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Clinical follow-up of the first SF-1 insufficient female patient

Suivi de la première patiente atteinte d'une insuffisance de SF-1

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Abstract

Objective. – Steroidogenic factor 1 (*SF-1/NR5A1*) plays a crucial role in regulating adrenal development, gonad determination and differentiation, and in the hypothalamic-pituitary control of reproduction and metabolism. In men (46, XY), it is known that mutations in *SF-1/NR5A1* gene cause a wide phenotypic spectrum with variable degrees of undervirilization. In recent years, the role of SF-1 in the ovarian function was increasingly discussed and alterations in the gene were related to primary ovarian insufficiency. We describe the follow-up of a 46, XX affected woman with a *SF-1* mutation and by comparing our case with the known manifestations reported in the literature, we try to further elucidate the function of SF-1 in the ovary. *Results.* – During infancy, adrenal insufficiency was the only clinical sign of the loss-of-function as ovarian development and function seemed normal. To date, this young woman aged 16.5 years shows normal growth, normal BMI and psychomotor development, has a normal puberty and regular menstruation. *Conclusion.* – This report shows one, to date uniquely described, phenotypic variant of *SF-1* mutation in a 46, XX affected person with adrenocortical insufficiency but no ovarian dysfunction nor disturbance of pubertal development. To follow the natural history of *SF-1* mutation in a 46, XX individual will further shed light on its role in the ovarian function and thus will help to counsel affected patients in future.

Keywords: Steroidogenic factor 1; Adrenal insufficiency; Primary ovarian insufficiency; Disorder of sexual development

Résumé

Objectif. – Le facteur 1 stéroïdogénique (*SF-1/NR5A1*) joue un rôle critique dans la régulation du développement des surrénales, la détermination et différentiation des gonades ainsi que dans le contrôle hypothalamo-pituitaire de la reproduction et du métabolisme. Chez les hommes (46, XY), il est connu que les mutations du gène *SF-1* peuvent entraîner de multiples phénotypes avec différents degrés d'impubérisme. Durant les dernières années, l'influence de SF-1 sur la fonction ovarienne a été étudié et les altérations de ce gène ont été mises en relation avec l'insuffisance ovarienne primaire. Nous décrivons le suivi d'une femme 46, XX porteuse d'une mutation du gène SF-1 et nous comparons notre cas clinique avec ceux de la littérature afin d'éclaircir l'influence du gène SF-1 sur la fonction ovarienne. *Résultats.* – Durant l'enfance, une insuffisance des surrénales fut le seul signe clinique de cette mutation, le développement des ovaires ainsi que leurs fonctions semblèrent alors normales. Actuellement cette adolescente de 16 ans a une croissance, un indice de masse corporelle et un développement psychomoteur normal, une puberté normale ainsi que des menstruations régulières. *Conclusion.* – Cette étude décrit une variante phénotypique, d'une personne 46, XX, atteinte d'une affection de SF-1 avec insuffisance surrénale mais sans dysfonctionnement ovarien ni perturbation de la puberté. Cette observation nous offre une opportunité unique de suivre l'histoire naturelle d'une mutation de SF-1 chez un individu 46, XX et peut contribuer à éclaircir son rôle dans la fonction ovarienne, afin de pouvoir conseiller de futurs patients.

Mots clés : Facteur 1 stéroïdogénique ; Insuffisance surrénale ; Insuffisance ovarienne primaire ; Anomalies du développement génital

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1. Introduction

Human SF-1/*NR5A1*/AdBP4 [1,2], a member of the nuclear receptor family (www.nursa.org), is an amino acid protein. Its crucial functional regions comprise an amino-terminal 2 zinc finger DNA-binding domain (DBD), an accessory DNA-binding region, a hinge region, and a ligand-binding domain (LBD), which forms an AF-2 structure [3,4].

SF-1/NR5A1 is essential for the regulation of adrenal development, gonad determination and differentiation, and in the hypothalamic-pituitary control of reproduction and metabolism. It activates key genes and interacts with transcription factors (DAX1, WT1, SRY, SOX9) involved in sex development. SF-1/NR5A1 thereby regulates the expression of anti-Müllerian hormone and the transcription of enzymes of the steroidogenesis (ACTHR, STAR, CYP11A1, CYP19, CYP17A1) and testosterone biosynthesis in human males [5]. In both sexes, SF-1/NR5A1 is expressed in the developing bipotential gonad and persists later in the testes, namely Leydig and Sertoli cells, as well as in the ovary [6,7].

