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Case Report

Different Clinical Manifestations Related to Subvirilization in Three XY Patients With the Same Pathogenic Variant of Steroidogenic Factor 1

Maria Fernanda Ochoa, MD [1], Francisca Yankovic, MD [2], Helena Poggi, Biochem [1], Alejandro Martinez, MD [1,*]

[1] Endocrinology Unit, División of Pediatric, Pontificia Universidad Católica, Santiago, Chile
[2] Urology Division of Clínica Santa María, Santiago, Chile

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ABSTRACT

Objective: During the prenatal period, steroidogenic factor 1 is required for the development of the adrenal glands and for gonadal determination and differentiation, and after birth, it regulates gonadal progenitor cell formation and their survival. Here, we describe the clinical phenotype of three 46,XY patients (2 brothers and an unrelated subject) with disorder of sex development due to the same genetic variant.
Methods: All patients underwent hormonal and pelvic ultrasound studies. Sequence analysis and deletion/duplication testing of a panel encompassing 8 genes (*AR, DHH, MAP3K1, NROB1, SRD5A2, SRY, WT1,* and nuclear receptor subfamily 5, group A, member 1 [*NR5A1*]) were performed in the index cases. All family members were tested for the presence of the *NR5A1* variant.
Results: A variant previously described as likely pathogenic in *NR5A1* (c.251G>A, p.Arg84His) that segregated in 1 family with different degrees of under-virilization was found. The family 1 index case (IV2) and his brother (IV3) had an external masculinization scale score of 5/12, but only the index case had Müllerian remnants; however, the family 2 patient had a milder score of 9/12. The older female relatives of family 1 who harbor this variant experienced premature menopause.
Conclusion: To our knowledge, this is the first report where the c.251G>A (p.Arg84His) variant is associated with the presence of Müllerian remnants in 46,XY subjects and primary ovarian insufficiency in 46,XX individuals. The segregation of this variant with clinical manifestations provides further evidence for considering it as pathogenic.

Introduction

Steroidogenic factor 1 (SF1) belongs to the family of nuclear transcription factors and regulates many genes involved in reproduction, steroidogenesis, and sexual differentiation.[1,2] SF1 is expressed early during fetal development in the adrenal cortex, where it regulates the synthesis of several enzymes, such as STAR, CYP11A1, and CYP17A1, and the biosynthesis of steroidal hormones.[3,4] Pathogenic variants in the gene coding for SF1 (nuclear receptor subfamily 5, group A, member 1 [*NR5A1*]; OMIM* 184757) represent the second most frequent genetic factor associated with disorder of sex development (DSD) in 46,XY subjects.[5]

SF1 protein expression is observed during fetal life, from the time that the bipotential gonad develops throughout sex determination. In the developing testis, SF1 is expressed by fetal Sertoli cells, where it upregulates the expression of 2 genes crucial for male sex determination and differentiation, *SRY*-box transcription factor 9, and anti-Müllerian Hormone (*AMH*), through synergistic interactions with SRY. SF1 is also expressed within Leydig cells, where it controls various factors involved in steroidogenesis, ultimately leading to male virilization in utero and in the prepubertal gonad. On the other hand, in postnatal life, SF1 regulates the

Abbreviations: AMH, anti-Müllerian hormone; DSD, disorder of sex development; NR5A1, nuclear receptor subfamily 5, group A, member 1; POI, primary ovarian insufficiency; SF1, steroidogenic factor 1.

* Address correspondence and reprint requests to Dr. Alejandro Martínez, División of Pediatric, Pontificia Universidad Católica, Diagonal Paraguay 362, Santiago, Región Metropolitana 8330077, Chile
E-mail address: alemarti@med.puc.cl (A. Martinez).

