Case Report
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The rare case of 46,XX testicular disorder of sex development carrying a heterozygous p.Arg92Trp variant in *NR5A1*

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The 46,XX testicular disorder of sex development (DSD) is a rare condition in which 46,XX individuals develop testicular differentiation and virilization. Translocation of the sex-determining region Y (*SRY*) onto the X chromosome is the main cause of 46,XX testicular DSD, whereas dysregulation between pro-testis and pro-ovarian genes can induce *SRY*-negative 46,XX testicular DSD. Nuclear receptor subfamily 5 group A member 1 (*NR5A1*), a nuclear receptor transcription factor, plays an essential role in gonadal development in XY and XX embryos. Herein, we report the first Korean case of *SRY*-negative 46,XX testicular DSD with a heterozygous *NR5A1* p.Arg92Trp variant. The patient presented with a small penis, bifid scrotum, and bilateral undescended testes. Whole exome sequencing revealed a heterozygous missense variant (c.274C>T) of *NR5A1*. Our case highlights that *NR5A1* gene variants need to be considered important causative factors of *SRY*-negative non-syndromic 46,XX testicular DSD.

Key words: Steroidogenic factor 1, 46,XX testicular disorders of sex development, Disorders of sex development.

Introduction

Human sex differentiation from bipotential gonads to testes or ovaries is a complex process involving the interaction of several genes [1,2]. In XY embryos, the expression of sex-determining region Y (*SRY*) initiates a cascade of testicular formation pathways via the upregulation of SRY-box-9 (*SOX9*), followed by other *SOX* family members, including SRY-box-3 (*SOX3*), SRY-box-8 (SOX8), and SRY-box-10 (*SOX10*) [1-3]. In XX embryos, in the absence of *SRY*, pro-ovarian factors, such as Wnt family member 4 (*WNT4*) and R-spondin 1 (*RSPO1*) stabilize β-catenin and antagonize *SOX9*, resulting in ovarian differentiation [2,3].

46,XX testicular or ovotesticular disorders of sex development (DSD) is a rare condition with a prevalence of approximately 1 per 20,000 newborn males [4]. Approximately 80-90% of these cases are caused by translocation of the *SRY* allele onto the X chromosome, known as *SRY*-positive [5]. Testicular development may also occur in *SRY*-negative conditions and is caused by increased expression of pro-testis genes or insufficient expression of pro-ovarian genes [1,3,6]. *SRY*-negative (ovo)testicular DSD has been reported in XX individuals with duplications of *SOX3*, *SOX9*, and *SOX10* [2]. In addition, loss-of-function variants of *WNT4* and *RSPO1* have been identified in a few individuals with syndromic 46,XX (ovo)testicular DSD [2].

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Nuclear receptor subfamily 5 group A member 1 (*NR5A1*), also known as steroidogenic factor-1 (SF1), is a nuclear receptor transcription factor that regulates adrenal and reproductive development [7]. NR5A1 mediates the initial step of gonadal development in both XY and XX embryos [2,7]. It upregulates *SOX9* synergistically with SRY in XY embryos, and induces *WNT4* and *RSPO1* expression in XX embryos [2-4]. Loss-of-function variants of *NR5A1* have been characterized in 46,XY DSD, gonadal dysgenesis and 46,XX primary ovarian insufficiency [2,3,7]. The case of 46,XY female who carried a missense variant of c.205C>G; p.R68G in *NR5A1* has been reported in Korea [8]. Recently, six reports have described a missense variant of *NR5A1* (c.274C>T; p.Arg92Trp) as the cause of 46,XX (ovo)testicular DSD [9-14] in 15 patients. Additional *NR5A1* variants, c.275G>A; p.Arg92Gln and c.779C>T; p.Ala260Val, have also been reported in 46,XX individuals with (ovo)testicular DSD [12,15].

Herein, we report the rare Korean case of 46,XX testicular DSD with a heterozygous *NR5A1* p.Arg92Trp variant, which presented with a small penis, bifid scrotum, and bilateral undescended testes.

