ETIOLOGIES, SEX ASSIGNMENT, AND TREATMENT OUTCOMES IN PATIENTS WITH DISORDERS OF SEX DEVELOPMENT

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Abstract. Managing patients with disorder of sex development (DSD) is challenging. The Siriraj Intersex Care Team has provided multidisciplinary care for DSD patients since 2006. In this study, we aimed to identify the etiologies, clinical manifestations, gender assignments, and treatment outcomes of DSD patients. A retrospective study of DSD patients seen between 2002 and 2014 was performed. Patients who presented with ambiguous genitalia or who had external genitalia discordant with their genotypes were recruited. 46,XX patients with congenital adrenal hyperplasia were excluded because they were not routinely cared for by the intersex care team. There was a total of 191 patients. 46,XY DSD was the most common classification (85.3%). The common causes of 46,XY DSD were unknown etiology (40.5%) and developmental defect (36.2%). 5-alpha reductase deficiency and androgen insensitivity syndrome were found in 9.2% and 4.9% of patients, respectively. The sex chromosome DSD was found in 10.5% of cases, and mixed gonadal dysgenesis was the most common diagnosis (65%). 46,XX DSD was found in 4.2% of the patients, and most of those had ovotesticular DSD (62.5%). The male sex had been assigned to 82.7% of the patients. The external masculinization score was significantly higher among patients with male-sex assignment ($p < 0.001$). In both male and female sex-assigned patients, the median frequency of surgery was 2 times. Fistulae were common (33.6%) among male assigned patients who had undergone urethroplasty. Diagnosis remained inconclusive for 40% of the 46,XY DSD patients, and more extensive genetic testing would be required to yield a diagnosis.

Keywords: disorders of sex development, etiologies, sex assignment, outcomes

INTRODUCTION

Previously, classification of the etiology of ambiguous genitalia was a challenge due to the use of confusing and vague medical terms (Hughes, 2008). In 2006, the Lawson Wilkins Pediatric Endocrine Society (LWPES) and the European Society for Paediatric Endocrinology (ESPE) created the consensus for the new classification and used the new term, "Disorders of Sex Development (DSD)" (Houk *et al*, 2006; Hughes *et al*, 2006; Hughes, 2008), defined as the congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical. DSD patients are classified into 3 groups

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according to karyotype, comprising: 1) 46,XY DSD; 2) 46,XX DSD; and 3) sex chromosome DSD, which included ovotesticular DSD, mixed gonadal dysgenesis (MGD), Turner Syndrome, and Klinefelter Syndrome. Although there have been many studies on DSD, those examining the incidence and etiologies of patients with DSD according to the new classification remain limited (Erdogan *et al*, 2011; Jaruratanasirikul and Engchaun, 2014).

Each year, many DSD patients are treated at our institute, the Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. Diagnosis and sex assignment are complicated. In each case, sex assignment depends upon the diagnosis, the external-genitalia appearance, the ease of surgical procedures for the assigned sex, the possibility of fertility and having sexual intercourse, and the parents' and/or patients' gender preferences. Early evaluation by a multidisciplinary team of specialists is needed for proper management and sex assignment (Ekenze *et al*, 2015). At our institute, the intersex care team, consisting of pediatric endocrinologists, pediatric surgeons, a geneticist, and pathologists, has been functioning since 2006. The team provides holistic care for DSD patients with regard to their diagnosis, investigation, sex assignment, treatment, and continuity of care. Over the past 5-10 years, genetic testing has been available for 46,XY DSD patients. Molecular diagnosis for the androgen receptor (*AR*) gene has been performed in cases suspected of androgen insensitivity syndrome (AIS), and for the *SRD5A2* gene in cases suspected of 5-alpha-reductase deficiency.