However, pathogenic significance of *SF-1/NR5A1* mutation became clear by analyzing SF-1 mouse-model. Deletion of the gene (*Nr5a1*) encoding SF-1 in XY mice resulted in impaired adrenal development, complete testicular dysgenesis with Müllerian structures, and female external genitalia as well as abnormal structure of the ventromedial hypothalamus, hyposplenism, hypogonadotropic hypogonadism and late onset obesity [8–10].

These studies in mice lead to the search for *SF-1/NR5A1* mutations in humans displaying primary adrenal failure, 46, XY gonadal dysgenesis and Müllerian structures. Indeed, in 1999, Achermann et al. identified such a mutation in a patient with the mentioned phenotype [11]. After this discovery, *SF-1/NR5A1* mutation related to adrenal insufficiency in human 46, XY men was only reported in one more similar case [12].

In men (46, XY) mutations in *SF-1/NR5A1* gene actually cause a wide phenotypic spectrum of 46, XY disorders of sexual development (DSD) without adrenal insufficiency that ranges from complete testicular dysgenesis with Müllerian structures and amenorrhoea, through persons with mild clitoromegaly or genital ambiguity, to severe penoscrotal hypospadias or anorchia and oligospermia [5]. To date, *SF-1/NR5A1* mutations are thought to be one of the most common monogenic causes of 46, XY DSD due to gonadal dysgenesis. However, having only been reported in two individuals with combined 46, XY DSD and primary adrenal insufficiency, its role in the etiology of the latter seems to be less crucial than initially thought [11–13]. Nevertheless, recently one further case of *SF-1* mutation associated with primary adrenal insufficiency has been reported in a 46, XX female patient harboring a homozygous p.R92Q mutation [14], the same homozygous affection reported in the 46, XY DSD individual with adrenal insufficiency in 2002.

In female mice knockout of SF-1 in granulosa cells results in hypoplastic ovaries and infertility [15]. In human women (46, XX), mutations in *SF-1/NR5A1* seem to be associated with impairment of ovarian development and function leading to primary ovarian insufficiency due to low ovarian reserve. Indeed, *SF-1* mutation has been reported in familial and sporadic cases of primary ovarian insufficiency (POI), albeit it is a rather rare cause in sporadic cases (1.4–8%) [16–18]. However, SF-1 alterations seem to lead to different degrees of severity of impairment of ovarian function, depending on the mutation. Therefore, the role of SF-1 in human ovarian function remains to be more deeply explored.

2. Materials and methods

We described the first case of a SF-1 insufficient 46, XX girl in whom, at the age of 14 months, adrenal insufficiency was the only clinical sign of the loss-of-function mutation [19]. Direct sequencing of PCR fragments amplified from genomic DNA of the patient revealed the presence of a heterozygote G to T transversion in exon 4 of the *NR5A1* gene, leading to the missense p.R255L in the hinge region of SF-1/NR5A1 protein. Functional studies demonstrated that the mutated *SF-1/NR5A1* did not bind properly to the known responsive element of the human CYP11A (cholesterol side-chain cleavage) promoter. At that time, ovarian development and function seemed normal on the base of normal ovarian morphology at ultrasound and MRI (showing an infantile uterus [2.6 cm] and infantile ovaries of normal size [maximal diameter 1.2 cm] and structure) and repeatedly normal gonadotropins and ovarian markers (inhibins). Nevertheless, it remained unknown then whether this mutation would interfere with ovarian function at onset of puberty and beyond.

Our follow-up was based on 6-monthly visits at our outpatient clinic, with measurements of auxological (height and weight) parameters and evaluation of pubertal development. Hormonal measurements were performed using commercially available immunoassays (AMH Gen II ELISA kit by Beckman Coulter; DELFIA hFSH and LH kit by PerkinElmer; ST AIA-PACK ACTH kit by TOSOH).

