RESEARCH

Clinical characteristics and genetic expansion of 46,XY disorders of sex development children in a Chinese prospective study

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Abstract

Diagnosis and management strategy of disorders of sex development (DSD) are difficult and various due to heterogeneous phenotype and genotype. Under widespread use of genomic sequencing technologies, multiple genes and mechanisms have been identified and proposed as genetic causes of 46,XY DSD. In this study, 178 46,XY DSD patients were enrolled and underwent gene sequencing (either whole-exome sequencing or targeted panel gene sequencing). Detailed clinical phenotype and genotype information were summarized which showed that the most common clinical manifestations were micropenis (56.74%, 101/178), cryptorchidism (34.27%, 61/178), and hypospadias (17.42%, 31/178). Androgen synthesis/action disorders and idiopathic hypogonadotropic hypogonadism were the most frequent clinical diagnoses, accounting, respectively, for 40.90 and 21.59%. From all next-generation sequencing results, 103 candidate variants distributed across 32 genes were identified in 88 patients. The overall molecular detection rate was 49.44% (88/178), including 35.96% (64/178) pathogenic/likely pathogenic variants and 13.48% (24/178) variants of uncertain significance. Of all, 19.42% (20/103) variants were first reported in 46,XY DSD patients. Mutation c.680G>A (p.R227Q) on *SRD5A2* (steroid 5-alpha-reductase 2) (36.67%, 11/30) was a hotspot mutation in the Chinese population. Novel candidate genes related to DSD (*GHR* (growth hormone receptor) and *PHIP* (pleckstrin homology domain-interacting protein)) were identified. Overall, this was a large cohort of 46,XY DSD patients with a common clinical classification and phenotype spectrum of Chinese patients. Targeted gene panel sequencing covered most of the genes contributing to DSD, whereas whole-exome sequencing detected more candidate genes.

Key Words

- 46,XY disorder of sex development
- hypogonadism
- next-generation sequencing
- clinical characteristics
- genetic characteristics

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Introduction

Disorder of sex development (DSD) is a general term for diseases with inconsistencies between chromosomal karyotypes, external genitalia, and gonadal development (1). According to the DSD etiology classification defined in the 2006 Chicago Expert Consensus meeting by the European Society for Pediatric Endocrinology and the Lawson Wilkins Pediatric Endocrine Society, patients can be divided into three categories according to karyotype:

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(i) sex chromosome DSD, (ii) 46,XY DSD, and (iii) 46,XX DSD. The worldwide incidence of DSD is between 1/500 and 1/10,000 (3). However, there are no relevant DSD incidence statistics for the Chinese population (4, 5).

Compared with sex chromosome DSD and 46,XX DSD, 46,XY DSD patients present with more remarkable clinical heterogeneity among different classifications and ages (6). The phenotypes vary from mild to severe as the clinical classification varies. Gene sequencing technologies can help confirm the pathogenesis of different classifications of 46,XY DSD at an early stage. However, to date, only about 20% of 46,XY DSD patients have been identified with a specific genetic cause (7). It is important to determine the diagnosis protocol and genetic pathogenesis of 46,XY DSD patients, which not only helps to establish specialized medical care for affected individuals but also helps to provide future genetic counseling for the next generation (8).

In this study, we intended to expand the clinical and genetic characteristics of 178 46,XY DSD patients between 2016 and 2022 and compare the efficiency of two different next-generation sequencing (NGS) methods, targeted gene panel sequencing (TPS), and whole-exome sequencing (WES). Through analysis and discussion of sequencing results, we provide a better overview of DSD diagnosis, especially in the Chinese population.

Materials and methods

Study design and participants

This study was conducted at the Shanghai Children's Medical Center, affiliated with the Shanghai Jiao Tong University School of Medicine. This single-center prospective study enrolled pediatric patients (aged ≤18 years) suspected of having DSD by experienced physicians from December 2016 to March 2022. The diagnosis of DSD was based on clinical manifestations, laboratory tests, and imaging examinations according to the 2006 Chicago consensus criteria (2). The inclusion criteria of suspected DSD were as follows: patients presenting clinical manifestations of DSD, such as ambiguous external genitalia, male-type external genitalia with bilateral cryptorchidism and/or micropenis and/or hypospadias, female-type external genitalia with clitoral enlargement and/or labia fusion and/or inguinal mass and/or inguinal hernia, gonadal dysplasia (GD), absent secondary sexual characteristics during puberty, gynecomastia, and gender inconsistency between gonads and chromosomes showing 46,XY. Complete clinical data and genetic analysis results were evaluated. Subjects with any one of the following conditions were excluded: (i) other chronic diseases or malnutrition (such as uremia and anorexia) and (ii) history of intracranial lesions or pituitary tumors. Patient characteristics were collected by clinicians, including age at diagnosis, sex of rearing, main concern, medical history, relative familial history, consanguinity, history of pregnancy and delivery, previous treatment, and surgical intervention.

Physical examination

Physical examination methods were based on articles previously published by our team (9, 10). The body weight and height of the patients were measured and recorded using the same type of apparatus and following the standard procedures recommended by Cameron (11), and then the body mass index was calculated. The secondary sexual characteristics of all patients were evaluated and recorded by qualified endocrinologists using the Tanner stage grade (12). The testicular volume was measured by endocrinologists using both the Prader measurement apparatus and ultrasonography. Clinical manifestations of DSD were evaluated and recorded by following terminology: ambiguous external genitalia, male-type external genitalia with bilateral cryptorchidism and/or micropenis and/or hypospadias, female-type external genitalia with clitoral enlargement and/or labia fusion and/or inguinal mass and/or inguinal hernia, GD, absent secondary sexual characteristics during puberty, gynecomastia, and gender inconsistency between gonad and chromosome.

Paraclinical examination

All suspected DSD patients underwent basic laboratory tests, including testosterone, estrogen, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and gonadotropin-releasing hormone (GnRH) stimulation tests, and imaging examinations including bone age (13), pelvic ultrasonography (14), and magnetic resonance imaging of the pituitary gland and olfactory bulb. All the patients underwent karyotyping and were recommended to undergo gene sequencing.

Chinese smell identification tests (CSITs) (15, 16, 17) were performed in patients with suspected hypogonadotropic hypogonadism and healthy control groups (1:1 matched). Inclusion criteria were as follows: (i) aged 6–18 years old; (ii) willing to participate in this study;

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(iii) no difficulty in language communication. Cases and controls were excluded if they had a history of respiratory allergies/upper respiratory infections/rhinitis within the past 14 days or had any chronic diseases. The control's data were initially collected as described in Zhang *et al.* (17).

Gonadorelin acetate (Gonadorelin, BBCA, Anhui, China) was used for the GnRH stimulation test, which was conducted between 08:00 and 10:00 h in fasting subjects by intravenous injection of gonadorelin acetate at a dose of 2.5 mg/kg body weight (the maximum total dose 100 mg) (18). Venous blood samples were collected to determine the serum concentrations of testosterone, estrogen, LH, and FSH at 0, 30, 60, 90, and 120 min after the injection of gonadorelin acetate. Serum was separated from the blood samples less than 1 h after collection, and testosterone, estrogen, LH, and FSH levels were tested immediately. The concentration of testosterone, estrogen, LH, and FSH were detected by chemiluminescence immunoassay (Abbott Diagnostics) on an Architect i2000SR (Abbott Diagnostics), with detection limits of 0.02 nmol/L, 10 pg/mL, 0.09 IU/L, and 0.05 IU/L, respectively; intra-assay and inter-assay coefficients of variation were less than 6.4% and 8.4%, respectively.

Genetic analysis

Subjects from December 2016 to December 2018 underwent TPS and subjects from January 2019 to March 2022 underwent WES. Genetic sequencing was performed as previously described by Xu *et al.* (19). Peripheral blood (2 mL) was collected from the children and their parents to extract DNA. The experimental process included the digestion of sample DNA fragments, library hybridization, and capture library amplification and purification. Agilent Sure Select method was used for exon capture (using All Exome V6 kit and panel kit including 2742 hereditary disease-causing genes (cat No. 5190–7519; Agilent Technologies Inc.), see Supplementary Table 1 (see section on supplementary materials given at the end of this article)). Hiseq2500 sequencing platform (Illumina, Inc., San Diego, CA, USA) was used for high-throughput sequencing. For data quality control and removal of the adaptor sequence, Fastqc (Babraham Research Institute, Cambridge, UK) and Fastp (Visible Genetics, Inc., Toronto, Canada) were applied. Speedseq (Ira Hall Lab, St. Louis, MO, USA) was aligned to the reference genome, and bamdst and mosdepth were used to count the sequencing indexes of BAM files after alignment. The Genome Analysis Toolkit (Broad Institute, Cambridge, MA, USA) was used to detect the variations in the BAM file that passed the quality control, and a VCF format file was generated. Variations in VCF files were annotated by Ingenuity Variant Analysis (Ingenuity Systems, Redwood City, CA, USA). These candidate variants were verified by Sanger sequencing and manually classified according to the American College of Medical Genetics and Genomics (ACMG) variant classification criteria guideline. We analyzed the allele frequencies of the detected variants and checked whether the variants had been reported before by retrieving relevant information from the population database Genome Aggregation Database (gnomAD) (http://gnomad.broadinstitute.org/). Alamut functional software was used to predict the functions of the mutated proteins. The accessible DNA of the patients' parents (94.32%, 83/88) was also isolated and subjected to Sanger sequencing to confirm the origin of the detected candidate variants.

