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Phenotypic spectrum and long-term outcomes of patients with 46,XX disorders of sex development

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Purpose: 46,XX disorders of sex development (DSD) involve atypical genitalia accompanied by a normal female karyotype. This study was performed to investigate the clinical characteristics and long-term outcomes of patients with 46,XX DSD.
Methods: The study included 34 patients with 46,XX DSD who presented with ambiguous genitalia or delayed puberty. Patients with congenital adrenal hyperplasia were excluded. Clinical phenotypes and overall outcomes were analyzed retrospectively.
Results: Age at presentation ranged from birth to 40 years (median, 0.6 years), and the follow-up period ranged from 0.3 to 29.7 years (median, 8.8 years). Twenty patients were assigned female (58.8%). Etiologies included disorders of gonadal development (n=22), exogenous androgen exposure during pregnancy (n=5), association with syndromic disorders or genital anomalies (n=2), and unclassified causes (n=5). Ovotestis was the most frequent gonadal pathology (41.7%). Müllerian duct remnants were usually underdeveloped (52.9%) or absent (23.5%). Spontaneous puberty occurred in 17 of the 21 patients of pubertal age, while 9 patients required sex hormone replacement therapy. Gonadal complications were observed in 4 patients (gonadal tumors [n=3], and spontaneous gonadal rupture [n=1]), and gender dysphoria occurred in 1 patient who was assigned male.
Conclusion: This study described the wide phenotypic spectrum and pubertal outcome of patients with 46,XX DSD. Long-term multidisciplinary monitoring for pubertal development, fertility, gender identity, and gonadal complications is recommended.

Keywords: Ambiguous genitalia, Disorder of sex development, Gonadal dysgenesis, Ovotestis

Highlights

- 46,XX disorders of sex development (DSD) are rare and caused by a diverse spectrum of genetic and phenotypic factors.
- This study described the diverse clinical phenotypes and long-term outcomes of 34 patients with 46,XX DSD.
- Multidisciplinary approaches and regular follow-up are recommended for pubertal development, fertility, gender identity, and gonadal complications.

Introduction

Disorders of sex development (DSD) are a spectrum of conditions that present with

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ambiguous genitalia in patients with atypical chromosomal, gonadal, or anatomical sex [1]. DSD can be classified as 46,XY DSD, 46,XX DSD, or sex chromosomal DSD [1]. 46,XX DSD involve atypical genitalia accompanied by a normal female karyotype (46,XX). The incidence of all genital anomalies, including cryptorchidism and hypospadias, is estimated to be one in 200–300 live births [2]. In contrast, the prevalence of 46,XX DSD is 3.5–4.7 per 100,000 newborns, and the incidence of males with 46,XX DSD is 0.4 per 1,000,000 live births; however, the exact frequency of specific DSDs remains unclear [2,3]. Given that specific DSD involve gonadal disorders or require corrective surgery, it is crucial to ascertain the exact etiology and assign the appropriate sex.

Several studies have examined the etiological spectrum and clinical outcomes in patients with 46,XX DSD [3-6]. 46,XX DSD can result from disorders of gonadal development, such as 46,XX testicular DSD (T-DSD) and ovotesticular DSD (OT-DSD), and other syndromic disorders involving Müllerian duct remnants [6]. The most common cause of 46,XX DSD is congenital adrenal hyperplasia (CAH), which accounts for more than 90% of cases [6]. Other major causes of 46,XX DSD are aromatase insufficiency, androgen-secreting tumors, and maternal source of androgens [6]. At the molecular level, environmental factors and defects in many genes involved in gonadal and/or genital development have been implicated as causes of 46,XX DSD [7].

