal dysgenesis, and XY complete gonadal dysgenesis (44,45).

Many studies have shown that variations in the *NR5A1* may be associated not only with gonadal dysgenesis and adrenal failure, but also with hypospadias, anorchia, male infertility, and premature ovarian failure, affecting both
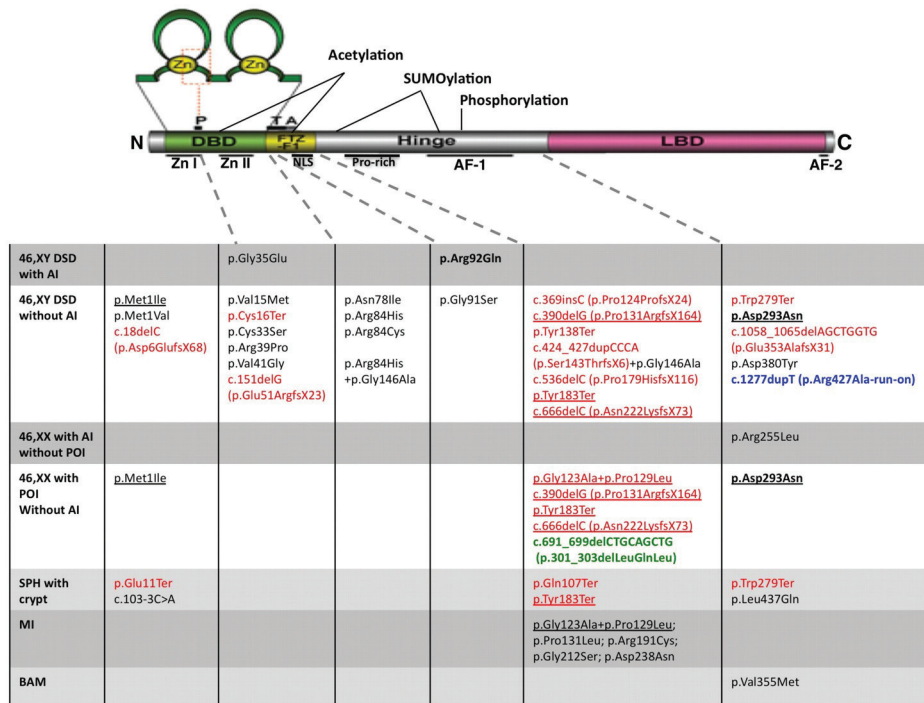

	Zn I	Zn II	NLS	Pro-rich	AF-1	LBD (AF-2)
46,XY DSD with AI		p.Gly35Glu		**p.Arg92Gln**		
46,XY DSD without AI	p.Met1Ile p.Met1Val c.18delC (p.Asp6GlufsX68)	p.Val15Met p.Cys16Ter p.Cys33Ser p.Arg39Pro p.Val41Gly c.151delG (p.Glu51ArgfsX23)	p.Asn78Ile p.Arg84His p.Arg84Cys p.Arg84His +p.Gly146Ala	p.Gly91Ser	c.369insC (p.Pro124ProfsX24) c.390delG (p.Pro131ArgfsX164) p.Tyr138Ter c.424_427dupCCCA (p.Ser143ThrfsX6)+p.Gly146Ala c.536delC (p.Pro179HisfsX116) p.Tyr183Ter c.666delC (p.Asn222LysfsX73)	p.Trp279Ter **p.Asp293Asn** c.1058_1065delAGCTGGTG (p.Glu353AlafsX31) p.Asp380Tyr **c.1277dupT (p.Arg427Ala-run-on)**
46,XX with AI without POI						p.Arg255Leu
46,XX with POI Without AI	p.Met1Ile				p.Gly123Ala+p.Pro129Leu c.390delG (p.Pro131ArgfsX164) p.Tyr183Ter c.666delC (p.Asn222LysfsX73) **c.691_699delCTGCAGCTG (p.301_303delLeuGlnLeu)**	**p.Asp293Asn**
SPH with crypt	p.Glu11Ter c.103-3C>A				p.Gln107Ter p.Tyr183Ter	p.Trp279Ter p.Leu437Gln
MI					p.Gly123Ala+p.Pro129Leu; p.Pro131Leu; p.Arg191Cys; p.Gly212Ser; p.Asp238Asn	
BAM						p.Val355Met