In the situation of either the patients with gender dysphoria or their parents disagreeing with the team's advice on the patients' gender of rearing, pediatric psychiatrists were consulted to evaluate the patients' gender identity and/or the parents' thoughts and beliefs. Then, the intersex care team, a psychiatrist, and the parents/patients jointly decided upon the patients' gender of rearing. However, before the establishment of the intersex care team, patients with DSD were primarily cared for by pediatric endocrinologists and pediatric surgeons. The lack of available genetic testing and extensive hormonal workup left many patients with an indefinite diagnosis. In addition, most of the time, the decision about the appropriate gender of rearing was made solely by primary physicians.

The primary objective of this study was to identify the etiologies of DSD in our patients. The secondary objectives were to 1) evaluate the clinical manifestations, gender assignments, and surgical-treatment outcomes of patients with DSD; and 2) compare the outcomes of the surgical treatments and gender assignments of the DSD patients who received treatment from our intersex care team, with those of patients who did not.

MATERIALS AND METHODS

Participants

DSD patients who had been evaluated by our institute between January 2002 and June 2014 were retrospectively reviewed. There were two groups of patients: those who had first been seen before the intersex care team's formation (2002-2005; non-intersex-care-team group), and those who were seen after its establishment (2006-2014; intersex-care-team group). The former group were treated by pediatric endocrinologists and surgeons, while the latter were cared for by the intersex care team.

Patients who were eligible for the study presented with ambiguous genitalia or external genitalia in discordance with their genotypes. Female patients with congenital adrenal hyperplasia (CAH) were excluded since those patients were not routinely cared for by the intersex care team.

Study methods

Data collection included details of history, physical examination, investigation, and medical

and/or surgical treatment. The history included the initial gender rearing (defined as the gender reared by the parent before the first visit to a physician); associated symptoms, such as dysmorphic features or congenital anomalies; family histories of consanguinity and ambiguous genitalia; and maternal and neonatal birth histories. The physical examination included the external appearance of the genitalia, the phallus size at first visit, the urethral or vaginal opening, the presence of hypospadias, the position and size of the gonads, and the external masculinization score (EMS) (Ahmed and Rodie, 2010). EMS is a scoring system to assess the severity of ambiguous genitalia, by evaluating the microphallus, urethral meatus, labioscrotal fusion, and location of the gonads. The maximum score is 12, as found in normal boys; the lower the total EMS score, the more severe the ambiguity.

The investigations included chromosome studies, hormone studies, pelvic ultrasonographies, and genitograms. To assess the Leydig cell function, the human Chorionic Gonadotropin (hCG) stimulation test was performed by intramuscular injection of 1,500 units of hCG every other day for three doses; the testosterone and dihydrotestosterone (DHT) levels were evaluated before the first dose and 24 hours after the third dose. The testosterone-to-DHT ratio was calculated, and the cut-off value for suspecting 5-alpha reductase deficiency was more than 10 after the hCG stimulation test (Maimoun *et al*, 2011). In some cases where the hCG stimulation test was not done, blood for the testosterone and DHT level-testing was collected during minipuberty to assess the Leydig cell function. As for AIS, a molecular analysis for the *AR* gene mutation was performed, but did not include exon 1. In the case of 5-alpha reductase deficiency, the *SRD5A2* gene mutation was tested for all three exons. Additional tests, such as *SRY* gene or 250-mcg Adrenocorticotropic (ACTH) stimulation, were performed in cases with relevant clinical manifestations.

Data on the gender assignment by the physicians or the intersex care team, and the types of surgery were obtained. The treatment outcomes focused on the frequency of surgery and fistula repair, the number of patients lost to follow-up, and the gender of rearing after sex assignment and/or sex reassignment.

In this study, we classified 46,XY DSD patients as having a "developmental defect" if they had normal Leydig cell function with spontaneously-increased phallus size without hormonal treatment, or if they presented with ambiguous genitalia as part of a syndrome, or if they had other anomalies and/or syndromes.

This study was approved by the Ethics Committee of Siriraj Hospital, Mahidol University.