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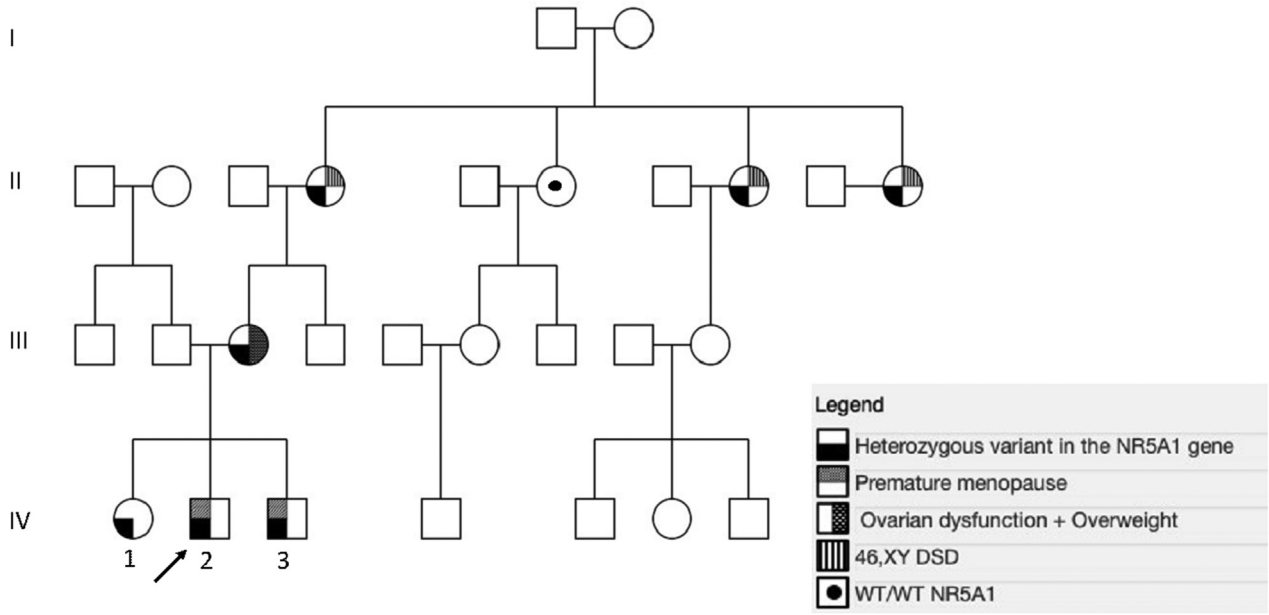

Fig. Pedigree family 1.

formation and survival of gonadal progenitor Sertoli and Leydig cells.[6,7]

Consistent with the multiple roles of SF1 in both testicular development and steroidogenesis, variants in *NR5A1* have been associated with a diverse and ever-growing spectrum of 46,XY DSD phenotypes. These include gonadal and testicular dysgenesis with or without Müllerian remnants, ambiguous genitalia, mild and severe forms of hypospadias, as well as varying degrees of undervirilization, such as micropenis and cryptorchidism.[6,7]

In 46,XX subjects, SF1 expression occurs during early ovarian development and sex differentiation, leading to normal ovarian morphogenesis. In granulosa cells, its expression is associated with follicular development, and in theca cells, it regulates ovarian steroidogenesis. Accordingly, alterations in SF1 expression have been demonstrated as a cause of primary ovarian insufficiency (POI) in women.[8]

We describe the clinical and genetic findings in 2 brothers and in another unrelated subject with 46,XY DSD with the same pathogenic variant in the SF1 gene, as well as in the female relatives.