3. Results

At the age of ten and a half years, puberty started spontaneously with thelarche and pubarche. Finally, at the age of 11 years and 7 months menstruation began spontaneously. At 13.3 years, her bone age was 14.7 years, her height was 153 cm (P10–25) and her weight 49 kg (P50–75) (Fig. 1). At age 14 and 16.8 years, LH and FSH serum levels were normal for puberty (LH 14.30 U/L, FSH 6.39 U/L [15th day of menstrual cycle] and LH 19.7 U/L, FSH 6.25 U/L [14th day of menstrual cycle], respectively, both times values compatible with midcycle peak). The marker of ovarian reserve anti-Müller-Hormone (AMH) showed normal ovarian function with a value of 17 pmo/L and 36 pmol/L, respectively (NV > 7.1 pmol/L) (Table 1). Furthermore, ACTH was within normal range (28 pg/mL and 11 pg/mL). However, at the age of thirteen years, ACTH level was markedly elevated (> 1250 pg/mL) due to malcompliance. At age 14, she deliberately lost weight by changing her eating habits and practicing more sports.

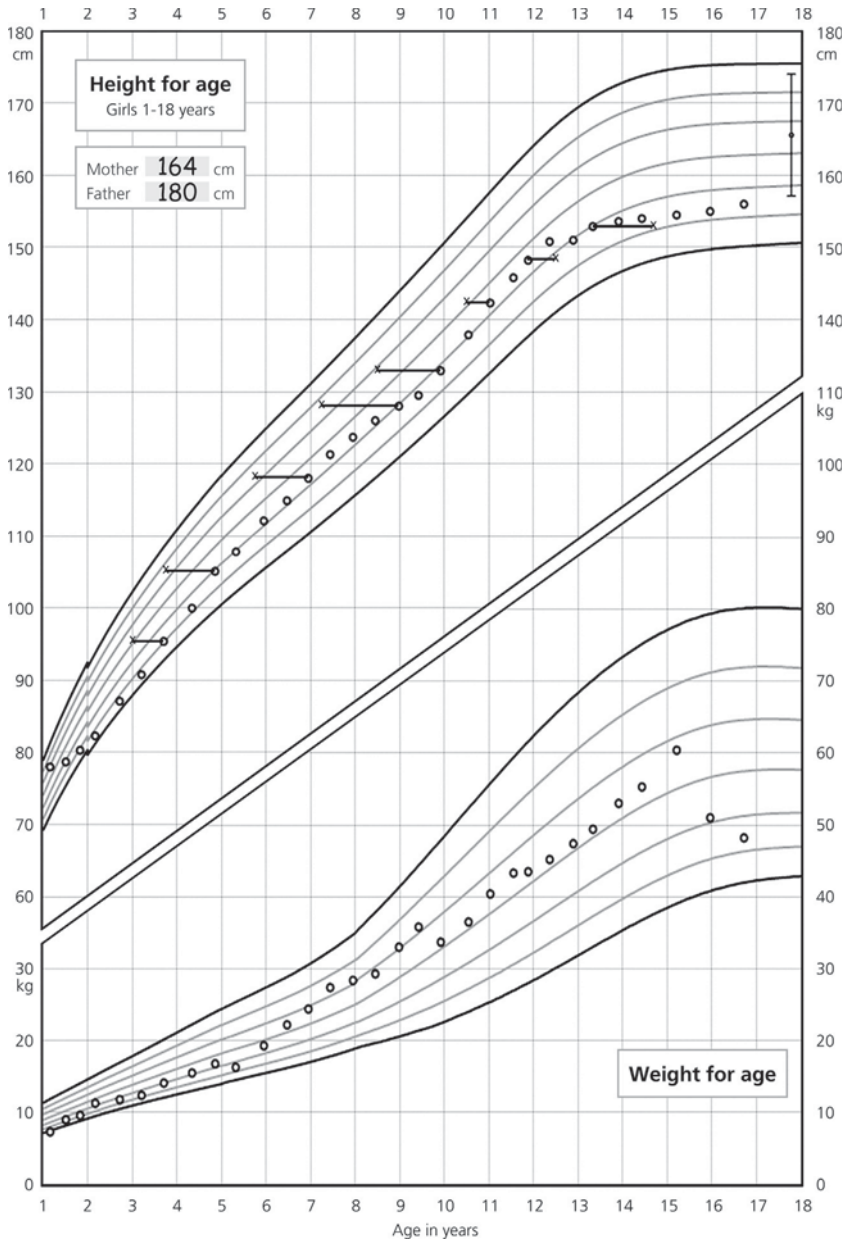

Fig. 1. Growth curve.