Case

1. Subject

A 27-month-old male was referred to the Seoul National University Hospital for evaluation of microphallus and small testicular size. He was the first child in dichorionic diamniotic twins of non-consanguineous healthy parents and was born through *in vitro* fertilization. On physical examination, his height, weight, and head circumference were 84.6 cm (6th percentile), 11 kg (10th percentile), and 44 cm (<3rd percentile), respectively. The patient had normal developmental milestones. He presented with male-type external genitalia with a micropenis, bifid scrotum, and bilateral undescended testes. Pelvic ultrasonography and magnetic resonance imaging showed bilateral normally shaped testes and epididymis in both supra-scrotal areas, and no uterus, ovary, or vagina was detected (Fig. 1). His gonadotropin levels were comparable to those of age- and sex-matched healthy controls: luteinizing hormone 0.07 mIU/mL and follicle stimulating hormone 0.03 mIU/mL. A proper testosterone response was observed after three days of stimulation with human chorionic gonadotropin (basal 0.02 ng/dL, peak 0.23 ng/dL). He underwent bilateral scrotal orchiopexy and scrotoplasty at the age of 2 years and 5 months. He also underwent testosterone replacement because of a small penis immediately after surgery (once at 4.8 years old) and later during childhood (four times during 8.1 and 9.0 years old) at the pediatric urology clinic. At the latest visit (at 9.2 years old), he showed an adequately sized penis, with a stretched penile length of 5 cm.

2. Cytogenetic and molecular genetic analysis

The research protocol was approved by the Institutional Review Board of Seoul National University Hospital (IRB no. 2206-190-1336). Written informed consent for genetic analysis was obtained from the parents of the patient.

Chromosome analysis of peripheral blood leukocytes revealed a 46,XX karyotype. Fluorescence *in situ* hybridization with an *SRY*-specific probe excluded *SRY* translocations on one X chromosome. Duplication of *SOX9* was ruled out using real-time PCR assay. After a diagnosis of 46,XX (*SRY*-negative) testicular DSD, chromosome analysis of peripheral blood leukocytes from his younger twin sibling with a female phenotype was performed and revealed a normal female karyotype (46,XX). Considering the possible confined blood chimerism that can occur

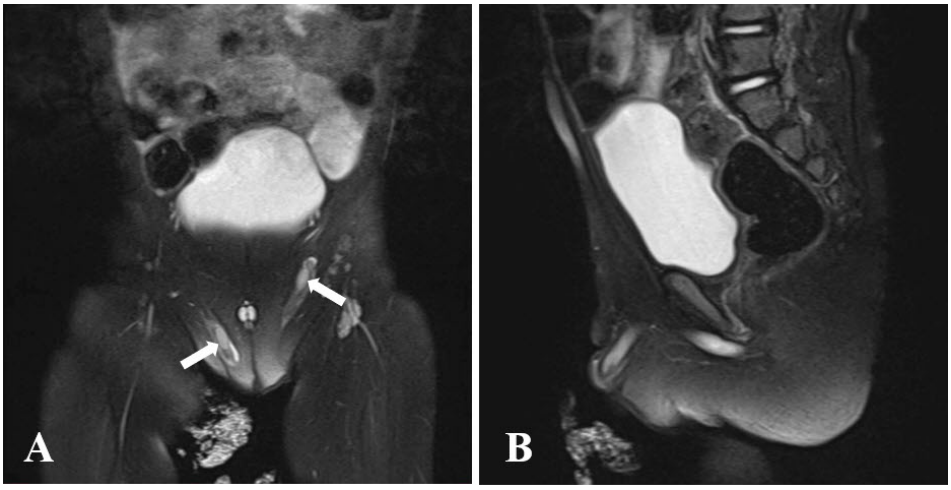

Fig. 1. T2-weighted magnetic resonance imaging of the pelvis. Coronal (A) and sagittal (B) images showing bilateral normally shaped testes (white arrows) and epididymis in both supra-scrotal areas without any evidence of female genital organs.