Statistical analysis

Data were analyzed using GraphPad Prism 9.4.0 (Macintosh Version by Software MacKiev GraphPad Software, LLC) and RStudio (2021.09.1+372, RStudio, PBC). Descriptive statistics were presented as mean ± standard deviation for continuous variables, and percentages were used for categorical variables. The Mann–Whitney U-test or the Kruskal–Wallis test was used for non-normal distribution, and the chi-square test was used to compare the proportion of manifestations between groups.

Results

A total of 178 patients with 46,XY DSD (163 boys and 15 girls) who underwent NGS were enrolled in this study (Fig. 1). Of these, 79 pathogenic/likely pathogenic (P/LP) variants were found in 64 subjects (35.96%, 64/178) under NGS (NGS-positive group). Twenty-four variants of uncertain significance (VUS) were detected in 24 subjects (13.48%, 24/178). The overall candidate variant detection rate was 49.44% (88/178).

Based on NGS results, all subjects were divided into the NGS-positive group (subjects with P/LP variants, $n=64$) and the NGS-negative group (subjects with no variants detected by current methods, $n=90$). Subjects with VUS or undefined variants ($n=24$) were not included in the NGS-positive groups during statistical analysis, since the pathogenicity of these variants was not clarified yet. Here, we mainly focus on the phenotype and genotype in the subjects who were diagnosed both clinically and

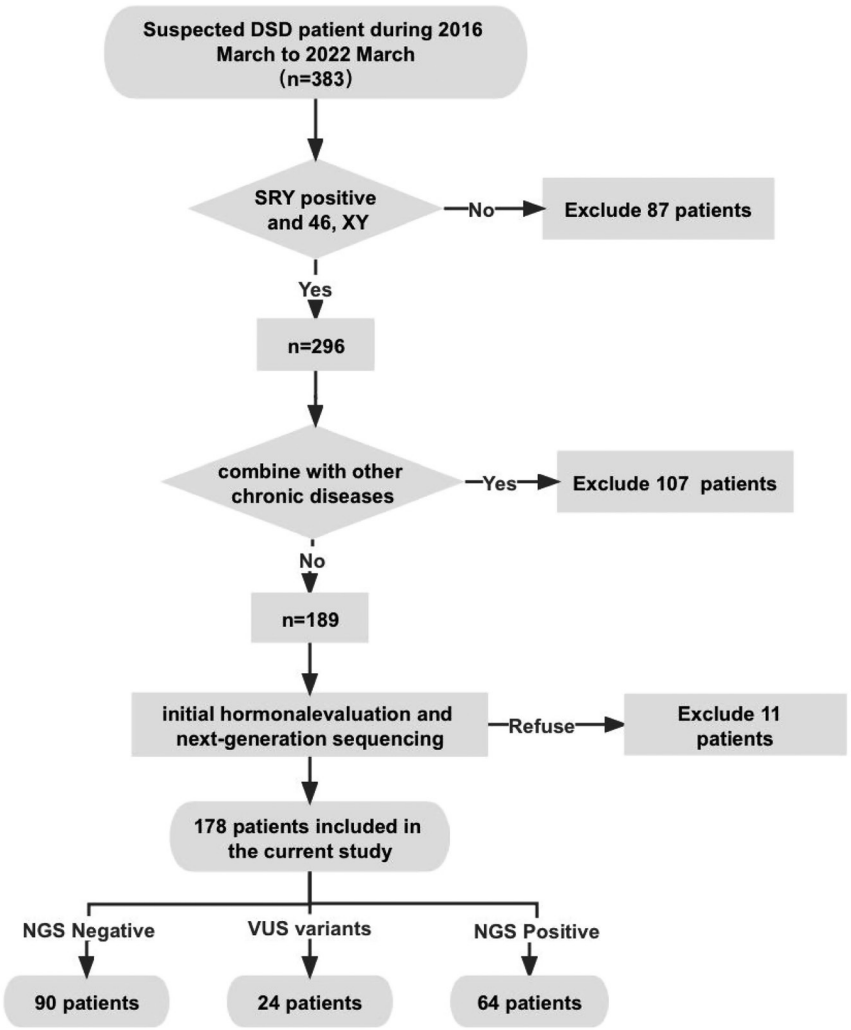

Figure 1
Flowchart of current study. In this study, 383 children suspected of DSD between 2016 and 2022 were initially included. Among these, 189 were SRY positive with A chromosome karyotype of 46,XY. A total of 107 subjects were excluded due to other chronic diseases, and 11 were excluded due to refusal of gene sequencing and incomplete examination results for personal reasons. Thus, a total of 178 subjects were finally included in this study.

genetically (NGS-positive group, $n = 64$). Furthermore, subjects were divided into the primary gonadal/genital disorders group ($n = 48$) and central hypogonadism group ($n = 16$) according to their phenotypes and paraclinical results.

Clinical characteristics

Age

Among all the 46,XY subjects, the median age at diagnosis was 8.63 (2.61, 12.00) years ($n = 178$), ranging widely from 1 month to 17 years. Most of these patients were diagnosed before puberty, and the youngest diagnosed patient was of 1 month.

A significant difference was observed ($P < 0.05$) in age at diagnosis between NGS-positive (median age 4.00 years old, $n = 64$) and NGS-negative patients (median age 10.04 years old, $n = 90$). In the NGS-positive group, the median age of primary gonadal/genital disorders subjects ($n = 48$) was 2.92 (0.67, 7.33) years old, and that of central cause subjects ($n = 16$) was 7.88 (2.69, 12.07) years old. A significant difference was observed in two groups ($P < 0.05$).

Clinical diagnosis

In primary gonadal/genital disorders group ($n = 48$), subjects were diagnosed as 5α reductase deficiency (5αRD, 35.42%, 17/48), androgen insensitivity syndrome (AIS, 27.08%, 13/48), GD (12.50%, 6/48), congenital adrenal hyperplasia (CAH, 4.17%, 2/48), persistent Mullerian duct syndrome (PMDS, 4.17%, 2/48), and syndromic hypogonadism (10.42%, 5/48).

In the central cause group ($n = 16$), idiopathic hypogonadotropic hypogonadism (IHH) subjects accounted for 68.75% (11/16), hypopituitarism accounted for 25.00% (4/16), and another Ulnar-mammary syndrome accounted for 6.25% (1/16), respectively.

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Clinical manifestations

46,XY DSD patients manifested various clinical manifestations, including bifid scrotum, penoscrotal transposition, penile curvature, ambiguous external genitalia, gynecomastia, and olfactory dysfunction. Overall, in all 178 subjects, the most frequent clinical manifestations were small testis (66.29%, 118/178), micropenis (56.74%, 101/178), unilateral or bilateral cryptorchidism (34.27%, 61/178), and hypospadias (30.34%, 54/178). Of all, 15 subjects were raised as girls from birth because of female external genitalia; the most common concern of these female patients was the predominance of female external genitalia (80.00%, 12/15), unilateral or bilateral cryptorchidism (73.33%, 11/15), clitoromegaly (53.33%, 8/15), ambiguous external genitalia (20.00%, 3/15), delayed mammary development in pubertal subjects (100.00%, 5/5), and primary amenorrhea in subjects >14 years old (100.00%, 3/3).

Among NGS-positive subjects ($n=64$), the most common clinical manifestations were micropenis (59.38%, 38/64), unilateral or bilateral cryptorchidism (50.00%, 32/64), and hypospadias (21.88%, 14/64). Most of them showed undeveloped testis volume and short stretched penile length (see Table 1). In comparison, in the NGS-negative group ($n=90$), a minor proportion of unilateral or bilateral cryptorchidism (21.11%, 19/90) and hypospadias (13.33%, 12/90) phenotypes was observed.

Specifically, in primary gonadal/genital disorders subjects, the main phenotype was micropenis (54.17%, 26/48), bilateral or unilateral cryptorchidism (52.08%, 25/48), and hypospadias (27.08%, 13/48) (see Table 1). Those subjects who presented hypospadias ($n=13$) could also manifest as cryptorchidism and/or micropenis. Details of different subgroups are shown in Table 2.

Among central DSD subjects, micropenis was the most common phenotype (75.00%, 12/16); unilateral or bilateral

Table 1 Clinical phenotype and biomarkers of NGS-positive subjects ($n = 64$).