Figure 2. Schematic overview of SF-1 and locations of each *NR5A1* mutation [adapted from Hoivik and cols. (20) and Ferraz-de-Souza and cols. (46)]. The main functional domains of the SF1 protein are shown, indicating the position of the DNA-binding domain (DBD) and the ligand-binding domain (LBD). FTZ-F1 domain that stabilizes protein binding to DNA is also illustrated. The two zinc fingers (Zn I, Zn II) of the DBD are highlighted on top. The P-box within zinc finger I is indicated by a dashed square. The hinge region between DNA- and ligand-binding domains is important for stabilizing the ligand-binding domains, and interacts with other proteins that control SF1 transcriptional activity. Post-translational modifications such as acetylation (in the DBD and Ftz-F1), SUMOylation in the residue K119 and K194, and phosphorylation in the residue S203 within the hinge region are indicated above. AF-1 and AF-2; activation function domains 1 and 2, NLS; nuclear localization signal, Pro-rich; proline-rich region, Ftz-F1; Fushi-tarazu factor-1 box, P/T/A; "DNA binding boxes", Zn I and Zn II; zinc fingers I and II are also denoted. Underlined mutations were detected in both 46,XX and 46,XY individuals. All changes were present in heterozygosis, except those denoted in bold. AI, adrenal insufficiency; DSD, disorder of sex development; POI, primary ovarian insufficiency; SPH, severe penoscrotal hypospadia; Crypt, cryptorchidism; MI, male infertility; BAM, bilateral anorchia with microphallus. Mutations in parenthesis are the presumed consequence on the protein sequence of nucleotide insertions or deletions. Missense mutations are in black; nonsense and frameshift mutations are in red. Mutations in green and blue are, respectively, an in-frame deletion and an insertion that eliminates the natural stop codon.

46,XY and 46,XX individuals. In some cases, it may be also associated with adrenal tumors and endometriosis (46).

The identification of *NR5A1* nucleotide changes in women with primary ovarian failure clearly indicates that SF1 is indeed a key factor in the development and function of ovaries in humans (44), although it was considered before that *NR5A1* mutations might not interfere with ovarian development (47). SF1 failure may affect the ovary at several levels: reducing the number of germ cells, damaging stroma integrity, and causing abnormal folliculogenesis. Phenotypic variability is verified for either 46,XY or 46,XX individuals carrying *NR5A1* mutations within families, probably as a result of multifunctional roles of SF1 protein (8,15,44,45,48). Another example of a mutated SF1 allele affecting both male and female development is the allele carrying the mutations p.Gly123Ala and p.Pro129Leu within the SF1 hinge region, which has been associated to either ovarian insufficiency or male infertility (44,49). In addition, p.Gly146Ala, also located in the hinge region, has been described as a missense associated with micropenis and cryptorchidism (50).

Finally, all the molecular studies reported so far indicate different clinical manifestations for different nucleotide changes in the *NR5A1* gene. Bilateral anorchia or testicular regression, in addition to all other manifestations, was reported as a result of p.Val355Met mutation in one out of 24 children evaluated (51). However, a recent study involving 26 patients with the same condition did not reveal any mutation (52), indicating that *NR5A1* mutation might be not a frequent cause of anorchia. It can be inferred from all data reported so far that mutations in the *NR5A1* gene are more frequent than in the *SRY* gene in cases of 46,XY DSD (53). However, more research on mutation screenings is necessary to indicate *NR5A1* gene mutations as frequent causes of either primary or premature ovarian failure without adrenal insufficiency.

The findings reviewed here indicate a complex expressivity of the phenotype, penetrance, and modes of inheritance of *NR5A1* mutations, and suggest that more research has to be done to figure out how *NR5A1* mutations correlate with different phenotypes, whether and how other genes modulate their expressivity, and to identify these genes for each affected phenotype.

Disclosure: no potential conflict of interest relevant to this article was reported.

REFERENCES

1. Swain A. Sex determination and differentiation. In: Neill JD, editor. Knobil and Neill's Physiology of Reproduction. 3rd ed. New York: Elsevier; 2006. p. 245-60.