Statistical analysis

Descriptive statistics were used for this study. In the case of the etiologies, clinical manifestation, sex assignment, and treatment outcomes, frequency and percentage values were used. As for the demographic and continuous data, the mean or median, as well as the minimum and maximum, values were used. The unpaired Student's *t*-test and chi-square test were used to compare the data between 2 groups (male- *vs* female-assigned patients and non-intersex-care-team *vs* intersex-care-team groups). A *p*-value <0.05 was considered statistically significant. All data analyses were performed using PASW Statistics for Windows, Version 18.0 (IBM, Armonk, NY).

RESULTS

A total of 191 patients was assessed. During the study period, 34 females with ambiguous genitalia were diagnosed as CAH, and all were excluded from the study.

The clinical characteristics of the DSD patients are shown in Table 1. Most patients presented with ambiguous genitalia, and most were initially raised as males. Some patients had a family history of ambiguous genitalia or consanguinity.

Table 1
Clinical characteristic of DSD patients (*N* = 191). Data were reported as number (percentage) or median (min, max).

Characteristics	Number (%)
Age when first seen (months)	8 (0, 178)[a]
Chief complaint	
Ambiguous genitalia	173 (90.6)
Undescended testis	3 (1.6)
Mass at genitalia	2 (1)
Isolated hypospadias	14 (7.3)
Family history of consanguinity	6 (3.1)
Family history of ambiguous genitalia	7 (3.7)
Maternal hormone use	6 (3.1)
Initial sex of rearing as male	155 (81.1)
Stretched phallus length (cm)	2.6 (0, 7.3)[a,b]
External masculinization score (EMS)	5.5 (0, 11.5)[a]

[a]Data were presented as median (min, max); [b]Patients with normal female external genitalia were given 0 for phallus length.

On physical examination, the median stretched phallus length was 2.6 cm, and the median EMS score was 5.5. There were 82 patients in the non-intersex-care-team group, and 109 patients in the intersex-care-team group.

Of the 191 cases, the prevalence of each classification was: 46,XY DSD: 85.3% (*n* = 163); 46,XX DSD: 4.2% (*n* = 8); and sex chromosome DSD: 10.5% (*n* = 20). The etiologies of each DSD classification and sex assignment are shown in Table 2. In the 46,XY DSD group, 40.5% of the patients did not have known causes. A developmental defect was found in 36.2%, 5-alpha reductase deficiency was found in 9.2%, and AIS was found in 4.9%. As for patients in the 46,XX DSD group, the most common diagnosis was ovotesticular DSD (62.5%). In the case of sex chromosomal DSD, the most common diagnosis was MGD (65%).

46,XY DSD

Unknown etiology. Among 66 cases of unknown etiology, 46 had normal Leydig cell function, 5 were without Leydig cell function (proven either by the hCG stimulation test or the blood samples for testosterone taken during the minipuberty period), and 15 were not tested. Of those with Leydig cell function, nine patients tested negative for the *AR* and *SRD5A2* gene mutations. As most patients in this group were lost to follow-up (*n* = 41; 62.1%), the diagnoses remained inconclusive.

Developmental defect. 46,XY DSD patients who had a spontaneous increase in penile length during minipuberty, or who had an associated

Table 2
Diagnosis and sex assignment of 191 DSD patients.