Given the wide phenotypic spectrum and diverse etiology of 46,XX DSD, diagnosis at the molecular level remains challenging, despite recent advances in next-generation sequencing (NGS) technologies that have facilitated the elucidation of molecular etiologies of DSD [7]. Because whole-exome sequencing (WES) and whole-genome sequencing (WGS) are expensive and time-consuming, targeted gene panels have been widely used for molecular genetic diagnosis [7], and several such panels have been proposed for DSD, with diagnostic yields ranging from 20% to 45% [8-10]. Recent studies using targeted gene panel sequencing identified pathogenic or likely pathogenic variants in 38%–43% of patients with 46,XY DSD; however, diagnostic efficiency is typically lower in patients with 46,XX DSD [8-11]. Conducting genetic studies of 46,XX DSD has proven to be challenging because of the rarity of this condition and the technical limitations of current NGS panels [11].

Determining the precise genetic etiologies of 46,XX DSD is crucial to establish strategies for sex assignment and predict long-term outcomes, including pubertal development, gender identity, potential fertility, and the risk of gonadal malignancies. However, the genetic etiologies of 46,XX DSD and the long-term outcomes of patients with 46,XX DSD remain unclear. Therefore, we performed this study to elucidate the clinical characteristics, etiological spectrum, and long-term outcomes of patients with 46,XX DSD.

Materials and methods

1. Subjects

This study included 34 Korean patients with 46,XX DSD diagnosed between December 1997 and April 2022 at the Department of Pediatrics, Asan Medical Center, Seoul, Korea. Patients who presented with ambiguous genitalia during the neonatal period or delayed puberty in adolescence were enrolled. Patients with CAH were excluded because the pathophysiology, clinical features, and outcomes of such patients are relatively well known. Ten previously reported patients with 46,XX DSD in our group were included to delineate the clinical and genetic spectra of the study cohort [9,12]. For patients who agreed to undergo molecular genetic analysis, WES and/or WGS was performed after obtaining written informed consent from patients or their guardians.

2. Clinical and endocrinological characteristics

The following parameters were retrospectively analyzed by medical chart review: presenting features, age at presentation, sex of rearing, presence of internal genitalia, shape of external genitalia, endocrine findings, pathological findings of gonads, spontaneous puberty, and surgical or hormonal interventions.

External genitalia were assessed using the external masculinization score (EMS) and Prader classification [13,14]. Micropenis was defined as a stretched penile length of <2.5 cm in a full-term newborn [15]. Internal genitalia were assessed using ultrasonography (US), magnetic resonance imaging, or laparoscopic findings.

Endocrinological findings included luteinizing hormone, follicle stimulating hormone, testosterone, dihydrotestosterone, and estradiol levels. A human chorionic gonadotropin (hCG) stimulation test was performed to assess the presence of functioning Leydig cells. Serum testosterone and dihydrotestosterone levels were measured at baseline and on the fourth day after intramuscular injections of hCG (IVF-C, LG Chemistry, Seoul, Korea) for 3 consecutive days at a dose of 1,000 units/day. The hCG stimulation test result was deemed positive when the serum testosterone level increased to more than twice the baseline level [14].

3. Molecular genetic testing

A targeted gene panel for DSD (n=2), WES (n=6), or WGS (n=5) was performed for select patients who agreed to undergo genetic testing. In this group, WGS was performed for 3 patients when no sequence variants were found in WES.

Targeted gene panel sequencing was performed using a customized panel, which included 67 genes previously reported to be associated with DSD in humans, as previously described [9].

For WES, SureSelect Human All Exon V5 (Agilent

Technologies, Santa Clara, CA, USA) or MGI Easy Exome (MGI Tech Co., San Jose, CA, USA) was used to construct the library. Sequencing was performed using the MGI DNBSEQ-G400 or MGI DNBSEQ-T7 platform (MGI Tech Co.). For WGS, the MGI Easy FS DNA Library Prep Set (MGI Tech Co.) was used to prepare the library, and sequencing was performed on the MGI DNBSEQ-T7 platform (MGI Tech Co.). Sequenced reads were aligned to the human reference genome (hg19) using the Burrow-Wheeler Alignment program (ver. 0.7.17). Variant calling was performed with the Genome Analysis Tool kit (GATK ver. 4.1.8), and annotation was conducted with the Variant Effect Predictor [16]. Sequence variants were classified according to the standards and guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [17].