Table 1
Clinical and biochemical findings.

Age	Bone age	Tanner stage	ACTH 10–55 pg/mL	LH/FSH (U/L)	Day of menstrual cycle	AMH >7.1 pmol/L
10Y6M		B2, P2				
11Y	10Y6M	B3, P3		0.7/3.2		
11Y7M		Menarche				
11Y10M	12Y6M	B4, P4				
12Y10M			>1250	6.92/5.45	17	
13Y4M	14Y8M					
13Y11M			28	14.3/6.4	15	17
16Y9M			11	19.7/6.3	14	36

Table 2
NR5A1 mutations in 46, XX.

Patient	Phenotype	Family member with 46, XY DSD	Mutation	rs	Frequency dbGap[e]	Occurence	Reference
1	AI	No	p.R255L	104894118	1/36499	Heterozygous	Biason-Lauber et al., 2000 [19]
2	POI	Yes[a]	p.N222Kfs	606231206	No data	Heterozygous	Lourenco et al., 2009 [16]
3	POI	Yes[b]	p.D293N	121918655	0/39071	Homozygous	Lourenco et al., 2009 [16]
4	POI	Yes[b]	p.M1I	121918656	0/37246	Heterozygous	Lourenco et al., 2009 [16]
5	POI	Yes[a]	p.P131Rfs	606231207	No data	Heterozygous	Lourenco et al., 2009 [16]
6	POI	NM	p.L231_L233del	606231208	No data	Heterozygous	Lourenco et al., 2009 [16]
7	HC	NM	p.G123A	200163795	46/36999	Heterozygous	Lourenco et al., 2009 [16]
			p.P129L	200749741	44/35919	Heterozygous	
8	No	Yes[a]	p.R92Q	104894119	0/37794	Heterozygous	Achermann et al., 2002 [12]
9	No	Yes[b]	p.R92Q	104894119	0/37794	Heterozygous	Achermann et al., 2002 [12]
10	AI	NM	p.R92Q	104894119	0/37794	Homozygous	Guran et al., 2015 [14]
11	Ovotesticular DSD	No	p.R92W	886039769	No data	Heterozygous	Igarashi et al., 2016 [22]
12	Ovotesticular DSD	No	p.R92W	886039769	No data	Heterozygous	Igarashi et al., 2016 [22]
13	Ovotesticular DSD	No	p.R92W	886039769	No data	Heterozygous	Baetens et al., 2016 [23]
14	Ovotesticular DSD	No	p.R92W	886039769	No data	Heterozygous	Baetens et al., 2016 [23]
15	Testicular DSD	No	p.R92W	886039769	No data	Heterozygous	Baetens et al., 2016 [23]
16	No	Yes[a]	p.R92W	886039769	No data	Heterozygous	Baetens et al., 2016 [23]
17	No	Yes[a]	p.R92W	886039769	No data	Heterozygous	Baetens et al., 2016 [23]
18	No	Yes[b]	p.R92W	886039769	No data	Heterozygous	Baetens et al., 2016 [23]
19	NM	Yes[a]	p.G91S	104894126	0/37721	Heterozygous	Lin et al., 2007 [25]
20	NM	Yes[a]	p.M78I	104894125	0/32254	Heterozygous	Lin et al., 2007 [25]
21	POI	No	p.A192F	No data	No data	Heterozygous	Janse et al., 2012 [17]
22	POI	No	p.R313H	No data	No data	Heterozygous	Janse et al., 2012 [17]
23	POI	NM	p.R255C	No data	No data	Heterozygous	Philibert et al., 2013 [21]
24	POI[a] NF, ↑FSH ↓AMH[b]	Yes[a,b]	p.Y183X	No data	No data	Heterozygous	Warman et al., 2010 [26]
26	NF, ↑FSH ↓AMH	Yes[a]	p.P179HfsX116	No data	No data	Heterozygous	Coutan et al., 2007 [27]
27	NM	Yes[a]	c.1277dupT	No data	No data	Heterozygous	Köhler et al., 2008 [28]
28	POI	NM	p.P235L	No data	No data	Heterozygous	Camats et al., 2012 [29]
29	Infertility	Yes[b]	p.H24TfsX51	No data	No data	Heterozygous	Camats et al., 2012 [29]
30	EM	Yes[a]	p.G90R	No data	No data	Heterozygous	Camats et al., 2012 [29]
29	NF, ↑FSH ↓AMH[a,c]	Yes[a,c]	p.R313H	No data	No data	Heterozygous	Ciaccio et al., 2012 [30]
30	POI	Yes[a]	9q33.3 microdel	No data	No data	Heterozygous	Harrison et al., 2013 [31]
31	POI	No	p.Y5D	No data	No data	Heterozygous	Jiao et al., 2013 [32]
32	POI[a,d] Dep, Anx[a]	Yes[a,d]	p.D257TfsX39	No data	No data	Heterozygous	Suwanai et al., 2013 [33]
33	Dep	Yes[a]	p.V424del	No data	No data	Heterozygous	Suwanai et al., 2013 [33]
33	POI	Yes[a]	p.C65Y	No data	No data	Heterozygous	Fabbri et al., 2014 [34]