Clinical Cases

Family 1

The index case (IV2) was a 4-year-old 46,XY boy with DSD, the second child of nonconsanguineous parents. On physical examination, he presented with an external masculinization scale score of 5/12[8] (labioscrotal fusion = 3, micropenis = 0, urethral meatus = 0, right gonad = 1, left gonad = 1), and a pelvic ultrasound showed Müllerian remnants. The concomitant hormonal profile was as follows: testosterone level of <2.5 ng/mL (0.2-13), luteinizing hormone level of <0.1 mIU/mL (0.02-0.3), follicle-stimulating hormone level of 0.8 mIU/mL (0.26-3), inhibin B level of 20.3 pg/mL (4-352), and AMH level of 16 ng/mL (32.7-262). His 46,XY brother (IV3) was born with atypical genitalia and an external masculinization scale score of 5/12 (labioscrotal fusion = 3, micropenis = 0, urethral meatus = 0, right gonad = 1, left gonad = 1), and his pelvic ultrasound showed no Müllerian remnants. The hormonal profile at 3 days of life was as follows: cortisol level of 19.9 μg/dL (>10), testosterone level of 82.2 ng/dL (75-400), and androstenedione level of 272 ng/dL (10-279).

The genetic study of a DSD panel (*AR, DHH, MAP3K1, NROB1, SRD5A2, SRY, WT1,* and *NR5A1*) showed a heterozygous variant in *NR5A1* c.251G>A (p.Arg84His, NM_004959.5), which has been previously described (rs375469069). This variant was also present in the affected brother as well as in his mother, sister, grandmother, and 3 of 4 great aunts. The mother was evaluated at the age of 32 years because she experienced secondary amenorrhea for 6 months. Her hormonal profile at that time was estradiol level of 91.7 pg/mL (12.4-233), AMH level of 5.33 ng/mL (0.7-7.5), luteinizing hormone level of 14.5 mIU/mL (2.4-12.6), and follicle-stimulating hormone level of 8.5 mIU/mL (3.5-12.5), which did not suggest premature ovarian failure, and she was considered to have ovarian dysfunction due to obesity and insulin resistance. The grandmother and 2 maternal great aunts had premature menopause at approximately 30 years, suggesting POI (Fig.).

Family 2

The index case was a 16-week-old 46,XY infant with DSD who was born with a micropenis, an external masculinization scale score of 9/12 (labioscrotal fusion = 3, micropenis = 0, urethral meatus = 3, right gonad = 1.5, left gonad = 1.5) and without Müllerian remnants. His concomitant hormonal profile showed the following results: cortisol level of 17.5 μg/dL (>10), testosterone level of 150 ng/dL (75-400), luteinizing hormone level of 8.8 mIU/mL (0.02-7), follicle-stimulating hormone level of 9.2 mIU/mL (0.16-4.1), and AMH level of 31 ng/mL (32.7-262). Analysis of the DSD panel showed the same pathogenic variant in *NR5A1* (p.Arg84His). This variant was found neither in the father nor in the mother, maternal grandmother, or maternal aunt.

Table
Clinical Manifestations in Patients With the Variant p. Arg84His in Steroidogenic Factor 1

Findings	First case 2008[10]	Second case 2018[11]	Third case 2018[11]	Family 1 (IV2)	Family 1 (IV3)	Family 2 index case
Karyotype	46,XY	46,XY	46,XY	46,XY	46,XY	46,XY
Age at the time of diagnosis	17 y	NA	NA	4 y	1 y	4 mo
Sex of rearing	Female	Female	Female	Male	Male	Male
Country of recruitment	Germany	Indonesia	Indonesia	Chile	Chile	Chile
Phenotype of external genitalia	Clitoromegaly urogenital sinus	Clitoromegaly vaginal pouch	Clitoromegaly 1 perianal opening	Proximal hypospadias micropenis	Micropenis proximal hypospadias	Webbed penis micropenis
Gonadal location	Inguinal bilateral	Inguinal bilateral	Inguinal bilateral	Inguinal bilateral	Inguinal bilateral	Scrotal
Müllerian structures	No	NA	No	Yes	No	No
Gonads	Bilateral testes	NA	Partial gonadal dysgenesis	NA	NA	NA
Basal follicle-stimulating hormone/ luteinizing hormone	NA	Elevated	Elevated	Normal	NA	Normal
Basal testosterone/post chorionic gonadotropin hormone	Normal/response	Elevated/NA	Decreased/no response	Normal/NA	Normal/NA	Normal/NA
Anti-Müllerian hormone	NA	NA	Decreased	Decreased	NA	Decreased
Adrenal function	Normal	Normal	Normal	Normal	Normal	Normal
Inheritance pattern mother/father	Normal/NA	NA	NA	Carrier/ normal	Carrier/normal	Normal/normal

Abbreviation: NA = not analyzed.

