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Heterozygous variant in NR5A1 gene as a monogenic form of gonadal dysgenesis: a case report

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Abstract

NR5A1 gene mutations are associated principally to 46,XY DSD (Disorders of Sex Development), with an extensive range of phenotypic variability in patients, comprising gonadal dysgenesis and male infertility. NR5A1 gene plays a crucial role in reproduction, steroidogenesis, and sexual differentiation. The present case describes an Italian male adolescent with ambiguous genitalia at birth and a 46,XY karyotype who was investigated for DSD genetic panel conditions due to slow pubertal progression. A heterozygous variant in NR5A1 (NM_004959.4: c.937C>T, p.Arg313Cys) was identified in this patient and in his father. The same variant was previously described in other studies showing the wide heterogeneity of genotype-phenotype correlation in DSD patients.

KeyWords:gonadal dysgenesis; NR5A1 gene; DSD; disorders of sex development

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Introduction

Disorders of Sex Development (DSD) are defined as conditions in which there are anomalies in chromosomal, gonadal, or anatomic sex development (1). DSD classification has a genetic basis and these defects are classified in three main categories:

- Sex Chromosomal DSD, comprising Turner and Klinefelter syndromes, which are the most frequent DSD conditions reported worldwide;
- 46,XX DSD, including patients with atypical gonadal differentiation, and excessive androgens excretion;
- 46,XY DSD, encompassing individuals with abnormal testicular differentiation, defects in testosterone biosynthesis, impaired testosterone action, and persistent Müllerian duct condition (2, 3).

Second and third DSD groups are often associated with defects in genes that are important in determining a precise synergic expression of a wide range of activating and repressing factors, some of them pivotal for sex development (2). Interestingly, phenotypes of individuals with the same monogenic defect can be heterogeneous, and an example of this situation is NR5A1 gene mutations, that are most commonly encountered in the form of 46,XY DSD (4, 5, 6).

NR5A1 gene (Nuclear Receptor Subfamily 5 Group A Member 1; MIM#184757), also known as SF-1, encodes for a transcription factor involved in reproduction, steroidogenesis, and sexual differentiation (7). This gene is expressed in steroidogenic tissues and in central nervous system (pituitary gland and ventromedial hypothalamus) (8). NR5A1 represents the first marker of gonadal and adrenal differentiation (9). During human development, this gene is expressed in gonad somatic cells (primitive gonad), in Leydig and Sertoli cells, and in steroid- secreting adrenal cortex (8). NR5A1 activates the transcription of genes involved in development and maintenance of male differentiation, such as LHCGR, STAR, CYP11A1, and CYP17A1. Furthermore, NR5A1 increases the expression of INSL3 (Insulin-like polypeptide 3), which regulates testicular descendent (10). NR5A1 also controls the expression of AMH (anti- Müllerian hormone) and its receptor (AMHR2), which are essential for male reproductive tract development. Moreover, this gene regulates the expression of SRY and SOX9 in Sertoli cells, and both genes are crucial for sex determining in males (11).

We report an Italian male adolescent with ambiguous genitalia at birth who was investigated for DSD genetic panel conditions due to slow pubertal progression.

Case report

Our patient is the second child born from non- consanguineous and healthy parents. He was born full- term after a physiological pregnancy (38 GW) with weight (2970 gr) and length (49 cm) at birth appropriate for date. In the first days of life, physical examination in association with imaging studies (ultrasonography and genitogram) showed ambiguous genitalia characterized by peno- scrotal hypospadias, bilateral abdominal cryptorchidism, prepenile and bifid scrotum characterized by posterior transposition of the scrotum and anterior transposition of the penis, Müllerian duct remnant (Prader stage 3). No facial dysmorphisms were observed and cardio-respiratory, digestive, and nervous systems were normal. Karyotype was 46,XY.

At 16 and 20 months he underwent surgical correction of bilateral cryptorchidism. At 4 years old, he underwent surgical correction of peno- scrotal transposition, urethra reconstruction according to Duplay technique and preputioplasty. Müllerian duct remnant persisted, causing sometimes urinary infection and urethral bleeding. After surgical corrections, he presented an adequate preputial sliding with a normal urethral orifice.

He presented regular growth in weight and height, with an appropriate bone age. He started puberty at 11 years of chronological age. However, he showed slow pubertal progression with low testicular volume. His endocrine profile demonstrated hypergonadotropic hypogonadism (FSH 74.10 mIU/ml; LH 17.30 mIU/ml; Testosterone 4 ng/ml), normal adrenal function, low serum

inhibin B (3.4 pg/ml) and normal value of AMH (Table 1). Adrenal ultrasonography was normal, while testicular ultrasonography showed a little (5 mm) right testicular cyst (unchanged in annual checks).