AI: adrenal insufficiency; POI: Primary ovarian insufficiency; HC: hypertrophic clitoris; NM: not mentioned; NF: normal fertility; EM: early menopause; Dep: depression; Anx: anxiety.

[a] Mother of patient with 46, XY DSD.
[b] Sister of patient with 46, XY DSD.
[c] Mother's sister of patient with 46, XY DSD.
[d] Maternal grandmother of patient with 46, XY DSD.
[e] Potential variants are reported according to dbGaP data (https://www.ncbi.nlm.nih.gov/clinvar/?term=NR5A1%5bgene).

To date at age 16, this young woman shows a normal growth and psychomotor development, has a normal puberty with normal breast development (Tanner stage 4) and regular menstruation. The current treatment of her adrenal insufficiency consists of hydrocortisone (17.4 mg/m^2/day) and fludrocortisone (0.05 mg/day).

4. Discussion

Over the last years, mutations in *SF-1/NR5A1* were shown to lead to different kind of phenotypes in 46, XX and XY affected individuals. The adrenals as well as the hypothalamo-pituitary-gonadal axes can be affected leading to several heterogeneous

clinical presentations. In fact, SF-1 is involved in regulating adrenal development, gonad determination/differentiation, and in the hypothalamic-pituitary control of reproduction and metabolism. Heterozygote mutations in SF-1 are one of the most common causes of 46, XY disorders/differences of sex development (DSD) due to gonadal dysgenesis. The role of *SF-1* mutations in adrenal and ovarian physiology is less clear.

Our patient was the first described affected 46, XX female patient with an *SF-1/NR5A1* loss of function mutation leading to adrenal insufficiency [19]. Adrenal insufficiency seems to be rare in SF-1 insufficiency. Although the first described patient was adrenal insufficient [11], since then only few more patients with adrenal insufficiency and *SF-1* mutations were identified, including ours. The first alteration found was a de novo p.G35E heterozygous dominant mutation in the P-box primary DNA-binding region, the second was a recessively inherited p.R92Q homozygous frameshift mutation in the A-box secondary DNA-binding region [12]. Recently, the same homozygous p.R92Q mutation was found in a 46, XX girl with early onset primary adrenal insufficiency [14]. The G to T transversion in exon 4 in our patient is predicted to be deleterious by Alamut Software alamut.interactive-biosoftware.com (comprehending the prediction programs PolyPhen-2, SIFT, Mutation taster, AlignGVGD and KD4v) and reported to be pathogenic. Even more relevant than predictions, our published data [19] demonstrated that the mutation lead to a loss of transactivation potential of the mutant SF-1. The fact that this heterozygous p.R255L variant was later found in 2/15056 South Asians (rs104894118) without any apparent disease, is most likely due to different penetrance of the mutation or modifying influences of other genes related to other genetic background in different populations.