2. Biason-Lauber A. Control of sex development. Best Pract Res Clin Endocrinol Metab. 2010;24(2):163-86.

3. Goodfellow PN, Darling SM. Genetics of sex determination. Development. 1988;102(2):251-8.

4. De-Mello MP, Soardi FC. Genes envolvidos na determinação sexual. In: Maciel-Guerra AT, Guerra-Júnior G, editores. Menino ou menina? Os distúrbios da diferenciação do sexo. 2. ed. São Paulo: Rubio; 2010. p. 3-14.

5. Guerra-Júnior G, Maciel-Guerra AT. The role of the pediatrician in the management of children with genital ambiguities. J Pediatr (Rio J). 2007;83(5 Suppl):S184-91.

6. Hughes IA, Houk C, Ahmed SF, Lee PA, Group LC, Group EC. Consensus statement on management of intersex disorders. Arch Dis Child. 2006;91(7):554-63.

7. Nabhan ZM, Lee PA. Disorders of sex development. Curr Opin Obstet Gynecol. 2007;19(5):440-5.

8. Kousta E, Papathanasiou A, Skordis N. Sex determination and disorders of the sex development according to the revised nomenclature and classification in 46,XX individuals. Hormones. 2010;9(3):218-31.

9. Mello MP, Assumpção JG, Hackel C. Genes involved in sex determination and differentiation. Arq Bras Endocrinol Metabol. 2005;49(1):14-25.

10. Ribeiro Scolfaro M, Aparecida Cardinalli I, Gabas Stuchi-Perez E, Palandi de Mello M, de Godoy Assumpção J, Matias Baptista MT, et al. Morphometry and histology of gonads from 13 children with dysgenetic male pseudohermaphroditism. Arch Pathol Lab Med. 2001;125(5):652-6.

11. Lipay MV, Bianco B, Verreschi IT. Disgenesias gonadais e tumores: aspectos genéticos e clínicos. Arq Bras Endocrinol Metabol. 2005;49(1):60-70.

12. Andrade JG, Guerra-Júnior G, Maciel-Guerra AT. 46,XY and 45,X/46,XY testicular dysgenesis: similar gonadal and genital phenotype, different prognosis. Arq Bras Endocrinol Metabol. 2010;54(3):331-4.

13. Gourlay WA, Johnson HW, Pantzar JT, McGillivray R, Crawford R, Nielsen W. Gonadal tumors in disorders of sexual differentiation. Urology. 1994;43(4):537-40.

14. Alvarez-Nava F, Puerta H, Soto M, Pineda L, Temponi A. High incidence of Y- chromosome microdeletions in gonadal tissues from patients with 45,X/46,XY gonadal dysgenesis Fertil Steril. 2008;89(2):458-60.

15. Biason-Lauber A. Control of sex development. Best Pract Res Clin Endocrinol Metab. 2010;24(2):163-86.

16. El-Khairi R, Martinez-Aguayo A, Ferraz-de-Souza B, Lin L, Achermann JC. Role of DAX-1 (NR0B1) and steroidogenic factor-1 (NR5A1) in human adrenal function. Endocr Dev. 2011;20:38-46.

17. Hanley NA, Ball SG, Clement-Jones M, Hagan DM, Strachan T, Lindsay S, et al. Expression of steroidogenic factor 1 and Wilms' tumour 1 during early human gonadal development and sex determination. Mech Dev. 1999;87(1-2):175-80.

18. Shen WH, Moore CCD, Ikeda Y, Parker KL, Holly AI. Nuclear receptor steroidogenic factor 1 regulates the Mullerian inhibiting substance gene: a link to the sex determination cascade. Cell. 1994;77(5):651-61.

19. Mlynarczuk J, Rekawiecki R. The role of the orphan receptor SF-1 in the development and function of the ovary. Reprod Biol. 2010;10(3):177-93.

20. Hoivik EA, Lewis AE, Aumo L, Bakke M. Molecular aspects of steroidogenic factor 1 (SF-1). Mol Cell Endocrinol. 2010;315(1-2):27-39.