4. Ethical statement

This study was approved by the Institutional Review Board (IRB) of Asan Medical Center, Seoul, Korea (IRB number: 2022-2440).

Results

1. Clinical and endocrinological characteristics at presentation

Median age at presentation was 0.6 years (range, birth to 40 years), and median duration of follow-up was 8.8 years (range, 0.3–29.7 years) (Table 1). Twenty patients (58.8%) were assigned female, and the other 14 patients (41.2%) were raised as male. Sixteen of the female-assigned patients presented during the

Table 1. Clinical characteristics at presentation

Characteristics	Total	Male-assigned	Female-assigned
No. of patients (%)	34 (100)	14 (41)	20 (59)
Age at presentation (yr)	0.6 (0–40)	0.9 (0–40)	0.3 (0–17)
Follow-up duration (yr)	8.8 (0.3–29.7)	15.8 (0.3–29.7)	6.8 (3.7–26.7)
Palpable gonad	20 (59)	13 (93)	7 (35)
EMS score	3.7±3.56 (0–12)	7.1±2.13 (3–12)	2.3±2.7 (0–9)
Prader score	2.8±1.55 (0–6)	4.4±0.82 (4–6)	2.0±1.12 (0–4)
Presenting features		Hypospadias (11, 79%), micropenis (8, 57%), cryptorchidism (6, 43%), bifid or poorly developed scrotum (6, 43%), gynecomastia (2, 14%), azoospermia (2, 14%)	Clitoromegaly (14, 70%), prominent or pigmented labia majora (7, 35%), urogenital anomaly (4, 20%), amenorrhea (4, 20%)
Patients undergoing sex hormone replacement therapy	9 (26)	5 (36)	4 (20)
Age at the start of sex hormone replacement therapy (yr)	17 (13–41)	18 (13–41)	15.5 (13–17)

Values are presented as number (%), median (range), or mean±standard deviation (range).
EMS, external masculinization score.

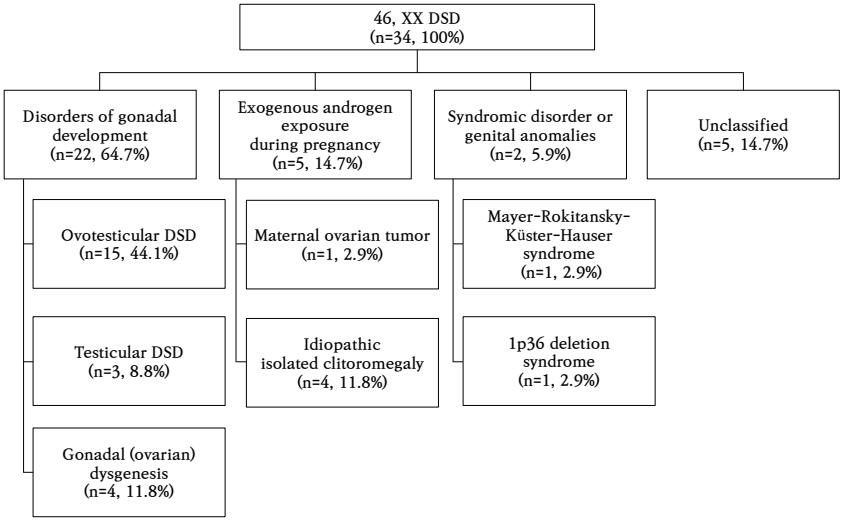

Fig. 1. Etiological classification of patients with 46,XX disorders of sex development (DSD).