In women, *SF-1* mutations have been related to POI, due to decreased expression of steroidogenic enzymes and compromised granulosa cell proliferation leading to impaired follicle development [20]. Mutations can be found in about 1.4–1.6% of women presenting with sporadic POI of unknown origin [16–18,21], with alterations of SF-1 including the hinge region, similarly to our patient.

In women, there is also a wide phenotypic spectrum of clinical impact from no apparent consequence to early primary ovarian insufficiency [16] (Table 2). Recently, in 46, XX individuals a heterozygous p.R92W mutation was found in association with several cases of ovotesticular DSD [22,23]. The fact that the same mutation can lead to different clinical pictures even in the same family suggests various degrees of penetrance due to co-modulating and/or epigenetic factors influencing SF-1 activity and integrity [16,21–24].

Our patient showed that despite impaired SF-1 integrity the ovaries are macroscopically normal and show a normal function before and after birth, throughout and beyond puberty. We suggest accordingly to previous data in humans [16] that SF-1 integrity is not essential for normal fetal ovarian development and early function, although it may play an important role in maintaining ovarian integrity. Careful clinical follow-up of this unique case will help to evaluate this hypothesis in an in vivo human clinical setting. Furthermore, it may help to predict the clinical course of similar cases and therefore better counsel and monitor future patients for potential risk of adrenal or gonadal insufficiency.

Disclosure of interest

The authors declare that they have no competing interest.

References

[1] Morohashi K, Honda S, Inomata Y, Handa H, Omura T. A common trans-acting factor, Ad4-binding protein, to the promoters of steroidogenic P-450s. J Biol Chem 1992;267:17913–9 [Epub 1992/09/05. PubMed PMID: 1517227].

[2] 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;6:1249–58, http://dx.doi.org/10.1210/mend.6.8.1406703 [Epub 1992/08/01. PubMed PMID: 1406703].

[3] Parker KL, Schimmer BP. Steroidogenic factor 1: a key determinant of endocrine development and function. Endocr Rev 1997;18:361–77 [Epub 1997/06/01. PubMed PMID: 9183568].

[4] Lin L, Achermann JC. Steroidogenic factor-1 (SF-1, Ad4BP, NR5A1) and disorders of testis development. Sex Dev 2008;2:200–9 [Epub 2008/11/07. doi:000152036 [pii] 10.1159/000152036. PubMed PMID: 18987494; PubMed Central PMCID: PMC2645687].

[5] Ferraz-de-Souza B, Lin L, Achermann JC. Steroidogenic factor-1 (SF-1, NR5A1) and human disease. Mol Cell Endocrinol 2011;336:198–205, http://dx.doi.org/10.1016/j.mce.2010.11.006 [PubMed PMID: 21078366; PubMed Central PMCID: PMC3057017].

[6] 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;87:175–80 [Epub 1999/09/24. doi: S0925-4773(99)00123-9 [pii]. PubMed PMID: 10495282].

[7] 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;80:2815–21, http://dx.doi.org/10.1210/jcem.80.9.7673429 [PubMed PMID: 7673429].

[8] 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 [Epub 1994/05/20. doi: 0092-8674(94)90211-9 [pii]. PubMed PMID: 8187173].

[9] Shinoda K, Lei H, Yoshii H, Nomura M, Nagano M, Shiba H, et al. Developmental defects of the ventromedial hypothalamic nucleus and pituitary gonadotroph in the Ftz-F1 disrupted mice. Dev Dyn 1995;204:22–9, http://dx.doi.org/10.1002/aja.1002040104 [Epub 1995/09/01. PubMed PMID: 8563022].

[10] Majdic G, Young M, Gomez-Sanchez E, Anderson P, Szczepaniak LS, Dobbins RL, et al. Knockout mice lacking steroidogenic factor 1 are a novel genetic model of hypothalamic obesity. Endocrinology 2002;143:607–14, http://dx.doi.org/10.1210/endo.143.2.8652 [PubMed PMID: 11796516].