46,XY DSD (*N* = 163; 85.3%)	*n* (%)	M	F	46,XX DSD (*N* = 8; 4.2%)	*n* (%)	M	F	Sex chromosome DSD (*N* = 20; 10.5%)	*n* (%)	M	F
Unknown etiology	66 (40.5)	59	7	Ovotesticular DSD	5 (62.5)	3	2	MGD	13 (65.0)		
Developmental defect	59 (36.2)	58	1	Unknown etiology	2 (25.0)	0	2	45,X/46,XY	7	4	3
46,XY DSD	57	57	0	Common cloaca	1 (12.5)	0	1	45,X/46X,del(Y)	1	0	1
45,XY, rob (21:22)(q10:q10)	1	1	0					45,X/46,X,+marker	3	1	2
47,XY,+21	1	0	1					45,X/47,XYY	1	1	0
5-alpha-reductase deficiency	15 (9.2)	14	1					45,X/46,XY/47,XYY	1	0	1
Partial AIS	6 (3.7)	5	1					Ovotesticular DSD	3 (15.0)		
Complete AIS	2 (1.2)	0	2					46,XX/48,XXYY	2	1	1
MGD	5 (3.0)	5	0					46,XX/46,XY	1	1	0
46,XY DSD	4	4	0					Klinefelter syndrome 47,XXY	1 (5.0)	1	0
46,XY der(12)t(?;12)(?;q22)	1	1	0					Klinefelter syndrome variant 49,XXXXY	1 (5.0)	1	0
Testicular regression syndrome	3 (1.8)	1	2					47,XYY	1 (5.0)	1	0
Extrophy of cloaca	4 (2.5)	1	3					Developmental defect due to 46,X, inv (Y) (p11.2q11.23)	1 (5.0)	1	0
Pure gonadal dysgenesis	2 (1.2)	0	2								
Acampomelic campomelic dysplasia	1 (0.6)	0	1								

M male, F female.

anomaly and/or syndrome, were classified as "developmental defect."

The group comprised 59 patients, and all had the 46,XY karyotype except for two: one with 47,XY+21, and the other with 45,XY, rob(21:22)(q10:q10). The former, with features of Down syndrome, presented with perineal hypospadias, bifid scrotum, and micropenis; the latter had perineal hypospadias, shawl and bifid scrotum, and micropenis.

There were 38 cases proven to have normal Leydig cell function, including 47,XY+21 and 45,XY, rob(21:22)(q10:q10) cases. Whether the developmental defect cases could enter puberty is not known due to the lack of follow-up appointments in 23 out of 59 cases (39%) and loss to follow-up in 27 out of 59 cases (45.8%).

Of all 59 cases, 28 (47.4%) had associated anomalies, including cardiac anomalies (13 cases), limb anomalies (4 cases), renal anomalies (3 cases), central nervous system anomalies (3 cases), cleft lip/cleft palate (2 cases), and gastrointestinal anomalies (2 cases). One patient had features of Russell Silver Syndrome.

Disorders of androgen synthesis/action. Fifteen patients had the *SRD5A2* gene mutation, and were diagnosed as having a 5-alpha reductase deficiency. However, 5 patients had heterozygous mutation of *SRD5A2* gene.

Six other patients were diagnosed as partial AIS, while there were two additional cases of complete AIS: one case was confirmed by mutation of the AR gene, and the second by clinical diagnosis. The latter case had no mutations in exons 2-8 of the AR gene; however, a mutation in exon 1 could not be excluded.

Testicular regression syndrome. Three patients were diagnosed with testicular regression syndrome, with EMS scores of 0, 3, and 4. The patient with the EMS score of 4 had a male-sex assignment, while the other two patients were assigned as females.

Two of the three patients underwent bilateral gonadectomy: one patient had atrophic testes and the other did not have testicular tissue.

Extrophy of cloaca. Of the four cases, three were raised as females before their first visit to the physician.

The first patient was assigned female at age 7 months after parental counseling.

The second patient was assigned female. However, the testes were removed around 13 years of age. Although having received female hormonal replacement, the patient developed male gender identity, had poor compliance in taking estrogen replacement, and had an interest in the female sex. Therefore, this patient requested male-sex reassignment at age 15 years, and has received testosterone replacement therapy since then.

The third patient was reassigned male at two years of age at the parents' request.

Only one case of cloacal extrophy was initially raised as male and also received male-sex assignment after seeing the intersex care team.

Pure gonadal dysgenesis. One patient with 46,XY karyotype had been raised as a girl, presented at 14 years of age with delayed puberty. A physical examination revealed breast Tanner stage I and normal external female genitalia. A genetic analysis revealed the presence of the *SRY* gene. She received bilateral gonadectomy with resection of the Mullerian-like structure; the pathology results revealed an immature uterus, left and right fallopian tubes, and atrophic testicular tissue. Pure gonadal dysgenesis was diagnosed.