Clinical characteristics	**NGS-positive subjects** ($n = 64$)	**Primary group** ($n = 48$)	**Central group** ($n = 16$)	***P*-value**[a]
Age (years)	4.00 (0.87, 10.00)	2.92 (0.67, 7.33)	7.88 (2.69, 12.07)	0.033
Micropenis, %	59.38%	54.17%	75.00%	0.142
Cryptorchidism, % (unilateral or bilateral)	50.00%	52.08%	43.76%	0.774
Hypospadias, n/N (%)	21.88%	27.08%	6.25%	0.159
Olfactory disorder, % (*n*/*N*)	NA	NA	62.50%	/
Other phenotypes	Prepenile scrotum, bifid scrotum, ambiguous external genitalia, penile curvature, blind perineal pouch resembling vagina, female external genitalia, transverse testicular ectopic, persistent Müllerian duct syndrome, gonadal dysplasia, enlarged clitoris, gynecomastia.	Prepenile scrotum, bifid scrotum, ambiguous external genitalia, penile curvature, blind perineal pouch resembling vagina, female external genitalia, transverse testicular ectopic, persistent Müllerian duct syndrome, gonadal dysplasia, enlarged clitoris, gynecomastia.	Intellectual disability, delayed motor development, skeletal deformity, bilateral pinky joint malformation, brain structure abnormalities, and hearing disorders	/
Average testis volume (mL)	1.00 (1.00, 2.00) ($n = 47$)	1.00 (1.00, 2.00) ($n = 33$)	1.00 (1.00, 3.00) ($n = 16$)	0.384
Stretched penis length (cm)	3.00 (1.00, 3.45) ($n = 48$)	3.00 (1.50, 3.50) ($n = 34$)	2.75 (2.00, 3.00) ($n = 16$)	0.643
Testosterone (ng/ml)	0.11 (0.09, 0.24) ($n = 51$)	0.12 (0.09, 0.66) ($n = 35$)	0.11 (0.09, 0.12) ($n = 16$)	0.652
DHT (pg/mL)	70.18 (40.85, 133.91) ($n = 51$)	54.85 (34.98, 110.32) ($n = 35$)	132.87 (66.65, 145.52) ($n = 14$)	0.001
FSH (mIU/mL)	2.77 (1.57, 4.88) ($n = 51$)	3.31 (1.78, 6.79) ($n = 35$)	0.91 (0.43, 1.71) ($n = 16$)	0.089
LH (mIU/mL)	0.80 (0.29, 2.07) ($n = =51$)	0.88 (0.30, 2.63) ($n = 35$)	0.19 (0.08, 0.31) ($n = 16$)	0.075

Sixty-fout subjects with pathogenic/likely pathogenic variants in this cohort were classified as NGS positive and were further divided into primary cause group ($n = 48$) and central cause group ($n = 16$) according to clinical diagnosis. The phenotypes (cryptorchidism, micropenis, and hypospadias), physical examination (testicular volume and penis length), concentrations of testosterone, estrogen, FSH, and LH in serum or plasma, and other clinical or laboratory findings are listed separately.

[a]The Mann–Whitney *U*-test and chi-square test were used between primary and central groups. $P < .05$ was considered statistically significant.

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Table 2 Clinical features of primary gonadal/genital disorders.

Clinical characteristics	**5αRD** (*n* = 17)	**AIS** (*n* = 13)	**Gonadal dysplasia** (*n* = 6)
Age (years)	2.00 (0.58, 4.46)	10.00 (0.92, 14.17)	2.75 (0.17, 6.00)
Female external genitalia, %	5.88% (1/17)	53.85% (7/13)	16.67% (1/6)
Micropenis, %	76.47% (13/17)	53.85% (7/13)	16.67% (1/6)
Cryptorchidism, %	29.41% (5/17)	53.85% (7/13)	66.67% (4/6)
Hypospadias, *n*/*N* (%)	35.29% (6/17)	23.08% (3/13)	50.00% (3/6)
Other phenotypes	Bifid scrotum, ambiguous external genitalia, penile curvature, penile curvature.	Penoscrotal transposition, bifid scrotum, enlarged clitoris, gynecomastia.	Penoscrotal transposition, bifid scrotum, ambiguous external genitalia, penile dysplasia
Average testis volume (mL)	1.00 (1.00, 2.00) (*n* = 13)	1.00 (0.75, 3.00) (*n* = 6)	1.00 (0.88, 1.25) (*n* = 4)
Stretched penis length (cm)	2.80 (1.25, 3.25) (*n* = 14)	2.50 (1.00, 3.50) (*n* = 6)	3.00 (2.23, 3.85) (*n* = 5)
Testosterone (ng/mL)	0.09 (0.09, 0.49) (*n* = 14)	0.19 (0.09, 2.32) (*n* = 13)	0.13 (0.09, 0.86) (*n* = 5)
DHT (pg/mL)	47.36 (38.89, 60.63) (*n* = 14)	100.11 (77.86, 141.51) (*n* = 13)	88.48 (33.18, 131.63) (*n* = 5)
FSH (mIU/mL)	2.07 (0.68, 2.97) (*n* = 14)	13.44 (2.94, 47.98) (*n* = 13)	6.40 (3.76, 9.54) (*n* = 5)
LH (mIU/mL)	0.78 (0.09, 1.58) (*n* = 14)	9.31 (2.75, 20.04) (*n* = 13)	1.05 (0.84, 1.59) (*n* = 5)

The top three classifications of primary cause DSD were 5α-reductase deficiency (*n* = 17), androgen insensitive syndrome (*n* = 13), and gonadal dysplasia (*n* = 6). The median age of 5α-reductase deficiency was lower than that of AIS subjects. The patients' testosterone and DHT levels in these diseases remained similar, but the gonadotropin levels in 5α-reductase-deficient subgroup were much lower than those in the AIS subgroup.

cryptorchidism was found in 43.75% (7/16) patients; and few subjects presented hypospadias (6.25%, 1/16) (see Table 1). Different from the primary cause group, subjects in the central cause group, mostly those IHH subjects, could be presented with other systemic manifestations, such as olfactory dysfunction (62.50%, 10/16), intellectual disability, delayed motor development, skeletal deformity, brain structure abnormalities, and hearing disorders. CSIT score, a toolkit specifically designed to assess olfactory function in the Chinese population, was analyzed (Supplementary Fig. 1).

Genetic characteristics

Overall distribution

The overall genetic diagnosis rate was 49.44% (88/178). A total of 103 candidate variants distributed across 32 genes were identified in 88 patients. Of all, 79 P/LP variants (79/103) were detected in 64 subjects in this cohort (see Fig. 2, Tables 3 and 4). The genetic diagnostic rate (including patients with P/LP variants only) of this cohort was 35.96% (64/178). According to specific phenotype, the genetic diagnostic rate of micropenis phenotype, cryptorchidism phenotype, and hypospadias phenotype was 67.09% (53/79), 79.25% (42/53), and 79.17% (19/24), respectively. According to the ACMG guidelines, all 103 variants were classified as pathogenic (41.75%, 43/103), likely pathogenic (34.95%, 36/103), and variants of uncertain significance (VUS, 24/103, 23.30%) (details of each variant in Tables 2, 3, and 4).

Of all the P/LP variants (*n* = 78), 68 were heterozygote (87.18%, 68/78) and 10 were homozygote (12.82%, 10/78). The variant types included missense (64.10%, 50/78), deletion (11.54%, 9/78), nonsense (11.54%, 9/78), frameshift (8.97%, 7/78), and splicing (3.85%, 3/78) mutations. The accessible DNA of these subjects' parents (95.31%, 61/64) was then isolated and subjected to Sanger sequencing to confirm the origin of the detected candidate variants. The verification test results showed that 21.79% variants (17/78) were found to be *de novo* mutations.

In primary gonadal/genital disorders subjects (*n* = 48), *SRD5A2* (steroid 5-alpha-reductase 2) and *AR* (androgen receptor) were the most common candidate genes. *SRD5A2* mutations were detected in 35.42% (17/48) of the patients, and *AR* mutations were detected in 27.08% (13/48) of the patients, accounting for 42.19% (27/64) and 21.88% (14/64) of all the variants detected in this group, respectively (Figure 2 and 3). Of the variants in *SRD5A2* (*n* = 27), most were located on exon 4. The same mutation site (c.680G>A, p.R227Q) was detected in 16 patients, which is considered a hotspot mutation in Chinese patients with 5αRD. Of the variants in the *AR* gene (*n* = 14), most were located on exon 1. To date, no clear hotspot mutations in *AR* have been found in a Chinese cohort.