Discussion

SF1 is a transcription factor that regulates gonadal differentiation and determination and can cause DSD when altered. This study reports three 46,XY subjects with different degrees of subvirilization harboring the same pathogenic variant (p.Arg84His) in *NR5A1*. Older female relatives with this variant reported premature menopause, suggesting POI. To our knowledge, these are the first reported 46,XY Chilean patients with DSD due to alterations of SF1.

This missense variant has been previously categorized as likely pathogenic in subjects with DSD[7] based on functional studies demonstrating a reduction in the ability of SF1 to activate SRY-box transcription factor 9 expression[6] on very low frequency in large databases and on in silico analysis, supporting a damaging effect on the protein. We also observed the segregation of this variant with under-virilization in 46,XY patients and with premature menarche in 46,XX subjects in 1 family, providing evidence for considering this variant as pathogenic based on the recommendations for variant classification.[9]

This variant, p.Arg82His, initially described in 2008[10] and then in 2 more cases in 2018,[11] was associated with different degrees of under-virilization, similar to that observed in our subjects (Table). In the index case (IV2) of family 1, atypical genitalia, which is the most frequent presentation associated with alterations in SF1, was observed. However, 1 patient showed Müllerian remnants, whose presence has been reported in only 24% of subjects with SF1 variants.[12] The pathogenic mechanism for the persistence of Müllerian remnants could be explained by the reduced expression of SRY-box transcription factor 9 that fails to promote gonadal determination and the differentiation of Sertoli cells and a deficient SF1 interaction in Sertoli cells that results in decreased anti-Müllerian hormone expression, which is important for the involution of Müllerian remnants.[6,7] To date, more than 160 pathogenic variants have been described in this gene,[13] but there is no clear correlation among the variant location, functional performance in vitro, and associated phenotype. Despite having the same pathogenic variant in *NR5A1* there is no clear explanation for the phenotypic heterogeneity observed in our patients, as in the cases of other variants reported. As other authors have hypothesized, this could be explained by epigenetic changes, environmental stimuli, or sequence variants in other genes.[13] Because SF1 has multiple roles in gonadal determination and also in gonadal and adrenal steroidogenesis, the resulting phenotypes when SF1 expression is altered can be difficult to categorize, either as gonadal dysgenesis or alterations in steroidogenesis, even if the same variant is present.

The majority of 46,XY subjects with DSD due to *NR5A1* variants are affected by a loss-of-function variant on 1 allele of the gene, leading to haploinsufficiency and the resulting phenotype.[7] They present most frequently de novo, as in our second family; however, in approximately 30% of children, the variant is dominantly inherited from the mother with sex-dependent traits, as in the first family.

In the 46,XX subjects of family 1, the variant p.Arg84His was found to segregate with premature menopause in the grandmother and maternal great aunts; the mother presented amenorrhea at age 32 but normal hormonal results. According to the Human Gene Variant Database, 22 variants in the SF1 gene are associated with POI.[14,15] In a recent report, also milder phenotypes characterized by decreased ovarian reserve in women with *NR5A1* variants have been described,[16] which suggests that mutations in this gene can cause a continuum of ovarian deficiency.

Because variants in the SF1 gene result in a variable phenotype and in the risk of premature ovarian failure, a genetic study that allows an integral diagnosis and genetic counseling for the index case and their families is important.

Conclusion

The c.251G>A (p.Arg84His) variant in the gene encoding SF1 is associated with different degrees of under-virilization in males with DSD and in women with premature menopause and diminished ovarian reserve. Because it segregates with phenotypic manifestations, this variant can be reclassified as pathogenic.

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Disclosure

The authors have no multiplicity of interest to disclose.

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