Another patient presented with ambiguous genitalia at birth and was raised as a girl. She was seen by the intersex care team at 3 years of age. A physical exam revealed a small phallus (0.9 cm) and a urethral opening at the tip of the phallus. An HCG stimulation test revealed no Leydig cell function. She underwent bilateral gonadectomy

and vaginal reconstruction. The pathology result reported dysgenetic gonads.

Sex reversal acampomelic campomelic dysplasia. One patient with 46,XY DSD presented with multiple anomalies: cleft palate, ventricular septal defect (VSD), right scapular hypoplasia, and patellar dislocation with marked joint laxity, but without congenital bowed limbs. She had normal external female genitalia and had been raised as a girl. This patient was diagnosed with acampomelic campomelic dysplasia clinically; testing for the *SOX-9* mutation was not performed. She underwent bilateral gonadectomy; however, tissue diagnosis revealed bilateral prepubertal testes with a normal male genital duct, and without dysgenesis of the gonads.

46,XX DSD

After excluding CAH in this study, there was a total of eight patients with the 46,XX karyotype. Of these, five had ovotesticular DSD (confirmed by tissue diagnosis); one had common cloaca; and two were without known causes, but were proven not to be CAH after showing a normal response to the 250-mcg ACTH stimulation test.

Sex chromosome DSD

MGD was the most common diagnosis found in patients with sex chromosome DSD (65%), followed by ovotesticular DSD (15%). Among the 13 patients diagnosed with MGD, the most common karyotype was 45,X/46,XY (53.8%).

The one patient with Klinefelter syndrome presented with ambiguous genitalia and had an EMS score of 5.5. This patient underwent surgery to repair hypospadias, and was assigned male.

Another patient with a Klinefelter syndrome variant presented with ambiguous genitalia and had an EMS score of 5.5. This patient did not undergo surgery at Siriraj Hospital, but was transferred to a provincial hospital for continuity of care.

Sex of rearing and sex assignment

The initial sex of rearing was male in 81.2% of all cases. Sex assignment was mostly male (82.7%). The sex assignments of the non-intersex-care-team and intersex-care-team groups were not significantly different: male-sex assignment accounted for 68 out of 82 (82.9%) cases in the non-intersex-care-team group, and 90 out of 109 (82.6%) cases in the intersex-care-team group ($p = 0.949$).

The EMS scores of patients with male versus female-sex assignments were significantly different (mean EMS = 5.36±2.36 and 2.57±2.77, respectively; $p < 0.001$).

Among the 191 patients, only three with 46,XY DSD underwent sex reassignment. Two were the patients with cloaca extrophy who had been reassigned from female to male (as previously mentioned); and one was a patient with 5-alpha reductase deficiency, who had requested male-sex reassignment at age 13 years after developing virilization during puberty. The third patient had been raised as female and had never been diagnosed with DSD until entering puberty and becoming virilized, following which the parents brought him for medical consultation.

Outcome of surgical treatment

Of the 191 patients, 165 underwent surgery, consisting of female-sex assignment in 26 cases and male-sex assignment in 139 cases (including 3 patients with male-sex reassignment). Among the female-assigned patients, the median frequency of surgery was 2 times (minimum 1; maximum 3). As for the male-assigned patients, the median frequency was also 2 (minimum 1; maximum 10).

Of the 139 male-assigned patients, 113 underwent urethroplasty (median frequency 2; minimum 1; maximum 3); 38 out of the 113 cases (33.6%) had persistent fistulae (median frequency of repair 1; minimum 1; maximum 6).

Of the 139 male-assigned patients who underwent surgery, 52 cases (37.4%) completed

surgery; 3 (2.2%) had a fistula; 2 patients (1.4%) were in the process of further surgery; 1 patient (0.7%) refused further surgery; and 81 patients (58.3%) were lost to follow-up before completing the surgical procedures.

Of the 26 female-assigned patients, 17 (65.4%) completed surgery, and 9 (34.6%) were lost to follow-up.