Table 2. Clinical features of patients who underwent an hCG stimulation test

No.	Age at presentation	Current age (yr)	Sex of rearing	Prader stage	EMS	Gonads, right/left	Müllerian duct remnants	Testosterone response to hCG stimulation test	Age at hCG stimulation test	Spontaneous puberty	Age at sex hormone replacement	Diagnosis
1	0 Mo	23	M	4	9	ImT/O	Yes	Yes	12 Yr	(-)	18 Yr	OT-DSD
3	0 Mo	14	F	3	7.5	OT/O	Yes	Yes	1 Mo	(+)	ND	OT-DSD
5	1 Mo	11	F	3	7	OT/O	No	Yes	6 Mo	PP	ND	OT-DSD
7	1 Mo	5	F	3	1	O/OT	Yes	Yes	4 Mo	PP	ND	OT-DSD
9	8 Mo	30	M→F	4	4.5	ImO/ImT	Yes	Yes	8 Mo	(+)	23 Yr	OT-DSD
12	9 Mo	19	F	3	5	OT/O	Yes	Yes	1 Yr	(+)	ND	OT-DSD
13	12 Mo	16	M	4	7.5	O/OT	Yes	Yes	1 Yr	(+)	13 Yr	OT-DSD
14	13 Mo	32	M	4	7	OT/OT	Yes	Yes	1 Yr	(+)	15 Yr	OT-DSD
19	1 Mo	6	M	5	7	NA	Yes	Yes	4 Mo	FUL	FUL	Unclassified
20	9 Mo	30	M	6	9	NA	Yes	Yes	9 Mo	(+)	FUL	Unclassified
21	9 Mo	22	M	4	6	NA	Yes	Yes	4 Mo	(+)	ND	Unclassified
22	3 Yr	23	M	4	6	NA	No	Yes	3 Yr	(+)	FUL	Unclassified
24	13 Yr	18	F	0	0	Not visible	Yes	No	13 Yr	(-)	13 Yr	Gonadal dysgenesis

hCG, human chorionic gonadotropin; EMS, external masculinization score; O, ovary; T, testis; OT, ovotestis; Im, immature; NA, not assessed; ND, not done; PP, prepubertal period; FUL, follow-up loss; OT-DSD, ovotesticular disorder of sex development.

infantile period with clitoromegaly (14 of 20, 70%), prominent labia majora (7 of 20, 35%), and/or a urogenital anomaly (4 of 20, 20%), while the remaining 4 female-assigned patients presented with primary amenorrhea during adolescence (4 of 20, 20%). Meanwhile, 12 of the male-assigned patients presented with hypospadias (11 of 14, 78.6%), micropenis (8 of 14, 57.1%), cryptorchidism (6 of 14, 42.9%), and/or bifid or poorly developed scrotum (6 of 14, 42.9%) during the infantile period, and the remaining 2 male-assigned patients were brought to medical attention during the adult period due to gynecomastia (2 of 14, 14.3%) or infertility with azoospermia (2 of 14, 14.3%). Mean EMSs of the male- and female-assigned patients were 7.1±2.13 and 2.3±2.70, respectively, and mean Prader stages of the male- and female-assigned patients were 4.4±0.82 and 2.0±1.12, respectively.

Gonads were palpable unilaterally or bilaterally in 20 patients (58.8%). Most of the male-assigned patients exhibited palpable gonads (13 of 14, 92.9%), and 7 female-assigned patients (7 of 20, 35%) also displayed palpable gonads unilaterally (n=4) or bilaterally (n=3).

The hCG stimulation test was performed in 13 of 27 patients with disorders of gonadal development or unclassified forms at a median age of 9 months (range, 1 month to 13 years) (Table 2). In this group, all 8 male-assigned patients showed elevated serum testosterone levels compared to baseline. Among the 5 female-assigned patients, 4 with OT-DSD showed a positive response, and all were found to have a unilateral ovary and unilateral ovotestis. However, they were subsequently assigned as female because of penile growth failure during infancy. One patient with ovarian dysgenesis showed a negative response following hCG stimulation.

2. Gonadal pathology and etiological classification

As shown in Fig. 1, 22 patients (64.7%) were found to have disorders of gonadal development: OT-DSD (n=15), T-DSD (n=3), and ovarian dysgenesis (n=4). Four patients were found to have idiopathic isolated clitoromegaly without palpable gonads, and 1 patient had been exposed to excessive androgen during pregnancy due to a maternal Krukenberg tumor. Two patients were found to have a syndromic disorder or genital anomalies; one had Mayer-Rokitansky-Küster-Hauser syndrome and the other had 1p36 deletion syndrome. In 5 patients, it was not possible to determine etiology. These patients had palpable gonads and underdeveloped Müllerian structures on US; however, gonadal biopsies were not performed.