In central DSD subjects (*n* = 16), variants in genes related to Kallmann syndrome were detected frequently in our cohort, including *ANOS1* (anosmin 1), *CHD7* (chromodomain helicase DNA-binding protein 7), *FGFR1* (fibroblast growth factor receptor 1), *GNRHR* (gonadotropin-releasing hormone receptor), *SOX2*

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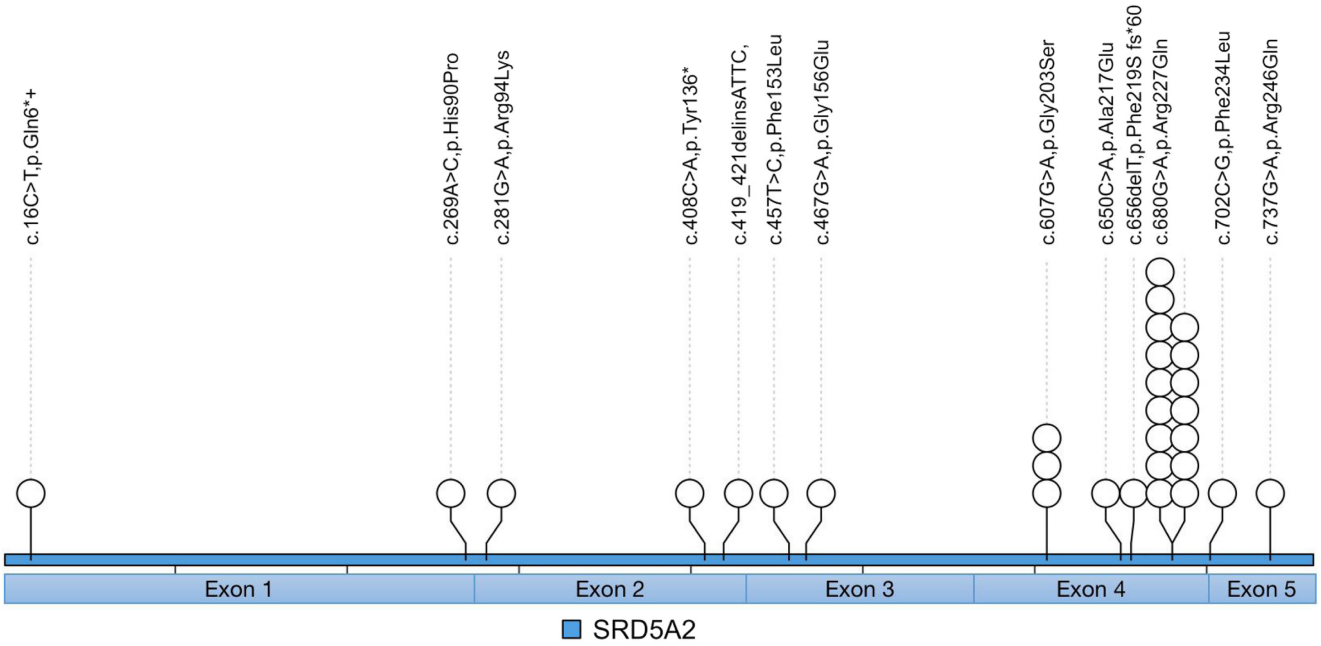

Figure 2
Mutations detected in *SRD5A2* gene in our cohort. All the variants and the hot spot (c.680G>A, p. Arg227Gln) detected in *SRD5A2* in 5α reductase deficiency patients of the current cohort.

(SRY-box transcription factor 2), *SOX10* (SRY-box transcription factor 10), *and TACR3* (tachykinin receptor 3) (see Table 3). Mutations in *ANOS1* accounted for 25.00% (4/16) in this section. Besides, *GLI2* (GLI family zinc finger 2) variants (18.75%, 3/16) were reported in three subjects diagnosed as hypopituitarism (Fig. 4).

Rare variants

To be mentioned, we have reported a rare variant in *TBX3* (T-box transcription factor 3). *TBX3* is a critical developmental regulator of heart, mammary glands, limbs, and lungs. It is considered a pathogenic gene for Ulnar-mammary syndrome according to limited literature from the HGMD database. In Ulnar-mammary syndrome, DSD phenotypes such as hypospadias, micropenis, or cryptorchidism could be observed (20). Here we report a 5-month-old boy presented with bilateral cryptorchidism and bilateral fifth finger joint malformation. Abnormal mammary development was not found up to now. After prediction of the variant's (c.932dupA, p. Glu312Glyfs*6) function by Alamut software, this subject was thus diagnosed as Ulnar-mammary syndrome with a *de novo* pathogenic *TBX3* mutation c.932dupA, p. Glu312Glyfs*6.

Another likely pathogenic variant (c.600+1G>C) in pleckstrin homology domain-interacting protein (*PHIP*) was found in a 12.5-year-old boy, presented with intellectual disability, unilateral renal agenesis, obesity, and bilateral cryptorchidism. *PHIP* is known as an implicated gene for Chung–Jansen syndrome, characterized by developmental delay, intellectual disability, obesity, and dysmorphism (21). According to the previous case, it is reported that genital disorders can be observed in Chung–Jansen syndrome (21), differed from our case.

Among VUS variants, we also noticed a homo variant (c.136+1G>A) in growth hormone receptor (*GHR*) which has not been verified to be related to 46,XY DSD. The 5-year-old patient presented with dominant female external genitalia, suspicious uterine-like structure between bladder and rectum, absent gonad structure and short stature. The paraclinical results showed low level of testosterone (<0.10 ng/mL), DHT (26.63 pg/mL), and estrogen (<20.00 pg/mL). The gonadotropin was augmented as LH 0.26 mIU/mL and FSH 10.73 mIU/mL. The evaluation of the adrenal axis showed normal function. The patient was clinically diagnosed as growth hormone insensitivity and 46,XY DSD. According to the literature review, other than growth hormone insensitivity, *GHR* could also cause Laron syndrome characterized by a typical phenotype including dwarfism, obesity, and hypogenitalism (22). However, no cases of dominant female external genitalia were reported, and functional experiments are still lacking. Whether *GHR* variant could cause sex

Table 3 Phenotype and genotype of primary gonadal/genital disorders subjects with P/LP variants.

Patient ID	Age	Phenotype	Transcript ID	Genetic variants	Zygosity	Family inheritance	Inheritance mode	Pathogenicity	gnomAD frequency	Popmax
1880	2 months	Penoscrotal transposition, bifid scrotum	NM_003140.2	*SRY* c.397C>T, p. Arg133Trp	Hemi	NA	YL	P	/	/
1886	6 months	Ambiguous genitalia, blind perineal pouch resembling vagina, cryptorchidism	NM_000348.3	*SRD5A2* c.419_421delinsATTC, p.Trp140Tyrfs*8	Het	F	AR	P	/	/
				SRD5A2 c.680G>A, p.Arg227Gln	Het	M	AR	P	0.0002235	0.004817 (East Asian)
2367	11 years	Predominantly female external genitalia	NM_000102.3	*CYP17A1* c.985_987delinsAA, p.Tyr329Kfs*90	Homo	F + M	AR	P	0.00004599	0.0006317 (East Asian)
2420	7 months	Micropenis, cryptorchidism	NM_000348.3	*SRD5A2* c.680G>A, p.Arg227Gln	Homo	F + M	AR	P	0.0002235	0.004817 (East Asian)
2439	2 months	Ambiguous external genitalia	NM_004959.4	*NR5A1* c.721C>T, p.Arg241Trp	Het	M	AD	LP	/	/
2540	17 years	Predominantly female external genitalia, cryptorchidism	NM_000044.3	*AR* c.1787G>A, p.Cys596Tyr	Hemi	*De novo*	XR	P	/	/
2752	1 year	Micropenis, hypogonadism	NM_000044.3	*AR* c.2528T>C, p.Ile843Thr	Hemi	M	XR	LP	/	
2782	2 years 9 months	Perineal hypospadias, micropenis	NM_000348.3	*SRD5A2* c.680G>A, p.Arg227Gln	Het	F	AR	P	0.0002235	0.004817 (East Asian)
				SRD5A2 c.281G>A, p.Arg94Lys	Het	M	AR	LP	/	/
2892	7 months	Ambiguous genitalia, micropenis, severe hypospadias, cryptorchidism	NM_000348.3	*SRD5A2* c.607G>A, p.Gly203Ser	Het	NA	AR	P	0.00002629	0.0001567 (East Asian)
				SRD5A2 c.737G>A, p.Arg246Gln	Het	NA	AR	LP	0.000126	0.0004087 (South Asian)
3167	6 years	Predominantly female external genitalia, cryptorchidism	NM_000233.3	*LHCGR* c.681-1Gly>Ala	Het	F	AR	P	/	/
		Predominantly female external genitalia, cryptorchidism		*LHCGR* c.1582_1585delATAT, p.Ile528*	Het	M	AR	P	/	/
4004	11 years	Micropenis, hypogonadism	NM_000044.3	*AR* c.1175C>G, p.Pro392Arg	Hemi	M	XR	LP	0.00001776	0
4119	5 years	Micropenis, bilateral cryptorchidism, gonadal dysplasia	NM_003309.3	*TSPYL1* c.67C>T, p.Gln23*	Het	*De novo*	AR	LP	0.0001441	0.001463 (East Asian)
4208	10 months	Female external genitalia, clitoromegaly	NM_000044.3	*AR* c.2256G>A, p.Trp752*	Hemi	M	XR	P	/	/
4622	1 year 7 months	Hypospadias, penile curvature, cryptorchidism	NM_000348.3	*SRD5A2* c.269A>C, p.His90Pro	Het	F	AR	LP	/	/
				SRD5A2 c.680G>A, p.Arg227Gln	Het	M	AR	P	0.0002235	0.004817 (East Asian)
4986	6 months	Ambiguous genitalia, hypospadias, cryptorchidism	NM_004959.4	*NR5A1* c.251G>A, p.Arg84His	Het	M	AD	P	/	/

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Table 3 Continued.