[11] 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. Nat Genet 1999;22:125–6 [Epub 1999/06/16. doi: 10.1038/9629. PubMed PMID: 10369247].

[12] 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;87:1829–33 [Epub 2002/04/05. PubMed PMID: 11932325].

[13] Lin L, Gu WX, Ozisik G, To WS, Owen CJ, Jameson JL, et al. 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 [doi: 10.1210/jc.2006-0603. PubMed PMID: 16684822; PubMed Central PMCID: PMCPMC1865080].

[14] Guran T, Buonocore F, Saka N, Ozbek MN, Aycan Z, Bereket A, et al. Rare causes of primary adrenal insufficiency: genetic and clinical characterization of a large nationwide cohort. J Clin Endocrinol Metab 2016;101:284–92 [doi: 10.1210/jc.2015-3250. PubMed PMID: 26523528; PubMed Central PMCID: PMCPMC4701852].

[15] Jeyasuria P, Ikeda Y, Jamin SP, Zhao L, De Rooij DG, Themmen AP, et al. Cell-specific knockout of steroidogenic factor 1 reveals its essential roles in gonadal function. Mol Endocrinol 2004;18:1610–9 [doi: 10.1210/me.2003-0404. PubMed PMID: 15118069].

[16] Lourenco D, Brauner R, Lin L, De Perdigo A, Weryha G, Muresan M, et al. Mutations in NR5A1 associated with ovarian insufficiency. N Engl J Med 2009;360:1200–10 [Epub 2009/02/28. doi: NEJMoa0806228 [pii] 10.1056/NEJMoa0806228. PubMed PMID: 19246354; PubMed Central PMCID: PMC2778147].

[17] Janse F, de With LM, Duran KJ, Kloosterman WP, Goverde AJ, Lambalk CB, et al. Limited contribution of NR5A1 (SF-1) mutations in women with primary ovarian insufficiency (POI). Fertil Steril 2012;97:141e2–6e2, http://dx.doi.org/10.1016/j.fertnstert.2011.10.032 [PubMed PMID: 22100173].

[18] Voican A, Bachelot A, Bouligand J, Francou B, Dulon J, Lombes M, et al. NR5A1 (SF-1) mutations are not a major cause of primary ovarian insufficiency. J Clin Endocrinol Metab 2013;98:E1017–21 [10.1210/jc.2012-4111. PubMed PMID: 23543655].

[19] 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 [Epub 2000/10/20. doi: S0002-9297(07)63224-8 [pii] 10.1086/316893. PubMed PMID: 11038323; PubMed Central PMCID: PMC1287931].

[20] Pelusi C, Ikeda Y, Zubair M, Parker KL. Impaired follicle development and infertility in female mice lacking steroidogenic factor 1 in ovarian granulosa cells. Biol Reprod 2008;79:1074–83 [10.1095/biolreprod.108.069435. PubMed PMID: 18703422; PubMed Central PMCID: PMCPMC2780474].

[21] Philibert P, Paris F, Lakhal B, Audran F, Gaspari L, Saad A, et al. *NR5A1 (SF-1)* gene variants in a group of 26 young women with XX primary ovarian insufficiency. Fertil Steril 2013;99:484–9, http://dx.doi.org/10.1016/j.fertnstert.2012.10.026 [PubMed PMID: 23153500].

[22] Igarashi M, Takasawa K, Hakoda A, Kanno J, Takada S, Miyado M, et al. Identical NR5A1 missense mutations in two unrelated 46, XX individuals with testicular tissues. Hum Mutat 2016 [doi:10.1002/humu.23116. PubMed PMID: 27610946].

[23] Baetens D, Stoop H, Peelman F, Todeschini AL, Rosseel T, Coppieters F, et al. NR5A1 is a novel disease gene for 46, XX testicular and ovotesticular disorders of sex development. Genet Med 2016 [doi: 10.1038/gim.2016.118. PubMed PMID: 27490115].

[24] Philibert P, Zenaty D, Lin L, Soskin S, Audran F, Leger J, et al. Mutational analysis of steroidogenic factor 1 (NR5a1) in 24 boys with bilateral anorchia: a French collaborative study. Hum Reprod 2007;22:3255–61 [Epub 2007/10/18. doi: dem278 [pii] 10.1093/humrep/dem278. PubMed PMID: 17940071; PubMed Central PMCID: PMC2990861].