Eighteen patients underwent a gonadal biopsy or gonadectomy at a median age of 2 years (range, 1 month to 40 years) (Table 3). Of these patients, 15 were diagnosed with OT-DSD and 3 were diagnosed with T-DSD. Among the 36 gonads, ovotestis was the most frequent finding (n=15, 41.7%), followed by ovary (n=8, 22.2%), immature testis (n=6, 16.7%), testis (n=4, 11.1%), and immature ovary (n=3, 8.3%).

Internal genital organs of all 34 patients were assessed, and Müllerian structures were detected in 26 cases (76.5%).

Table 3. Location and pathological findings of gonads

No.	Age at presentation	Sex of rearing	Location	Müllerian duct remnants	Age at biopsy	Gonadal pathology (right/left)	Preserved gonad	*SRY*	Diagnosis
1	0 Mo	M	Scrotum/ scrotum	Yes	14 Yr	ImT/O	T	-	OT-DSD
2	0 Mo	F	Inguinal/ abdomen	No	6 Mo	OT/O	O	-	OT-DSD
3	0 Mo	F	Scrotum/ abdomen	Yes	3 Mo	OT/O	O	-	OT-DSD
4	1 Mo	F	Scrotum/ scrotum	Yes	1 Mo	ImO/OT	None	-	OT-DSD
5	1 Mo	F	Inguinal/ abdomen	No	11 Mo	OT/O	O	-	OT-DSD
6	1 Mo	F	Scrotum/ scrotum	Yes	6 Mo	OT/T	None	-	OT-DSD
7	1 Mo	F	Abdomen/ abdomen	Yes	2 Yr	O/OT	O	-	OT-DSD
8	1 Mo	F	Inguinal/ inguinal	No	9 Yr	OT/OT	None	-	OT-DSD
9	1 Mo	M→F	Abdomen/ scrotum	Yes	2 Yr	ImO/ImT	None	-	OT-DSD
10	1 Mo	M	Abdomen/ inguinal	Yes	4 Mo	O/T	T	-	OT-DSD
11	4 Mo	F	Abdomen/ abdomen	No	27 Yr	ImO/OT	None	-	OT-DSD
12	9 Mo	F	Scrotum/ inguinal	Yes	12 Mo	OT/O	O	-	OT-DSD
13	12 Mo	M	Abdomen/ scrotum	Yes	13 Mo	O/OT	T	-	OT-DSD
14	13 Mo	M	Scrotum/ scrotum	Yes	2 Yr	OT/OT	None	-	OT-DSD
15	39 Yr	M	Inguinal/ abdomen	No	40 Yr	OT/OT	None	-	OT-DSD
16	21 Mo	M	Scrotum/ scrotum	Yes	2 Yr	ImT/T	T	-	T-DSD
17	23 Mo	M	Inguinal/ inguinal	No	3 Yr	ImT/T	T	+	T-DSD
18	29 Yr	M	Scrotum/ scrotum	No	29 Yr	ImT/ImT	T	+	T-DSD

O, ovary; T, testis; OT, ovotestis; Im, immature; OT-DSD, ovotesticular disorder of sex development; T-DSD, testicular disorder of sex development.

Müllerian structures were underdeveloped in 18 patients. Normal female internal genital structures were identified in 8 patients: 5 with isolated clitoromegaly, 1 with 1p36 deletion, 1 with 46,XX pure gonadal dysgenesis, and 1 with OT-DSD. There were no Müllerian duct remnants in 3 of the female-assigned patients with OT-DSD and 5 of the male-assigned patients: 3 with a condition with an unclassified etiology and 2 with T-DSD. Urethrovaginal fistulas were observed in 4 patients with OT-DSD.