Patient ID	Age	Phenotype	Transcript ID	Genetic variants	Zygosity	Family inheritance	Inheritance mode	Pathogenicity	gnomAD frequency	Popmax
5015	8 months	Cryptorchidism, inguinal fallopian tubes	NM_000479.3	*AMH* c.585C>A, p.Tyr195*	Het	F	AR	LP	/	/
				AMH c.1165G>T, p.Glu389*	Het	M	AR	LP	0.0000198	/
6791	2 years 9 months	Micropenis	NM_000348.3	*SRD5A2* c.680G>A, p.Arg227Gln	Homo	F+M	AR	P	0.0002235	0.004817 (East Asian)
7847	9 years 9 months	Hypospadias, micropenis, penile curvature	NM_000348.3	*SRD5A2* c.656delT, p.Phe219Serfs*60	Het	F	AR	P	0.00000657	0
7929	6 years	Penoscrotal hypospadias, cryptorchidism	NM_004959.4	*NR5A1* c.848G>T, p.Cys283Phe	Het	M	AD	LP	/	/
8065	10 months	Female external genitalia, cryptorchidism	NM_000044.3	*AR* del Exon5-8	Hemi	NA	XR	P	/	
8803	10 years	Micropenis, hypospadias	NM_000044.4	*AR* c.2270A>G, p.Asn757Ser	Hemi	M	XR	LP	0.000008892	0
8945	1 month	Hyperpigmentation, cryptorchidism, electrolyte disturbance	NM_000475.4	*NR0B1* c.1169-2Ala>Gly	Hemi	*De novo*	XL	P	/	/
9052	1 year	Female external genitalia, cryptorchidism	NM_000044.4	*AR* c.2495G>A, p.Arg832Gln	Hemi	M	XR	LP	0.000008987	0
9242	1 year 3 months	Perineal hypospadias, bifid scrotum	NM_000348.3	*SRD5A2* c.650C>A, p.Ala217Glu	Het	F	AR	LP	/	/
				SRD5A2 c.680G>A, p.Arg227Gln	Het	M	AR	P	0.0002235	0.004817 (East Asian)
9431	3 years 1 month	Ambiguous genitalia, cryptorchidism	NM_000348.3	*SRD5A2* c.607G>A, p.Gly203Ser	Het	F	AR	P	0.00002629	0.0001567 (East Asian)
				SRD5A2 c.680G>A, p.Arg227Gln	Het	M	AR	P	0.0002235	0.004817 (East Asian)
9471	12 years	Predominantly female external genitalia, clitoromegaly, cryptorchidism	NM_000044.4	*AR* c.4G>A, p.Glu2Lys	Hemi	M	XR	LP	/	
9482	5 years	Micropenis, cryptorchidism	NM_000348.3	*SRD5A2* c.680G>A, p.Arg227Gln	Homo	F + M	AR	P	0.0002235	0.004817 (East Asian)
10066	5 months	Micropenis, cryptorchidism	NM_000044.4	*AR* c.899G>C, p.Gly300Ala	Hemi	M	XR	LP	0.00002657	0.00001494 (European)
10239	2 years	Bilateral cryptorchidism, motor development retardation, ptosis, short stature, hydrocephalus	/	7q33-q35Del	Het	M	-	P	/	/
10482	7 years 6 months	Bilateral cryptorchidism, short stature, funnel chest	NM_002934.4	*PTPN11* c.1472C>A, p.Pro491His	Het	*De novo*	AD	P	/	/

(*Continued*)

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Table 3 Continued.

Patient ID	Age	Phenotype	Transcript ID	Genetic variants	Zygosity	Family inheritance	Inheritance mode	Pathogenicity	gnomAD frequency	Popmax
10800	2 months 12 days	Hypospadias, penoscrotal transposition, micropenis, bifid scrotum	/	2q33.1Del	Het	*De novo*	-	LP	/	/
10892	3 years 5 months	Unilateral cryptorchidism, short stature	NM_002934.4	*PTPN11* c.922A>G, p.Asn308Asp	Het	*De novo*	AD	P	0.00001193	0
10908	14 years 6 months	Female external genitalia, cryptorchidism, gynecomastia	NM_000044.4	*AR* c.1737delC, p.Cys580Alafs*46	Hemi	M	XR	P	/	/
11072	15 years 6 months	Female external genitalia, cryptorchidism, clitoromegaly	NM_000044.4	*AR* c.2113C>T, p.Leu705Phe	Hemi	M	XR	LP	/	
11757	8 months	Hypospadias, micropenis	*NM_000348.3*	*SRD5A2* c.16C>T, p.Gln6*	Het	M	AR	P	/	/
				SRD5A2 c.680G>A, p.Arg227Gln	Het	F	AR	P	0.0002235	0.004817 (East Asian)
11867	6 months	Micropenis	NM_000348.3	*SRD5A2* c.680G>A, p.Arg227Gln	Homo	F + M	AR	P	0.0002235	0.004817 (East Asian)
12480	5 years	Bilateral cryptorchidism, mental retardation, intelligence disability, ptosis, strabismus	/	18p11.31-p11.21del	Het	*De novo*	-	P	/	/
12888	13 years	Bilateral cryptorchidism, micropenis, intelligence disability	/	16p11.2del	Het	*De novo*	-	P	/	/
13048	5 years	Micropenis, mental retardation	/	17p11.2del	Het	*De novo*	-	P	/	/
13421	4 years	Micropenis, hypospadias, penoscrotal transposition	NM_000044.4	*AR* c.2020T>C, p.Phe674Leu	Hemi	M	XR	LP	/	/
14250	4 months 3 days	Micropenis, hypospadias, bifid scrotum	NM_000348.3	*SRD5A2* c.607G>A, p.Gly203Ser	Het	F	AR	P	0.00002629	0.0001567 (East Asian)
				SRD5A2 c.680G>A, p.Arg227Gln	Het	M	AR	P	0.0002235	0.004817 (East Asian)
15362	1 year 9 months	PMDS, transverse testicular ectopia	NM_000479.3	*AMH* c.259-270del, p.Val187-Ala90del	Het	M	AR	LP	/	/
				AMH c.1432C>T, p.Leu478Phe	Het	F	AR	LP	/	/

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Table 3 Continued.

Patient ID	Age	Phenotype	Transcript ID	Genetic variants	Zygosity	Family inheritance	Inheritance mode	Pathogenicity	gnomAD frequency	Popmax
16213	3 years 11 months	Micropenis	NM_000348.3	*SRD5A2* c.680G>A, p.Arg227Gln	Homo	F + M	AR	P	0.0002235	0.004817 (East Asian)
16746	5 years 4 months	Micropenis	NM_000348.3	*SRD5A2* c.680G>A, p.Arg227Gln	Homo	F + M	AR	P	0.0002235	0.004817 (East Asian)
18423	6 years 10 months	Bilateral cryptorchidism, dysmorphic facial features (ocular hypertelorism, low nose bridge, flapping ear, ptosis), chicken breast, lumbar lateral curvature, brachydactylia, pulmonic stenosis, atrial septal defect, patent foramen ovale, lymphangioma of right lower limb	NM_006912.6	*RIT1* c.170C>G, p.Ala57Gly	Het	F	AD	P	/	/
18553	11 years 9 months	Micropenis	NM_000348.3	*SRD5A2* c.680G>A, p.Arg227Gln	Homo	F + M	AR	P	0.0002235	0.004817 (East Asian)
19697	2 years 8 months	Micropenis	NM_000348.3	*SRD5A2* c.467G>A, p.Gly156Glu	Het	M	AR	LP	0.000006677	0
20472	13 years 10 months	Hypospadias, penoscrotal transposition	NM_000044.6	*AR* c.2521C>T, p.Arg841Cys	Hemi	M	XR	LP	/	/
				AR c.1175C>G, p.Pro392Arg	Hemi	M	XR	LP	0.00001776	0

AD, autosomal dominant; AR, autosomal recessive; F, father; Hemi, hemizygote; Het, heterozygote; Homo, homozygote; LP, likely pathogenic; M, mother; P, pathogenic; PMDS, persistent Müllerian duct syndrome; XL, X chromosome linked; YL, Y chromosome linked.

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Table 4 Phenotype and genotype of central hypogonadotropic DSD subjects with P/LP variants.