[25] Lin L, Philibert P, Ferraz-de-Souza B, Kelberman D, Homfray T, Albanese A, et al. 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 [doi: 10.1210/jc.2006-1672. PubMed PMID: 17200175; PubMed Central PMCID: PMC1872053].

[26] 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;75:70–7 [Epub 2010/09/22. doi: 10.1159/000320029. PubMed PMID: 20861607].

[27] Coutant R, Mallet D, Lahlou N, Bouhours-Nouet N, Guichet A, Coupris L, et al. Heterozygous mutation of steroidogenic factor-1 in 46, XY subjects may mimic partial androgen insensitivity syndrome. J Clin Endocrinol Metab 2007;92:2868–73 [Epub 2007/05/08. doi: 10.1210/jc.2007-0024. PubMed PMID: 17488792].

[28] Köhler B, Lin L, Ferraz-de-Souza B, Wieacker P, Heidemann P, Schroder V, et al. Five novel mutations in steroidogenic factor 1 (SF1, NR5A1) in 46, XY patients with severe underandrogenization but without adrenal insufficiency. Hum Mutat 2008;29:59–64, http://dx.doi.org/10.1002/humu.20588 [PubMed PMID: 17694559; PubMed Central PMCID: PMC2359628].

[29] Camats N, Pandey AV, Fernandez-Cancio M, Andaluz P, Janner M, Toran N, et al. Ten novel mutations in the *NR5A1* gene cause disordered sex development in 46, XY and ovarian insufficiency in 46, XX individuals. J Clin Endocrinol Metab 2012;97:E1294–306 [Epub 2012/05/01. doi: 10.1210/jc.2011-3169. PubMed PMID: 22549935].

[30] Ciaccio M, Costanzo M, Guercio G, De Dona V, Marino R, Ramirez PC, et al. Preserved fertility in a patient with a 46, XY disorder of sex development due to a new heterozygous mutation in the *NR5A1/SF-1* gene: evidence of 46, XY and 46, XX gonadal dysgenesis phenotype variability in multiple members of an affected kindred. Horm Res Paediatr 2012;78:119–26, http://dx.doi.org/10.1159/000338346 [Epub 2012/08/14. PubMed PMID: 22907560].

[31] Harrison SM, Campbell IM, Keays M, Granberg CF, Villanueva C, Tannin G, et al. Screening and familial characterization of copy-number variations in NR5A1 in 46, XY disorders of sex development and premature ovarian failure. Am J Med Genet 2013;161A:2487–94, http://dx.doi.org/10.1002/ajmg.a.36084 [Epub 2013/08/05. PubMed PMID: 23918653; PubMed Central PMCID: PMC3788034].

[32] Jiao X, Qin Y, Li G, Zhao S, You L, Ma J, et al. Novel NR5A1 missense mutation in premature ovarian failure: detection in Han Chinese indicates causation in different ethnic groups. PLoS One 2013;8:e74759, http://dx.doi.org/10.1371/journal.pone.0074759 [eCollection 2013. PubMed PMID: 24073220; PubMed Central PMCID: PMC3779243].

[33] Suwanai AS, Ishii T, Haruna H, Yamataka A, Narumi S, Fukuzawa R, et al. A report of two novel NR5A1 mutation families: possible clinical phenotype of psychiatric symptoms of anxiety and/or depression. Clin Endocrinol (Oxf) 2013;78:957–65, http://dx.doi.org/10.1111/cen.12054 [Epub 2013/04/01. Pubmed PMID: 23095176].

[34] Fabbri HC, de Andrade JG, Soardi FC, de Calais FL, Petroli RJ, Maciel-Guerra AT, et al. The novel p.Cys65Tyr mutation in *NR5A1* gene in three 46, XY siblings with normal testosterone levels and their mother with primary ovarian insufficiency. BMC Med Genet 2014;10:7 [doi: 10.1186/1471-2350-15-7. PubMed PMID: 24405868; PubMed Central PMCID: PMC3900668].