3. Molecular genetic analysis

The *SRY* gene was detected in 2 of the 3 patients with T-DSD. One patient with intellectual disability and prominent labia majora (patient 33) was diagnosed with 1p36 deletion syndrome by multiple ligation-dependent probe amplification analysis. Targeted gene panel sequencing resulted in the identification of a novel variant of uncertain significance in RSPO1 [c.542A > G (p.H181R)] in a female-assigned patient (patient 11) with OT-DSD and dysgerminoma and in *WWOX* [c.359G > A (p.R120Q)] in a female-assigned patient (patient 26) who presented with primary amenorrhea at the age of 17 years [9]. Among the 8 patients who underwent WES and/or WGS, no pathogenic or likely pathogenic variants were identified.

4. Pubertal development and long-term outcomes

At the time of the study, the median age of the patients was 17 years (range, 4–46 years). Surgery was performed on the external genitalia of 29 of the 34 patients (85.3%) at a median age of 1.5 years (range, 6 months to 40 years) and consisted of clitoroplasty or vulvovaginoplasty (n=17) or urethroplasty with

peno-scrotal repair (n=12). Four patients underwent additional surgeries to treat urethrovaginal fistulas.

Among the 15 patients with OT-DSD, 9 patients (60%) were raised as female and the other 6 (40%) were raised as male. Six patients underwent a bilateral gonadectomy, and 9 patients underwent a unilateral gonadectomy according to their assigned sex. Four patients required additional gonadectomy due to the following gonadal complications: seminoma (patient 9), dysgerminoma (patient 11), mucinous cystadenoma (patient 13), and spontaneous gonadal rupture (patient 14).

One patient with OT-DSD (patient 9) who was initially assigned as male underwent a right oophorectomy and preserved left testis at 2.9 years of age. However, the patient changed his sex to female due to gender dysphoria at 20 years of age and has been treated with estrogen replacement therapy. This patient planned to undergo sex reassignment surgery. After a period of 8 years of follow-up loss, the patient underwent a radical orchiectomy due to the occurrence of a seminoma in the left immature testis. Another patient with OT-DSD (patient 11) was initially diagnosed with Mayer-Rokitansky-Küster-Hauser syndrome and underwent external genitoplasty without gonadal biopsies at 6 months of age and experienced spontaneous puberty. The patient was diagnosed with bilateral dysgerminoma at 26.7 years of age and underwent a bilateral salpingo-oophorectomy; the presence of a right immature ovary and left ovotestis confirmed the diagnosis of OT-DSD.

All 3 patients with T-DSD and 5 patients with conditions with unclassified forms were assigned as male. Four patients with 46,XX gonadal dysgenesis, 5 patients with isolated clitoromegaly, and 2 patients with genital anomalies were assigned as female. All 5 patients with isolated clitoromegaly required clitoroplasty.

Ten female-assigned patients and 11 male-assigned patients reached pubertal age (female > 10 years, male > 12 years), while 2 female-assigned patients were lost to follow-up before puberty. Spontaneous puberty occurred in 17 of the 21 patients (81.0%) (Fig. 2). Four patients who did not undergo spontaneous puberty were treated with sex hormone replacement therapy: 3 female-assigned patients with primary ovarian insufficiency and 1 male-assigned patient with OT-DSD. A further 5 patients were treated with sex hormone replacement therapy due to premature ovarian failure (n=1), lack of virilization (male-assigned OT-DSD; n=3), and sex re-assignment (n=1). Azoospermia was documented in 2 male-assigned patients with T-DSD.

Discussion

This study provides further evidence of the broad clinical phenotypes of patients with 46,XX DSD and describes their long-term outcomes. Among the 34 patients included in this study, most were assigned as female (58.8%), and gender dysphoria was reported in 1 patient with OT-DSD who was initially assigned as male. In terms of genetic etiologies, disorders of gonadal development were the most common causes identified (64.7%); molecular genetic causes were not identified. Gonadal tumors were observed in 3 patients. Spontaneous puberty occurred in the majority of the patients (81.0%), with some requiring sex hormone replacement therapy.