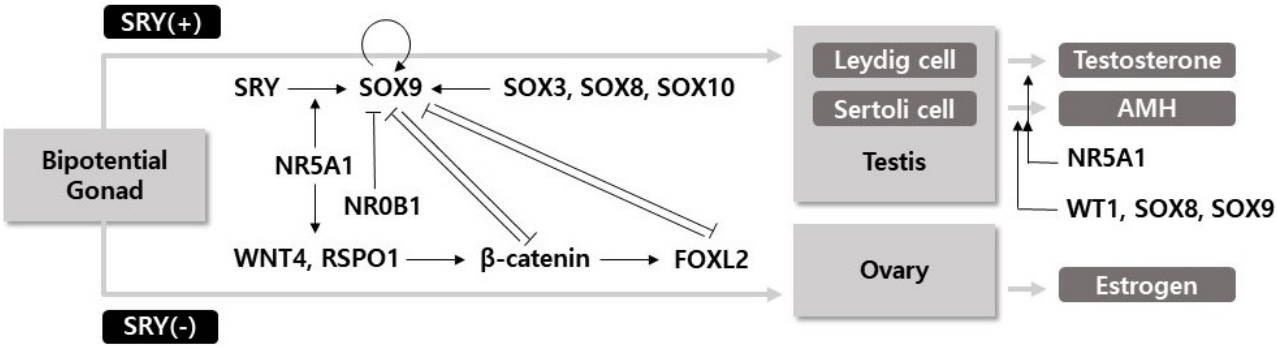

Fig. 2. Genetic pathway of human sex differentiation. The bipotential gonad may differentiate into the testis or ovary, depending on the delicately balanced expression of the genes involved in testicular and ovarian differentiation. *SRY* upregulates *SOX9* and stimulates testis-specific pathways subsequently. In the absence of *SRY*, ovary-specific genes, such as *WNT4* and *RSPO1*, stimulate downstream signaling pathways via β-catenin and FOXL2. *SRY*, sex-determining region Y; *NR5A1*, Nuclear receptor subfamily 5 group A member 1; AMH, anti-mullerian hormone.

in diamniotic twins sharing a common placenta [16], short tandem repeat (STR) polymorphism analysis was performed. Skin fibroblasts of the proband and blood leukocytes from younger siblings and both parents were used to confirm the dizygosity of the twins. The twins were determined as dizygotic because of the nine discordant genotypes among the 16 STR markers. In the proband, karyotyping using skin fibroblasts also revealed 46,XX, and the patterns of all 16 STR markers were identical between the skin and peripheral blood samples, ruling out the possibility of mosaicism.

Whole exome sequencing was subsequently performed, and a previously reported heterozygous missense variant of *NR5A1* (c.274C>T, p.Arg92Trp) was identified as the best candidate for the patient's phenotype (likely pathogenic, PM1+PM2+PP3+PP4+PP5). There were no other pathogenic or likely pathogenic variants of sex differentiation-related genes, including *SRY*, *SOX3, SOX9*, and *DAX1*. Genetic analysis was also performed on his parents, and the parents did not harbor this variant, indicating his variant as *de novo*.

Discussion

Human gonads are bipotential until about six weeks of gestation, then differentiate into testes or ovaries [1,2]. In developing testes, *SRY* leads to the upregulation of *SOX9* and subsequent stimulation of testis-specific pathways. During normal ovarian development, ovary-specific genes, such as *RSPO1* and *WNT4*, modulate ovary-specific signaling pathways via β-catenin by antagonizing *SOX9*, and *NR0B1* represses the *NR5A1*-induced transactivation of *SOX9* (Fig. 2) [1-3]. Dysregulation of these delicately balanced processes of testicular and ovarian differentiation can induce 46,XX (ovo)testicular DSD [4,10]. In the absence of *SRY*, gain-of-function variants of pro-testicular genes, such as *SOX3*, *SOX9*, and *SOX10*, or loss-of-function variants of pro-ovarian genes, such as *RSPO1, WNT4*, and *NR0B1*, can lead to *SRY*-negative 46,XX sex reversal [1,10].