Patient ID	Age	Phenotype	Transcript ID	Genetic variants	Zygosity	Family inheritance	Inheritance mode	Pathogenicity	gnomAD frequency	Popmax
2061	15 years	Hypogonadotropic hypogonadism, hyposmia	NM_000216.2	*ANOS1* del Exon 4-5	Hemi	M	XR	LP	/	/
2356	7 years	micropenis, small testes	NM_000406.2	*GNRHR* c.806C>T, p.Thr269Met	Homo	F + M	AR	LP	0.00001194	0.00000293 (European non-Finnish)
2955	6 years	Hypogonadotropic hypogonadism, bilateral cryptorchidism, small testes	NM_000216.2	*ANOS1* c.1712G>A, p.Trp571*	Hemi	*De novo*	XR	P	/	/
3879	15 years	Hypogonadotropic hypogonadism, hyposmia, absence of olfactory bulb	NM_000216.2	*ANOS1* c.984C>G, p.Tyr328*	Hemi	M	XR	LP	/	/
3973	11 years	Gonadal dysplasia, cryptorchidism, short stature	NM_005634.2	*SOX3* c.424C>A, p.Pro142Thr	Hemi	M	XL	LP	/	/
4136	11 years	Gonadal dysplasia, hypogonadotropic hypogonadism	NM_001059.2	*TACR3* c.196dupG, p.Ala66fs*84	Het	F + M	AR	LP	/	/
5175	1 year	Micropenis, short stature	NM_005270.4	*GLI2* c.3463_3464delGA, p.Asp1155Arg fs*39	Het	*De novo*	AD	P	/	/
5589	2 years 3 months	Micropenis, cryptorchidism	NM_005270.4	*GLI2* c.3137delG, p.Gly1046Ala fs*84	Het	M	AD	LP	/	/
6606	3 years	Micropenis, small testes	NM_005270.4	*GLI2* c.3640C>T, p.Gln1214*	Het	M	AD	LP	/	/
12343	10 years	Micropenis, gonadal dysgenesis, short stature	NM_017780.3	*CHD7* c.5211-2A>G	Het	*De novo*	AD	P	/	/
14317	12 years 5 months	Micropenis, hypogonadotropic hypogonadism, mild intelligence disability, absence of olfactory bulb (normal olfactory function)	NM_003106.3	*SOX2* c.259A>G, p.Lys87Glu	Het	*De novo*	AD	LP	/	/
14376	8 years 9 months	Hypogonadotropic hypogonadis, micropenis, bilateral cryptorchidism, hyposmia	NM_000216.2	*ANOS1* c.515G>A, p.Cys172Tyr	Hemi	M	XR	LP	/	/
15071	4 years 8 months	Micropenis, motor development retardation	NM_006941.3	*SOX10* c.506C>T, p.Pro169Leu	Het	M	AD	LP	/	/
15128	5 months	Bilateral cryptorchidism, bilateral pinky joint malformation	NM_005996.3	*TBX3* c.932dupA, p.Glu312Glyfs*6	Het	*De novo*	AD	P	/	/
15562	1 year 1 month	Micropenis, bilateral cryptorchidism, hyposmia	NM_006941.3	*SOX10* c.506C>T, p.Pro169Leu	Het	*De novo*	AD	LP	/	/

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Table 4 Continued.

Patient ID	Age	Phenotype	Transcript ID	Genetic variants	Zygosity	Family inheritance	Inheritance mode	Pathogenicity	gnomAD frequency	Popmax
16255	13 years 3 months	Bilateral cryptorchidism, micropenis, absence of olfactory bulb, intelligence disability, dysmorphic facial features (long philtrum, congenital cup ear, ocular hypertelorism), brachydactyly (finger), syndactyly (toe), abnormal brain structure	NM_023110.2	*FGFR1* c.2173T>C, p.Cys725Arg	Het	*De novo*	AD	LP	/	/
2061	15 years	Hypogonadotropic hypogonadism, hyposmia	NM_000216.2	*ANOS1* del Exon 4-5	Hemi	M	XR	LP	/	/
2356	7 years	Micropenis, small testes	NM_000406.2	*GNRHR* c.806C>T, p.Thr269Met	Homo	F + M	AR	LP	0.00001194	0.00000293 (European non-Finnish)
2955	6 years	Hypogonadotropic hypogonadism, bilateral cryptorchidism, small testes	NM_000216.2	*ANOS1* c.1712G>A, p.Trp571*	Hemi	*De novo*	XR	P	/	/
3879	15 years	Hypogonadotropic hypogonadism, hyposmia, absence of olfactory bulb	NM_000216.2	*ANOS1* c.984C>G, p.Tyr328*	Hemi	M	XR	LP	/	/
3973	11 years	Gonadal dysplasia, cryptorchidism, short stature	NM_005634.2	*SOX3* c.424C>A, p.Pro142Thr	Hemi	M	XL	LP	/	/
4136	11 years	Gonadal dysplasia, hypogonadotropic hypogonadism	NM_001059.2	*TACR3* c.196dupG, p.Ala66fs*84	Het	F + M	AR	LP	/	/
5175	1 year	Micropenis, short stature	NM_005270.4	*GLI2* c.3463_3464delGA, p.Asp1155Arg fs*39	Het	*De novo*	AD	P	/	/
5589	2 years 3 months	Micropenis, cryptorchidism	NM_005270.4	*GLI2* c.3137delG, p.Gly1046Ala fs*84	Het	M	AD	LP	/	/
6606	3 years	Micropenis, small testes	NM_005270.4	*GLI2* c.3640C>T, p.Gln1214*	Het	M	AD	LP	/	/
12343	10 years	Micropenis, gonadal dysgenesis, short stature	NM_017780.3	*CHD7* c.5211-2A>G	Het	*De novo*	AD	P	/	/
14317	12 years 5 months	Micropenis, hypogonadotropic hypogonadism, mild intelligence disability, absence of olfactory bulb (normal olfactory function)	NM_003106.3	*SOX2* c.259A>G, p.Lys87Glu	Het	*De novo*	AD	LP	/	/

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Table 4 Continued.

Patient ID	Age	Phenotype	Transcript ID	Genetic variants	Zygosity	Family inheritance	Inheritance mode	Pathogenicity	gnomAD frequency	Popmax
14376	8 years 9 months	Hypogonadotropic hypogonadis, micropenis, bilateral cryptorchidism, hyposmia	NM_000216.2	*ANOS1* c.515G>A, p.Cys172Tyr	Hemi	M	XR	LP	/	/
15071	4 years 8 months	micropenis, motor development retardation	NM_006941.3	*SOX10* c.506C>T, p.Pro169Leu	het	M	AD	LP	/	/
15128	5 months	Bilateral cryptorchidism, bilateral pinky joint malformation	NM_005996.3	*TBX3* c.932dupA, p.Glu312Glyfs*6	Het	*De novo*	AD	P	/	/
15562	1 year 1 month	Micropenis, bilateral cryptorchidism, hyposmia	NM_006941.3	*SOX10* c.506C>T, p.Pro169Leu	Het	*De novo*	AD	LP	/	/
16255	13 years 3 months	Bilateral cryptorchidism, micropenis, absence of olfactory bulb, intelligence disability, dysmorphic facial features (long philtrum, congenital cup ear, ocular hypertelorism), brachydactyly (finger), syndactyly (toe), abnormal brain structure	NM_023110.2	*FGFR1* c.2173T>C, p. Cys725Arg	Het	*De novo*	AD	LP	/	/
2061	15 years	Hypogonadotropic hypogonadism, hyposmia	NM_000216.2	*ANOS1* del Exon 4-5	Hemi	M	XR	LP	/	/
2356	7 years	Micropenis, small testes	NM_000406.2	*GNRHR* c.806C>T, p.Thr269Met	Homo	F + M	AR	LP	0.00001194	0.00000293 (European non-Finnish)
2955	6 years	Hypogonadotropic hypogonadism, bilateral cryptorchidism, small testes	NM_000216.2	*ANOS1* c.1712G>A, p. Trp571*	Hemi	*De novo*	XR	P	/	/
3879	15 years	Hypogonadotropic hypogonadism, hyposmia, absence of olfactory bulb	NM_000216.2	*ANOS1* c.984C>G, p.Tyr328*	Hemi	M	XR	LP	/	/
3973	11 years	Gonadal dysplasia, cryptorchidism, short stature	NM_005634.2	*SOX3* c.424C>A, p.Pro142Thr	Hemi	M	XL	LP	/	/
4136	11 years	Gonadal dysplasia, hypogonadotropic hypogonadism	NM_001059.2	*TACR3* c.196dupG, p.Ala66fs*84	Het	F + M	AR	LP	/	/
5175	1 year	Micropenis, short stature	NM_005270.4	*GLI2* c.3463_3464delGA, p.Asp1155Arg fs*39	Het	*De novo*	AD	P	/	/
5589	2 years 3 months	Micropenis, cryptorchidism	NM_005270.4	*GLI2* c.3137delG, p.Gly1046Ala fs*84	Het	M	AD	LP	/	/

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Table 4 Continued.