More than 90% of cases of 46,XX DSD are attributable to CAH, which can result in excessive androgen exposure and the subsequent virilization of the external genitalia [5,6]. Maternal malignant tumors (e.g., sex-cord stromal tumors, adrenal cortical carcinomas, cystadenocarcinomas, and hilar cell tumors) and benign tumors (e.g., luteomas, adrenal adenomas, thecomas, Brenner tumors, mature cystic teratomas, and serous cystadenomas) can also induce an excess of fetoplacental androgen [6]. In addition, maternal exposure to androgenic drugs may also induce fetus virilization [6]. It has also been shown that rare syndromic disorders may be associated with multiple deformities, with abnormal genitalia

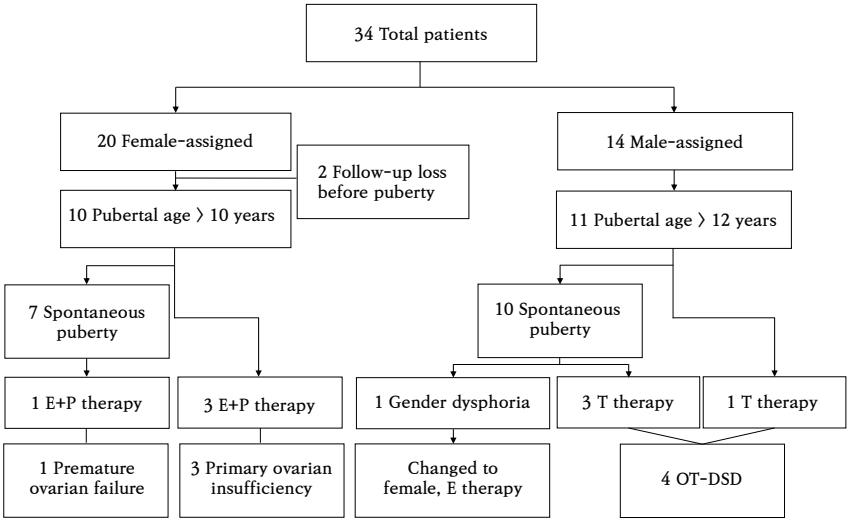

Fig. 2. Pubertal outcomes of the 34 patients included in this study and the hormonal replacement therapies applied. E, estrogen; P, progesterone; T, testosterone; OT-DSD, ovotesticular disorder of sex development.

reported in up to 25% of cases [18]. In the present study, a patient with 1p36 deletion syndrome exhibited prominent labia majora. In previous studies, patients with disorders of gonadal development accounted for 50%–78% of non-CAH 46,XX DSD [19,20], and hypergonadotropic hypogonadism was the main presentation of 46,XX DSD males [4].

Due to the diverse etiologies and heterogeneous clinical phenotypes of 46,XX DSD, it is difficult to establish a definitive cause in most cases despite the availability of comprehensive diagnostic data derived from detailed history taking, physical examinations, radiological findings, hormone testing, gonad biopsies, and NGS. It is vital to perform a gonadal biopsy in cases of suspected gonadal dysgenesis; however, this procedure is not routinely performed because of its invasive nature.

It was recently recommended that NGS be performed during the initial investigations of suspected cases of DSD to obtain a molecular diagnosis [21]. In the present study, the use of both WES and WGS did not result in identification of molecular etiologies of 46,XX DSD, and this finding is consistent with those of previous studies [4,5,21-23]. Approximately 80%–90% of T-DSD cases are attributable to the translocation of *SRY* to the X chromosome [24]. OT-DSD and T-DSD without *SRY* can result from cryptic mosaicism, mutations that cause overexpression of *SOX9*, *SOX3*, or *SOX10*, or inactivating mutations in *WNT4* or *RSPO1* [21]. Recently, it was proposed that the p.R92W variant of *NR5A1* is a monogenic cause of 46,XX OT-DSD/T-DSD, acting by upregulating *MAMLD1* and *SOX9* enhancer elements [25,26]. The phenotype of 46,XX individuals with pathogenic *NR5A1* variants includes primary ovarian insufficiency, early menopause, and OT-DSD/T-DSD [26]. Heterozygous variants affecting the fourth zinc finger of *WT1* may induce the upregulation of endogenous Sertoli cell transcripts [27]. Overall, the diagnostic yield of 46,XX DSD for NGS technologies ranges from 17% to 25% [8,10].