NR5A1 plays an essential role in both male and female gonadal development and maintenance by upregulating *SOX9* in the XY embryo, while upregulating pro-ovarian factors such as *WNT4* and *RSPO1*, and anti-testis factors such as *NR0B1* in the XX embryo [1-3]. To date, over 80 *NR5A1* variants have been reported to be associated with various gonadal development disorders, ranging from primary ovarian failure in 46,XX individuals to (ovo)testicular 46,XX DSD and oligospermia in 46,XY individuals [7,17]. The p.Arg92Trp variant observed in our study has been previously reported in 15 patients from 13 families with 46,XX (ovo)testicular DSD (Table 1). A recurrent heterozygous p.Arg92Trp variant of *NR5A1* was first reported in six individuals from four unrelated families of different ancestries who presented with ambiguous genitalia of variable degrees [9]. Five of them had 46,XX karyotype, but a sibling in one family raised as a girl was found to have 46,XY partial gonadal dysgenesis [9]. Another study reported three unrelated Caucasian patients with 46,XX (ovo)testicular DSD; two inherited *NR5A1* p.Arg92Trp variant from their unaffected mothers, suggesting incomplete penetrance [10]. The same *NR5A1* variant has been reported in two unrelated Japanese males with 46,XX (ovo)testicular DSD [11,13] and an Iranian infertile male with 46,XX testicular DSD [14]. An additional *NR5A1* variant, c.779C>T; p.Ala260Val, has been reported in a patient with 46,XX ovotesticular DSD [12]. A paternally inherited *NR5A1* c.275G>A; p.Arg92Gln variant in the same codon as c.274C>T; p.Arg92Trp variant, has also been reported in a patient with 46,XX ovotesticular DSD [15].

Several studies have suggested that the *NR5A1* p.Arg92Trp

Table 1. Phenotypic features of individuals with 46,XX (ovo)testicular DSD caused by *NR5A1* variant

Case	Karyotype	*SRY*	*NR5A1*	Sex of rearing	Presentation	Gonad position	Gonad histology	Uterus	Diagnosis	Reference
1	46,XX	–	R92W	M	Micropenis, retractile testes	Inguinal	NA	No uterus	T-DSD	This report
2-1	46,XX	–	R92W	F	Ambiguous genitalia	L: inguinoscrotal, R: inguinal	L: dysgenetic testis, R: ovotestis	Hemiuterus	OvT-DSD	9
2-2	46,XX	–	R92W	F	Ambiguous genitalia	Intra-abdominal	Bilateral ovotestes	Uterus	OvT-DSD	9
3	46,XX	–	R92W	M	Micropenis, retractile testes	Scrotal (retractile)	NA	No uterus	T-DSD	9
4	46,XX	–	R92W	M	Small testes	Scrotal	NA	No uterus	T-DSD	9
5-1	46,XY	+	R92W	F	Clitoromegaly, labial fusion	Inguinal	Dysgenetic testes	No uterus	Partial gonadal dysgenesis	9
5-2	46,XX	–	R92W	M	Penoscrotal hypospadias	Scrotal	Dysgenetic testes	No uterus	T-DSD	9
6	46,XX	–	R92W	F	Mild clitoris hypertrophy	Inguinal	L: infantile testis, R: fibrous streak	Hemiuterus	OvT-DSD	10
7	46,XX	–	R92W	F	Ambiguous genitalia	NA	Bilateral ovotestes	NA	OvT-DSD	10
8	46,XX	–	R92W	M	Micropenis, hypospadias	NA	Bilateral infantile testes	NA	T-DSD	10
9	46,XX	–	R92W	F	Ambiguous genitalia	NA	L: ovotestis, R: testis-like	No uterus	OvT-DSD	11
10	46,XX	–	R92W	M	Hypospadias, bifid scrotum	NA	Bilateral testes	No uterus	T-DSD	11, 13
11	46,XX	–	R92W	M	Micropenis, hypospadias	Scrotal	Bilateral testes	No uterus	T-DSD	12
12	46,XX	–	R92W	F	Micropenis	Inguinal	Bilateral ovotestes	No uterus	OvT-DSD	12
13	46,XX	–	R92W	M	Micropenis, hypospadias	Scrotal	NA	No uterus	T-DSD	12
14	46,XX	–	A260V	F	Micropenis, vaginal opening	Intra-abdominal	L: ovary, R: ovotestis	Uterus	OvT-DSD	12
15	46,XX	–	R92W	M	Infertility, hypospadias	Scrotal	NA	NA	T-DSD	14
16	46,XX	–	R92Q	F	Ambiguous genitalia	Intra-abdominal	Bilateral ovotestes	Uterus	OvT-DSD	15