Patient ID	Age	Phenotype	Transcript ID	Genetic variants	Zygosity	Family inheritance	Inheritance mode	Pathogenicity	gnomAD frequency	Popmax
6606	3 years	Micropenis, small testes	NM_005270.4	*GLI2* c.3640C>T, p.Gln1214*	Het	M	AD	LP	/	/
12343	10 years	Micropenis, gonadal dysgenesis, short stature	NM_017780.3	*CHD7* c.5211-2A>G	Het	*De novo*	AD	P	/	/
14317	12 years 5 months	Micropenis, hypogonadotropic hypogonadism, mild intelligence disability, absence of olfactory bulb (normal olfactory function)	NM_003106.3	*SOX2* c.259A>G, p.Lys87Glu	Het	*De novo*	AD	LP	/	/
14376	8 years 9 months	Hypogonadotropic hypogonadis, micropenis, bilateral cryptorchidism, hyposmia	NM_000216.2	*ANOS1* c.515G>A, p.Cys172Tyr	Hemi	M	XR	LP	/	/
15071	4 years 8 months	Micropenis, motor development retardation	NM_006941.3	*SOX10* c.506C>T, p.Pro169Leu	Het	M	AD	LP	/	/
15128	5 months	Bilateral cryptorchidism, bilateral pinky joint malformation	NM_005996.3	*TBX3* c.932dupA, p.Glu312Glyfs*6	Het	*De novo*	AD	P	/	/

AD, autosomal dominant; AR, autosomal recessive; F, father; Hemi, hemizygote; Het, heterozygote; Homo, homozygote; LP, likely pathogenic; M, mother; P, pathogenic; XL, X chromosome linked.

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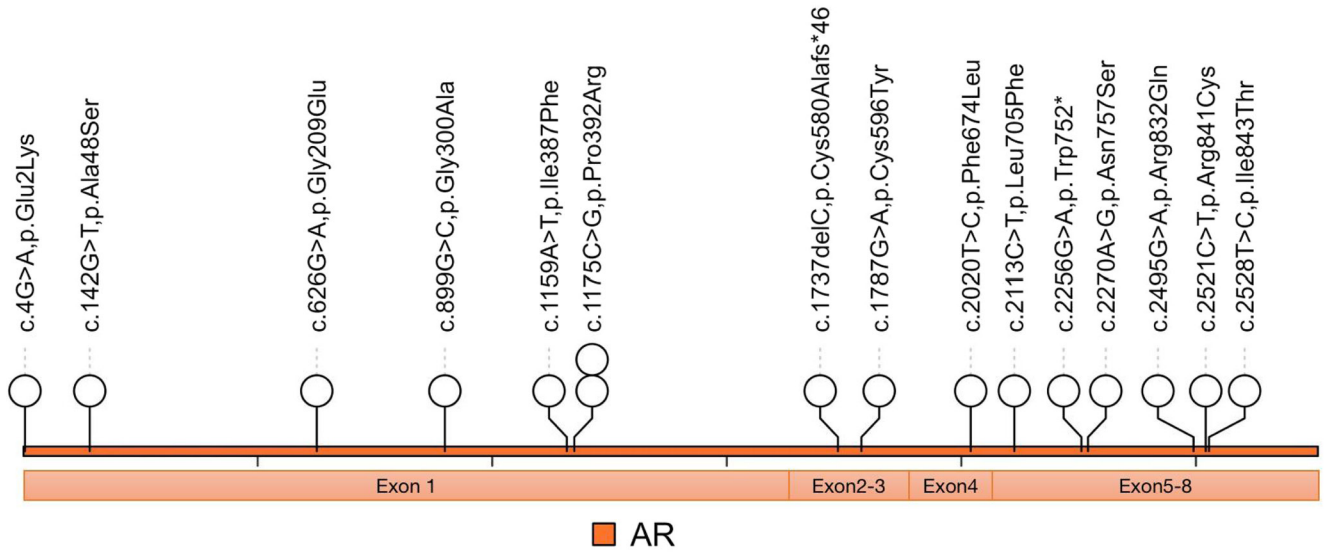

Figure 3
Mutations detected in *AR* gene in our cohort. Most of the variants detected in *AR* located on Exon1 in our androgen insensitivity syndrome patients.

differential or sex development disorder remains a question.

A VUS variant (c.2129A>G, p. Tyr710Cys) in *NOTCH3* (Notch Receptor 3) was found in a 2-month-old boy who presented with short stature, penile curvation, hypospadias, bilateral cryptorchidism, micropenis, penile scrotal translocation, scrotal division, special features (high hairline, forehead protrusion), premature delivery, and small for gestational age. The patient showed a remarkable DSD phenotype. However, at the moment, we only found a VUS variant in *NOTCH3* by WES. Cases related to *NOTCH3*, for example, CADASIL patients, do not include sexual disorders (23). Thus, the pathogenicity of NOTCH3 in sex development is still under-researched. Further genetical evaluation and functional verification are required to determine the pathogenesis mechanism.

Comparison of TPS and WES

After all, we intended to compare the efficacy and results of two different sequencing technologies in the current cohort. As mentioned earlier, subjects between 2016 and 2018 ($n=92$) underwent TPS (group TPS), while those during 2019 and 2022 ($n=86$) underwent WES (group WES). We compared the characteristics of these two groups to different degrees (see Table 5).

No significant differences were observed in the average age of the subjects at diagnosis. Second, the proportion of the DSD phenotype among the 46,XY DSD patients slightly increased. The proportion of patients with micropenis increased from 50.00 to 63.95% and that of patients with cryptorchidism increased from 32.61 to 36.05%. However, the proportion of patients with hypospadias decreased from 19.57 to 15.12%. This might have contributed to the early surgical correction in these patients, which led to a decrease in the number of patients in the endocrinology department. In addition, the proportion of patients with single phenotype (micropenis, cryptorchidism, hypospadias, GD, hypogonadism, undeveloped testis) has increased since 2019 (46.2 vs 58.8%, $P=0.288$). When the composition and clinical diagnosis between the two groups were compared, the percentage of AIS and 5αRD remained similar (22.64 vs 22.85% for AIS; 18.90 vs 17.14% for 5α RD), while the percentage of IHH slightly increased from 20.75 to 22.86%. Among all the variants detected, pathogenic and likely pathogenic variants accounted for 39.68 and 33.33% in group 2016–2018, respectively, while the percentage of pathogenic variants in group 2019–2022 has increased to 45.00% and that of likely pathogenic variants increased to 37.50%. Since 2019, under the application of WES technology, we have diagnosed much more complex syndromic DSD due to the accurate interpretation of variants at our center.

Discussion

Patients with 46,XY DSD present with various clinical manifestations and complex pathogenesis, which increases the difficulty of diagnosis (24, 25). To understand