In cases of children with DSD, sex assignment should take into account the etiological diagnosis, external and internal genitalia, risk of gonadal tumors, and the parents' predilection [28,29]. External genitalia can be assessed by Prader staging or the EMS. Prader staging was initially created to evaluate patients with CAH, whereas the EMS was designed for male neonates and infants [30,31]. Since both scoring systems have limitations in evaluating a male-female binary phenotype, the external genital score, which incorporates 5 anatomical landmarks of the genitalia (degree of labioscrotal fusion, length of the genital tubercle, position of the urethral meatus, and locations of the gonads), was proposed [32]. However, sex of rearing should not be determined solely based on external genitalia. A multidisciplinary approach should be applied, involving experts in urology, gynecology, medical genetics, pediatric psychiatry, and pediatric endocrinology [23,29]. In the present study, 60% of patients with 46,XX OT-DSD were assigned as female. In previous studies, 62.5%–93.8% of such patients were assigned as male [22,23]. Considering the potential for fertility, the presence of female internal reproductive organs may contribute to a higher proportion of patients being assigned as female [28]. Age at diagnosis is also important because gender identity typically begins to develop around 18 months and becomes established by approximately 30 months of age [33]. Changes in the sex of rearing after this period increase the risk of gender dysphoria.

The prevalence and outcomes of gender dysphoria in patients with 46,XX DSD remain unclear. Studies conducted in Thailand and South Africa found that sex reassignment had occurred in 9%–11% of patients with 46,XX DSD [23,29]. Gender identity may be influenced by the presence of the Y chromosome and the duration and degree of androgen exposure. It is essential to monitor gender dysphoria and provide neuropsychological support [23].

The reported incidence of malignancy in patients with OT-DSD is 2.6%–4.6%; it is particularly low when the Y chromosome is absent [28]. However, it is worth noting that in the present study, 3 *SRY*-negative patients with 46,XX OT-DSD experienced tumors. A previous study reported that while no histological abnormalities were detected in ovarian tissue samples, signs of dysgenesis were present in every testicular tissue sample examined [34]. Therefore, the incidence and types of tumors associated with 46,XX dysgenetic gonads have yet to be determined, and continuous follow-up of preserved gonads is recommended.

Spontaneous puberty can occur in patients with OT-DSD [23,35]. Previous studies have reported that most of the gonads of male-assigned patients with 46,XX OT-DSD/T-DSD are preserved for testosterone production during puberty; however, testosterone production may decline as a result of gradual dysgenetic changes in testicular tissues, and some patients require testosterone replacement therapy [4,35]. Girls who undergo spontaneous puberty can experience primary amenorrhea due to underdeveloped uterine and/or vaginal structures [35].

This study has several limitations due to its retrospective nature. Some data were missing, and the duration of the follow-up varied, as well as adherence to medications. Additionally, hCG stimulation testing and WES/WGS were conducted in a small number of patients. The hCG stimulation test was not performed in patients with complete female external genitalia who presented with primary amenorrhea, those whose parents refused the test, or patients diagnosed prior to when the test was available in our institute. However, this study provides a comprehensive overview of the clinical and genetic characteristics and long-term outcomes of patients with 46,XX DSD who attended a single academic center.

In conclusion, the phenotypic spectra and pubertal outcomes of patients with 46,XX DSD are diverse and difficult to predict. Given the irreversible nature of surgical intervention and the possibility of patients developing gender dysphoria, sex assignment should be meticulously performed and take into account the etiological diagnosis. A multidisciplinary approach must be used when decisions are being made about preserving gonads to induce puberty and preserve fertility. Long-term regular follow-up is required to monitor pubertal development, fertility, gender identity, and gonadal complications.

Notes

Conflict of interest: The authors have no potential conflicts of interest relevant to this article to report.

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