DSD, disorders of sex development; *NR5A1*, Nuclear receptor subfamily 5 group A member 1; *SRY*, sex-determining region Y; M, male; F, female; L, left; R, right; NA, not available; T-DSD, testicular DSD; OvT-DSD, ovotesticular DSD.

variant activates testis development in the XX background. Human *NR5A1* is expressed in ovarian somatic cells and can regulate cell fate at an early stage [9]. *In silico* analysis predicted that the *NR5A1* p.Arg92Trp variant affects a highly conserved amino acid in the RGGR motif of the A-box, involving DNA-binding specificity and stability [9-11]. The *NR5A1* p.Arg92Trp variant showed reduced ability to upregulate *SOX9* and reduced synergy with β-catenin, resulting in reduced activation of the pro-ovary canonical WNT/RSPO1 signaling pathway [9,10]. *In vitro* assays demonstrated that the *NR5A1* p.Arg92Trp variant was less reactive to *NR0B1* [11]. In addition, the *NR5A1* variant caused increased repression of ovarian-specific WNT/β-catenin signaling, resulting in reduced *NR0B1* expression and decreased testis pathway inhibition [12]. Although the exact pathophysiology of *NR5A1* variants causing 46,XX sex reversal has not been clearly elucidated, these findings provide evidence that *NR5A1* is involved in sex differentiation and its variants lead to testis differentiation in the XX gonad.

The *NR5A1* gene variants are also known to be related to primary adrenal insufficiency with or without sex reversal [18,19]. Adrenal insufficiency without sex reversal is extremely rare, and in only one report, a 46,XX girl with primary adrenal insufficiency has been reported to have a heterozygous *NR5A1* p.R255L variant [20]. Accompanying adrenal insufficiency in *NR5A1* variants also has been reported in 46,XY DSD, while not yet in 46,XX DSD [18,19]. Our case patient also had no symptom or sign of adrenal insufficiency, and we did not perform an ACTH stimulation test.

To the best of our knowledge, this is the first report of a Korean patient with 46,XX testicular DSD caused by a heterozygous *NR5A1* p.Arg92Trp variant, although there has been a previous case report of 46,XY DSD with *NR5A1* variant in Korea [8]. Our case report can be additional evidence that *NR5A1* is involved in a series of gonadal development processes from bipotential gonads. *NR5A1* gene variants need to be considered as important causative factors of *SRY*-negative non-syndromic 46,XX testicular DSD. Further investigations are necessary to demonstrate the exact molecular mechanism of *NR5A1* variants in the development of 46,XX (ovo)testicular DSD.

Authors' Contributions

Conception and design: JMK. Acquisition of data: LK, HYK. Analysis and interpretation of data: LK, HYK. Drafting the article: LK. Critical revision of the article: HYK, JMK. Final approval of the version to be published: all authors.

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