The loss-of-follow-up rates in the non-intersex-care-team and the intersex-care-team groups were not significantly different (58.5% *vs* 44.9%; $p = 0.144$).

DISCUSSION

There are previous reports on the etiologies of DSD from other countries (Thyen *et al*, 2006; Mazen *et al*, 2008; Erdogan *et al*, 2011). Two studies of Thai children are available: one from our institute (Nimkarn *et al*, 2002), and the other from southern Thailand (Jaruratanasirikul and Engchaun, 2014). A comparison of the DSD etiologies described in those studies and in ours is shown in Table 3. A previous study by our institute, which examined 104 DSD patients, originally classified patients into 3 categories: true hermaphrophitism (5 patients; 4.8%), female pseudohermaphroditism (52 patients; 50.0%), and male pseudohermaphroditism (47 patients; 45.2%). Among the patients with true hermaphroditism, there were 2 with chromosome mosaicism, 2 with 46,XX, and 1 with 46,XY. Thus, when classified according to the new consensus, the etiologies were: 46,XX DSD: 51.9%, 46,XY DSD: 46.2%, and sex chromosome DSD: 1.9% (Nimkarn *et al*, 2002). At that time, molecular testing for the *AR* and *SRD5A2* mutations was not available, so a definite diagnosis in patients with 46,XY DSD with hCG responsiveness could not be performed.

In 2013, Jaruratanasirikul and Engchaun retrospectively studied 117 DSD patients in southern part of Thailand; they found the causes of DSD to be sex chromosome abnormalities (53.0%), 46,XX DSD (29.9%), and 46,XY DSD (17.1%) (Jaruratanasirikul and Engchaun, 2014). Unlike our study, the most common cause of DSD in that study was Turner syndrome (36.8%) and CAH (29.9%). In our study, we only recruited patients with ambiguous genitalia or having discordance between external genitalia and sex genotype. Based on our findings, there were 13 patients with Turner syndrome mosaicism karyotype who presented with ambiguous genitalia, and all of them had pathologically-proven MGD.

For clarity of comparison, we also included CAH in the 46,XX DSD group. If the 34 female patients with 21-hydroxylase deficient CAH were included, 46,XY DSD would still be the most prevalent cause of DSD (72.4%). This proportion is higher than in previous studies, which reported prevalence from 47.0% to 65.9% (Thyen *et al*, 2006; Mazen *et al*, 2008; Erdogan *et al*, 2011).

In our study, the majority of 46,XY DSD cases were of unknown etiology. At least 69.6% of them had normal Leydig cell function, and only a small number had molecular analysis for *AR* and *SRD5A2*; thus, partial AIS or 5-alpha reductase deficiency might still be the cause of ambiguous genitalia in some patients in this group. A few cases of 46,XY DSD of unknown cause did not have normal testosterone production. That smaller group of patients might have had disorders of androgen synthesis, either due to an enzymatic defect (*eg*, 17-beta hydroxysteroid dehydrogenase deficiency) or gonadal dysgenesis arising from various mutations of the genes involved in testicular development [*eg*, steroidogenic factor 1 (*SF1*), Wilm tumor 1 (*WT1*)]. All patients with unknown causes would benefit from further genetic testing of the relevant genes; however, many of the patients have been lost to follow-up.

In our study, we classified 46,XY DSD patients with a spontaneous increase in penis size during minipuberty, or patients with an associated anomaly/syndrome, as a "developmental

Table 3
Etiologies of DSD in various studies.