21. Büdefeld T, Tobet SA, Majdic G. Steroidogenic factor 1 and the central nervous system. J Neuroendocrinol. 2011 Jun 11. doi: 10.1111/j.1365-2826.2011.02174.x. [Epub ahead of print].

22. Lala DS, Rice DA, Parker KL. Steroidogenic factor I, a key regulator of steroidogenic enzyme expression, is the mouse homolog of fushi tarazu-factor I. Mol Endocrinol. 1992;6(8):1249-58.

23. Parker KL, Rice DA, Lala DS, Ikeda Y, Luo X, Wong M, et al. Steroidogenic factor 1: an essential mediator of endocrine development. Recent Prog Horm Res. 2002;57:19-36.

24. Zimmermann S, Schwarzler A, Buth S, Engel W, Adham IM. Transcription of the Leydig insulin-like gene is mediated by steroidogenic factor-1. Mol Endocrinol. 1998;12(5):706-13.

25. Lin L, Achermann JC. Steroidogenic factor-1 (SF-1, Ad4BP, NR5A1) and disorders of testis development. Sex Dev. 2008;2(4-5):200-9.

26. Takayama K, Sasano H, Fukaya T, Morohashi K, Suzuki T, Tamura M, et al. Immunohistochemical localization of Ad4 binding protein with correlation to steroidogenic enzyme expression in cycling human ovaries and sex cord stromal tumors. J Clin Endocrinol Metab. 1995;80(9):2815-21.

27. Parker KL, Schimmer BP. Steroidogenic factor 1: a key determinant of endocrine development and function. Endocr Rev. 1997;18(3):361-77.

28. Wong M, Ramayya MS, Chrousos GP, Driggers PH, Parker KL. Cloning and sequence analysis of the human gene encoding steroidogenic factor 1. J Mol Endocrinol. 1996;17(2):139-47.

29. Oba K, Yanase, T, Nomura M, Morohashi K, Takayanagi R, Nawata H. Structural characterization of human Ad4bp (SF-1) gene. Biochem Biophys Res Commun. 1996;226(1):261-7.

30. Little TH, Zhang Y, Matulis CK, Weck J, Zhang Z, Ramachandran A, et al. Sequence-specific deoxyribonucleic acid (DNA) recognition by steroidogenic factor 1: a helix at the carboxy terminus of the DNA binding domain is necessary for complex stability. Mol Endocrinol. 2006;20(4):831-43.

31. Wilson TE, Fahrner TJ, Milbrandt J. The orphan receptors NGFI-B and steroidogenic factor 1 establish monomer binding as a third paradigm of nuclear receptor-DNA interaction. Mol Cell Biol. 1993;13(9):5794-804.

32. Kimura R, Yoshii H, Nomura M, Kotomura N, Mukai T, Ishihara S, et al. Identification of novel first exons in Ad4BP/SF-1 (NR5A1) gene and their tissue- and species-specific usage. Biochem Biophys Res Commun. 2000;278(1):63-71.

33. Demura M, Wang F, Yoneda T, Karashima S, Mori S, Oe M, et al. Multiple noncoding exons 1 of nuclear receptors NR4A family (nerve growth factor-induced clone B, Nur-related factor 1 and neuron-derived orphan receptor 1) and NR5A1 (steroidogenic factor 1) in human cardiovascular and adrenal tissues. J Hypertens. 2011;29(6):1185-95.

34. Wood MA, Mukherjee P, Toocheck CA, Walker WH. Upstream stimulatory factor induces Nr5a1 and Shbg gene expression during the onset of rat sertoli cell differentiation. Biol Reprod. 2011;85(5):965-76. Epub 2011 Jul 6.

35. Hoivik EA, Bjanesoy TE, Mai O, Okamoto S, Minokoshi Y, Shima Y, et al. DNA methylation of intronic enhancers directs tissue-specific expression of steroidogenic factor 1/adrenal 4 binding protein (SF-1/Ad4BP). Endocrinology. 2011;152(5):2100-12.