Table1 Clinical and Laboratory Findings in our patient

Chronological age (years)	**11**	**12**	**13**	**14**
Bone age, years	13	13	-	-
Height, cm	141	149	155,5	159
Tanner stages (with testis volume)	G2/P2 (R:4cc;L:3cc)	G2/P4 (R:4cc;L:5cc)	G2/P4 (R:6cc;L:6cc)	G2/P5 (R:7cc;L:6cc)
Testosterone (ng/ml) [1.19 – 3.49]	2,7	3,7	4	4,2
Basal FSH (mIU/ml) [1.5 – 12.4]	45,6	63	74,10	61,2
Basal LH (mIU/ml) [1.7 – 8.6]	7,34	14,40	17,30	13,2
Inhibin B (pg/ml) [25- 325]	-	-	3,40	-
AMH (ng/ml) [0.0 – 2.4]	-	-	0,2	-

R= right testis; L: left testis
*Normal values for age on brackets

We collected blood samples from our patient and his parents, and analysed DNA using a DSD 18-gene panel, with two different procedures:

1) Multiple Ligation-dependent Probe Amplification (MLPA), that was negative for deletion or duplication in DMRT1, CYP17A1, SRD5A2, HSD17B3, SOX9, NR0B1, WNT4, and NR5A1 genes;

2) Next Generation Sequencing (NGS), that was negative for pathogenic variants in AR, CBX2, DHH, MAP3K1, NR0B1, SOX9, SRY, WT1, AMH, AMHR2, HSD17B3, MAMLD1, and SRD5A2 genes. For NGS, a Miseq Illumina and SureSelectXT kit (Agilent) were used according to the manufacturer instructions.

The last procedure (NGS) identified a heterozygous missense variant in NR5A1 gene (NM_004959.4: c.937C>T, p.Arg313Cys). The same variant was found in his father who apparently was in healthy status. The father did not undergo to clinical and endocrine evaluations. However, this variant (rs1057517779) is reported in ClinVar database (ClinVar: last release on 27th of April) as pathogenic/ likely pathogenic, and it has been observed in individuals affected with autosomal dominant Disorders of Sex Development (DSD). Experimental studies have shown that the corresponding missense change results in a NR5A1 protein that lacks transcriptional transactivation activity (12, 13).

The revaluation of the family history allowed showing some peculiarities in the family tree of our patient: his paternal aunts have a condition of amenorrhea (the older one a primary amenorrhea; the younger one a secondary amenorrhea with an onset at 18 years old); his paternal uncle is married and childless. These family members refused to undergo to clinical, endocrine and genetic

evaluations. No genital alterations were observed in the older brother of our patient.

Table 2 Clinical and Laboratory Findings in children with p.Arg313Cys mutation in NR5A1 gene

Authors	**Allali et al. (2011) (14)**	**Malikova et al. (2014) (13)**	**Mazen et al. (2016) (12)**	**Our Case**
Origin	France	Spain	Egypt	Italy
Patients (n.)	1	3	1	1
Karyotype	46,XY	46,XY	46,XY	46,XY
Assigned sex	M	M	F	M
Genitalia at birth	Distal hypospadias, Normal phallus length	Scrotal hypospadias (3/3), Micropenis (3/3), Unilateral Cryptorchidism (1/3), NO Müllerian ducts (3/3)	Ambiguous genitalia, Clitoromegaly, Bilateral Cryptorchidism (inguinal canal)	Ambiguous genitalia, Peno-scrotal hypospadias, Bilateral abdominal cryptorchidism, Peno-scrotal transposition, Müllerian duct remnant
Endocrine data (age)	At 4 days: Normal Testosterone, LH and FSH slightly decreased, Low AMH, Inhibin B normal	At birth: Testosterone slightly decreased (1/3), High Testosterone (2/3), Low AMH (3/3)	At 4 years: Low Testosterone, Low basal serum FSH and LH, Low AMH	At 11-14 years: Normal Testosterone, High basal serum FSH and LH, Low Inhibin B, Normal AMH
Andrenal function	NR	Normal (3/3)	Normal	Normal

NR= not reported

Discussion

Our case describes a heterozygous variant in NR5A1 as a cause of monogenic gonadal dysgenesis in an Italian male adolescent, who presented at birth ambiguous genitalia and for what underwent three surgical corrections. At 11 years old, he started puberty showing slow pubertal progression with low testicular volume. Endocrine data demonstrated hypergonadotropic hypogonadism, normal adrenal function, low serum inhibin B and normal value of AMH. He was investigated for DSD genetic panel, which confirmed a heterozygous missense mutation in NR5A1 gene (NM_004959.4:c.937C>T). This variant was previously described in individuals with 46,XY gonadal dysgenesis (Table 2), in association with a wide spectrum of clinical findings and endocrine data alterations, mainly represented by ambiguous genitalia and hypospadias, with alteration in Testosterone and basal FSH/LH levels (12, 13, 14). However, NR5A1 mutations have a wide range of phenotypes, ranging from DSD to oligo/azoospermia in 46,XY individuals to ovarian failure in 46,XX individuals. In 46,XY patients, the most frequent phenotype is ambiguous genitalia with female external genitalia and clitoromegaly and palpable gonads. Phenotypic variability in these patients may be related to the association with genetic modifiers or pathogenic variant on other gonadal- determining genes (8). Interestingly, *Mazen et al.* (12) reported this mutation in a 4-years old child with ambiguous genitalia, a 46,XY karyotype, but a female assigned sex at birth.

In conclusion, this paper confirms the association between c.937C>T variant in NR5A1 and 46, XY gonadal dysgenesis. Previous studies have shown similar anomalies in gonadal differentiation. We recommend including NR5A1 genetic screening in patients with DSD features, particularly in case of ambiguous genitalia and hypospadias at birth. In fact, the assigned sex at birth could have important psychological implications, especially during adolescence.

Conflicts of Interest: There is no potential conflict of interest, and the authors have nothing to disclose. This work was no supported by anygrant.

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