Study		Nimkarn *et al*	Thyen *et al*	Mazen *et al*	Erdogan *et al*	Jaruratanasirikul and Engchuan	This study
Year		2002	2006	2008	2011	2014	2017
Country		Thailand	Germany	Egypt	Turkey	Thailand	Thailand
Number of patients		104[a]	80[b]	208	95	117	225[e]
Overall prevalence	46,XY DSD	46.2%	51.3%	65.9%	47.0%	17.1%	72.4%
	46,XX DSD	51.9%	28.8%	28.0%	25.0%	29.9%	18.7%
	Sex chromosome DSD	1.9%	12.5%[c]	0.96%	27.0%	53.0%	8.9%
Etiology in each classification	46,XY DSD	Suspected AIS or 5-alpha reductase deficiency 45.8%	AIS 31.7%	Suspected defect in androgen synthesis or action 60.0%[d]	Defect in androgen action 44.4%	Gonadal dysgenesis 35.0% Complete AIS 30.0%	Unknown 40.5% Developmental defect 36.2% 5-alpha reductase deficiency 9.2% AIS 4.9%
	46,XX DSD	CAH 96.3%	CAH 52.2%	CAH 75.4%	CAH 66.7%	CAH 88.6%	CAH 81.0% Ovotesticular DSD 11.9%
	Sex chromosome DSD	Ovotesticular DSD 100%	MGD 40.0%	MGD 100%	TS 80.7%	TS 69.4%	MGD 65.0% Ovotesticular DSD 15.0%

[a] Originally classified as true hermaphroditism, female pseudohermaphroditism, and male pseudohermaphroditism (Nimkarn *et al*, 2002).
[b] Karyotype not known in 6 cases (Thyen *et al*, 2006).
[c] Including chromosomal aberrations (*eg*, 45,X/46,XY) or deletion syndromes (*eg*, 6q-, 13q-) (Thyen *et a*l, 2006).
[d] Calculated from 75 patients who completed the investigations (Mazen *et al*, 2008).
[e] For comparison with other studies, 34 patients with 21-hydroxylase deficient CAH were included in the 46,XX DSD group.

defect," which represented one-third of causes of 46,XY DSD. This group mainly comprised patients with an abnormal development of the urogenital premordia without endocrine causes. Included also in this group was a patient with Down syndrome, with features of ambiguous genitalia. Male patients with Down syndrome have a low prevalence of genital anomaly, with most of those cases being hypospadias (Stoll *et al*, 2015).

However, whether some of the patients in the developmental defect group that did not have syndromic association/anomaly actually have a developmental defect is unknown. There is the possibility that some patients in this group might have a mild form of androgen synthesis or action defect. Thus, those patients need genetic testing, especially for the *SRD5A2* and *AR* mutations; also, long-term follow-up is needed to assess pubertal development. Unfortunately, the majority of those patients were not followed up long-term. In addition, some patients with an unknown etiology might have ambiguous genitalia due to environmental factors (*eg*, exposure to endocrine disruptors in utero) (Gaspari *et al*, 2011).

Two cases of 46,XY DSD were diagnosed as pure gonadal dysgenesis; whether those patients had a mutation of the *SRY* gene or abnormalities of other genes involved in testicular development (*eg*, *NR5A1, WT1, WNT4, NROB1, MAP3K1*, etc; Ostrer, 2014) is not known, and further genetic testing would be required.

As for the 46,XX DSD group, if the female patients with CAH were excluded, the most common diagnosis was ovotesticular DSD (62.5%); however, if they were included, the most common diagnosis would be CAH at 81%, similar to previous studies (Thyen *et al*, 2006; Mazen *et al*, 2008; Erdogan *et al*, 2011). In our study, all patients in the 46,XX DSD group were initially reared as females before their first visit, and were also assigned as females by the physicians.

Based on our results, MGD was the most common diagnosis in sex chromosome DSD (65%); this was similar to studies from Germany and Egypt (Thyen *et al*, 2006; Mazen *et al*, 2008), in which 45,X/46,XY was the most prevalent karyotype. The number of cases of chromosome DSD in our study was limited because we did not include Turner syndrome or Klinefelter syndrome cases with normal genitalia. Thus, our prevalence of sex chromosome DSD was lower than the studies from Turkey and Southern Thailand (Erdogan *et al*, 2011; Jaruratanasirikul and Engchaun, 2014).