36. Luo X, Ikeda Y, Parker KL. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. Cell. 1994;77:481-90.

37. Sadovsky Y, Crawford PA, Woodson KG, Polish JA, Clements MA, Tourtellotte LM, et al. Mice deficient in the orphan receptor steroidogenic factor 1 lack adrenal glands and gonads but express P450 side-chain-cleavage enzyme in the placenta and have normal embryonic serum levels of corticosteroids. Proc Natl Acad Sci USA. 1995;92(24):10939-43.

38. Achermann JC, Ito M, Hindmarsh PC, Jameson JL. A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. Nature Genetics. 1999;22(2):125-6.

39. Ito M, Achermann JC, Jameson JL. A naturally occurring steroidogenic factor-1 mutation exhibits differential binding and activation of target genes. J Biol Chem. 2000;275(41):31708-14.

40. Achermann JC, Ozisik G, Ito M, Orun UA, Harmanci K, Gurakan B, et al. 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(4):1829-33.

41. Correa RV, Domenice S, Bingham NC, Billerbeck AEC, Rainey WE, Parker KL, et al. A microdeletion in the ligand binding domain of human steroidogenic factor 1 causes XY sex reversal without adrenal insufficiency. J Clin Endocrinol Metab. 2004;89(4):1767-72.

42. Mallet D, Bretones P, Michel-Calemard L, Dijoud F, David M, Morel Y. Gonadal dysgenesis without adrenal insufficiency in a 46,XY patient heterozygous for the nonsense C16X mutation: a case of SF1 haploinsufficiency. J Clin Endocrinol Metab. 2004;89:4829-32.

43. Tajima T, Fujiwara F, Fujieda K. A novel heterozygous mutation of steroidogenic factor-1 (SF-1/Ad4BP) gene (NR5A1) in a 46, XY disorders of sex development (DSD) patient without adrenal failure. Endocr J. 2009;56(4):619-24.

44. Lourenço D, Brauner R, Lin L, De Perdigo A, Weryha G, Muresan M, et al. Mutations in the NR5A1 associated with ovarian insufficiency. New Eng J Med. 2009;360(12):1200-10.

45. Soardi FC, Coeli FB, Maciel-Guerra AT, Guerra-Júnior G, De-Mello MP. Complete XY gonadal dysgenesis due to p.D293N homozygous mutation in the NR5A1 gene: a case study. J Appl Genet. 2010;51(2):223-4.

46. Ferraz-de-Souza B, Lin L, Achermann JC. Steroidogenic factor-1 (SF-1, NR5A1) and human disease. Mol Cell Endocrinol. 2011;336(1-2):198-205.

47. 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(6):1563-8.

48. Warman DM, Costanzo M, Marino R, Berensztein E, Galeano J, Ramirez PC, et al. Three new SF-1 (NR5A1) gene mutations in two unrelated families with multiple affected members: within-family variability in 46,XY subjects and low ovarian reserve in fertile 46,XX subjects. Horm Res Paediatr. 2011;75(1):70-7.

49. Bashamboo A, Ferraz-de-Souza B, Lourenço D, Lin L, Sebire NJ, Montjean D, et al. Human male infertility associated with mutations in NR5A1 encoding steroidogenic factor 1. Am J Hum Genet. 2010;87(4):505-12.

50. Wuqiang F, Yanase T, Wei L, Oba K, Nomura M, Okabe T, et al. Functional characterization of a new human Ad4BP/SF-1 variation, G146A. Biochem Biophys Res Commun. 2003;311(4):987-94.

51. Philibert P, Zenaty D, Lin L, Soskin S, Audran F, Léger J, et al. Mutational analysis of steroidogenic factor 1 (NR5a1) in 24 boys with bilateral anorchia: a French collaborative study. Hum Reprod. 2007;22(12):3255-61.

52. Brauner R, Neve M, Allali S, Trivin C, Lottmann H, Bashamboo A, et al. Clinical, biological and genetic analysis of anorchia in 26 boys. PLoS One. 2011;6(8):e23292. Epub 2011 Aug 10.

53. Philibert P, Leprieur E, Zenaty D, Thibaud E, Polak M, Frances AM, et al. Steroidogenic factor-1 (SF-1) gene mutation as a frequent cause of primary amenorrhea in 46,XY female adolescents with low testosterone concentration. Reprod Biol Endocrinol. 2010;8:28.