Since the intersex care team was established in 2006, patient gender has been assigned as a consensus among the physicians in the team and the parents and/or patients themselves. In our practice, gender assignment is performed after the cause of ambiguous genitalia is determined, in conjunction with evaluating the external genitalia and the internal sex organs, assessing the difficulty and possible outcomes of surgical procedures to correct the ambiguity to the assigned sex, and the possibility of having spontaneous puberty and fertility. Patients with a micropenis receive a trial of testosterone treatment to increase the penis size, and patients who respond to the treatment are considered for possible male-sex assignment.

The majority of patients in our study had 46,XY DSD, and most were reared and assigned as male after being seen by specialists. That result is similar to a study from Hong Kong, where 53 out of 64 patients with 46,XY DSD were raised as male (Chan *et al*, 2015).

In contrast to most 46,XY DSD patients, the majority of patients with extrophy of the cloaca and testicular regression syndrome were raised as females, and they also had female-sex assignment. The female sex preference was due to severe undervirilization in the patients. In the past, there was a tendency to raise patients with cloaca extrophy as females; however, previous

reports showed that patients initially raised as female chose to have male-sex reassignment or had a male sexual identity (Meyer-Bahlburg, 2005; Reiner, 2005). Moreover, a more recent report by a pediatric urologist favored male gender assignment of 46,XY cloaca extrophy, changing past trends favoring female-sex assignment (Diamond *et al*, 2011). In our study, three out of four patients with cloaca extrophy were assigned female; however, two patients were reassigned as male, one at his own request, and the other at the request of his parents. The end result was that three out of the four patients with cloaca extrophy were eventually assigned the male gender.

In DSD patients, the purpose of surgery is to make the phenotypic sex correlate with the sex of rearing, to maintain reproductivity, to remove the gonad at risk for malignancy, and to correct the cosmetic problem (Lee *et al*, 2012; Wisniewski, 2012). The psychological outcome should also be considered. In the previous reports, the male-sex-assigned patients underwent multiple surgical procedures (Wisniewski, 2012); also, the female-sex-assigned patients who underwent early surgery had a tendency to have poorer outcomes than patients who had surgery at puberty (Hurwitz, 2011). In our study, patients with male and female-sex assignments appeared to have a similar frequency of surgical procedures (median: two times), however, one-third of the patients who underwent masculinizing surgery had fistulae, some requiring multiple surgery for fistula repair. Thus, the difficulty and complexity of the surgical procedures should be considered and discussed with the parents and/or patients.

To date, there have been a limited number of studies on the impact of a care team on gender assignment. A study of 47 DSD patients evaluated by a gender care team found that the initial sex assignment at birth correlated with the genotype and phenotype in 63.8% and 86.4% of cases, respectively; after evaluation by the gender care team, the sex assignment correlated with the genotype and phenotype in 76.6% and 97.7% of cases, respectively (Suresh *et al*, 2013). In our study, there was no significant difference in the distribution of sex assignment in the intersex-care-team and non-intersex-care-team groups. Also, the gender assignment was similar to the initial sex of rearing. Although the intersex care team provides a one-stop service for patients, approximately half of the patients were lost to follow-up. There is a need to develop a way to improve patients' adherence in order to provide long-term follow-up, care and support, since many DSD patients require hormonal treatment as well as counseling/disclosure of the disease when they reach adolescence.

Our study has several limitations: the retrospective nature of the study meant the data was incomplete in some cases; the limited availability of genetic testing prevented forming a definite diagnosis in certain cases; and cases that were lost to follow-up made their diagnosis and treatment incomplete.

In conclusion, most of our DSD patients presented with ambiguous genitalia, and the most common classification was 46,XY DSD, of which 40% of patients did not have a definite diagnosis. In the future, more extensive genetic testing using next-generation sequencing will be highly beneficial in discovering the genetic abnormalities in many patients.

Although the intersex care team provided holistic care for DSD patients, the lost-to-follow-up rate was very high; methods to improve adherence are needed.

Further studies on the physical satisfaction and psychological outcomes of DSD patients are also required in order to improve our care for this challenging group of patients.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

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