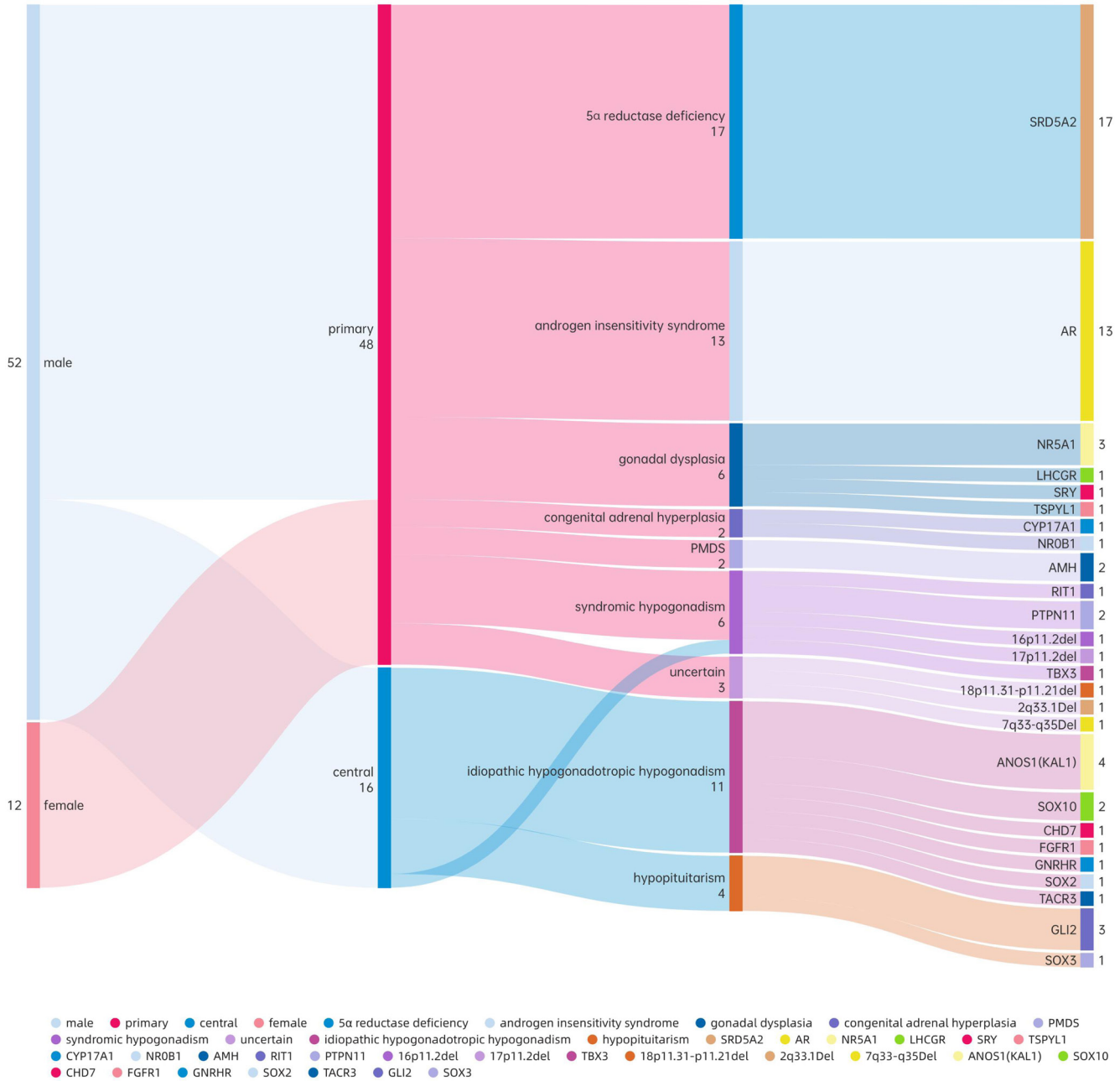

Figure 4
Genetic expansion of primary gonadal/genital disorders and central hypogonadism in this cohort. 79 variants accross 21 genes were detected in 65 subjects of the current study. AMH, anti-Mullerian hormone; ANOS1,anosmin 1; AR, androgen receptor; CHD7, chromodomain helicase DNA binding protein 7; CYP17A1, cytochrome P450 family 17 subfamily A member 1; FGFR1, fibroblast growth cactor receptor 1; GLI2, GLI family zinc finger 2; GNRHR, gonadotropin releasing hormone receptor; LHCGR, luteinizing hormone/choriogonadotropin receptor; NR0B1, nuclear receptor subfamily 0 group B member 1; RIT1, Ras like without CAAX 1; SOX10, SRY-box transcription factor 10; SOX2, SRY-box transcription factor 2; SOX3, SRY-box transcription factor 3; SRD5A2=Steroid 5 Alpha-reductase 2; SRY, sex determining region Y; TACR3,tachykinin receptor 3; TBX3, T-box transcription factor 3; TSPYL1, TSPYlike 1.

the diagnosis and pathogenesis and to improve the infertility consequences for patients, research is needed to expand the phenotype and genotype (26).

Overall speaking, the most common symptoms in 46,XY DSD patients are cryptorchidism, hypospadias, and micropenis. In our study, the main symptoms of patients in different age groups were different. Mini-pubertal and prepubertal children mostly presented with cryptorchidism and micropenis. Adolescent patients usually present with less cryptorchidism or hypospadias

Table 5 Comparison of targeted panel sequencing group (group TPS) and whole-exome sequencing group (group WES) in our cohort.

Features	TPS ($n = 92$)	WES ($n = 86$)	*P*-value
Clinical manifestation			
Age (years)	8.00 (1.58, 12.00)	8.67 (3.23, 11.79)	0.808
Micropenis, %	50.00%	63.95%	0.060
Cryptorchidism, %	32.61%	36.06%	0.629
Hypospadia, %	19.57%	15.12%	0.434
Genetic characteristics			
Diagnosis rate, % (*n*/*N*)	57.61% (53/92)	40.70% (35/86)	0.024[a]
Pathogenic mutation, % (*n*/*N*)	39.68% (25/63)	45.00% (18/40)	0.594
Likely pathogenic mutation, % (n/N)	33.33% (21/63)	37.50% (15/40)	0.666
VUS mutation, % (*n*/*N*)	25.87% (18/63)	15.00% (6/40)	0.267
De novo mutation, % (*n*/*N*)	6.34% (4/63)	32.50% (13/40)	0.001[a]
Missense mutation, % (*n*/*N*)	71.43% (45/63)	72.50% (29/40)	0.906
Nonsense mutation, % (*n*/*N*)	11.11% (7/63)	5.00% (2/40)	0.284
Deletion mutation, % (*n*/*N*)	15.87% (10/63)	20.00% (8/40)	0.591

Subjects between 2016 and 2018 ($n = 92$) underwent targeted gene panel sequencing (group TPS), those between 2019 and 2022 ($n = 86$) underwent whole-exome sequencing (group WES). We compared the characteristics of these two groups to both clinical phenotype and genetic degrees. *[a]Significant difference between group TPS and group WES, *P* value by the Pearson Chi-square, $P < 0.05$ was considered statistically significant.
[a]Significant difference between group TPS and group WES, *P*-value by the Pearson chi-square, $P < .05$ was considered statistically significant.

but are more likely to undergo micropenis and delayed puberty (characterized by undeveloped testicular volume and lack of secondary sex characteristics at the age of 14 in boys). A family history of delayed pubertal development is an important factor in distinguishing between DSD and a constitutional delay of growth and puberty (27). In other studies (28, 29), some children with DSD were reported to have spontaneous pubertal development. For patients with mild manifestations, it becomes more difficult to detect the symptoms accurately early, making the initial intervention difficult (30).

Currently, the age of diagnosis of 46,XY DSD has advanced as early as the neonatal period due to prenatal genetic testing and typical genital differences or associated symptoms observed soon after birth (31, 32). In our group, the overall average diagnosis of Chinese 46,XY patients was 8.63 (2.61,12.00) years, ranging from 1 month to 17 years. The median age of primary gonadal/genital disorders subjects was 2.92 (0.67, 7.33) years old. It is known that the detection of 46,XX CAH patients can be performed as early as 0.25 months old based on specific salt-losing features, electrolyte disturbances, and abnormal hormonal profiles. One of the early diagnosed patients (0.08 years) was a 46,XY atypical CAH boy (Patient ID 8945) whose symptoms manifested as hyperpigmentation, cryptorchidism, and electrolyte disturbance, indicating that early detection of CAH in 46,XY males is required and can be identified using NGS.

5αRD and AIS accounted for an important part in primary DSD (33). AIS was the most frequent cause of 46,XY DSD in the previous large Asian cohort, and 5αRD was ranked as the second. These two subgroups accounted for approximately 50.5% of all cases (33). AIS and 5αRD patients both presented abnormal androgen function. We have found that the median age of 5αRD was 2.00 (0.58, 4.46) years old and that of AIS subjects was 10.00 (0.92, 14.17) years old ($P = 0.06$) (see Table 2). The patients' testosterone and DHT levels in these diseases remained similar, but the gonadotropin levels in 5α-reductase-deficient patients were much lower than those in AIS patients, thus indicating that gonadotropin may be a marker for distinguishing between these two diseases.

On the other side, among central hypogonadism subjects, the median age at diagnosis was 7.88 (2.69, 12.07) years old. The specific phenotypes of these pre-pubertal and adolescent subjects were micropenis and small tests (average testis volume). And the most common clinical diagnosis of central hypogonadism is IHH and isolated hypopituitarism (hypogonadotropin). In IHH patients, multi-system manifestation evaluations are important for disease detection and diagnosis, such as olfactory dysfunction, intellectual disability, delayed motor development, skeletal deformities, brain structure abnormalities, and hearing disorders. These non-endocrinological manifestations in elder children probably refer to IHH.

To further explore the distribution of 46,XY DSD, we then concluded the clinical characteristics in those who had a P/LP genetic variant ($n = 64$) and those whose genetic results were negative ($n = 90$). First, we found a significant

age difference at diagnosis between the NGS-positive group (4.00 years old) and the NGS-negative group (8.75 years old) ($P < 0.05$). Second, the proportion of subjects presenting with unilateral or bilateral cryptorchidism in the NGS-negative group (21.11%) was smaller than that in the NGS-positive group (50.00%) ($P < 0.05$); the proportion of subjects presenting with isolated micropenis (28.89%, 26/90) was larger than that of NGS-positive group (9.38%, 6/64). Thus, we think that the value of gene sequencing in those young-age and cryptorchidism patients is especially important. And on the other hand, the mechanism of micropenis should be further explored in the future, since quite a part of subjects with isolated micropenis have not found a genetic explanation.

In the genetic aspect, with the development and application of NGS, the molecular detection rate of P/LP variants in 46,XY DSD has become easier and more efficient (34). To date, there are more than 300 candidate genes associated with DSD phenotypes (35), and the efficiency of molecular diagnosis of DSD patients has improved from 33% to 74.3% (36, 37, 38). The total molecular detection rate of the current cohort was 49.44% and the genetic diagnostic rate (including patients with P/LP variants only) was 35.96% (64/178).

In our study, among the genes associated with 46,XY DSD, the most common pathogenic variants in primary gonadal/genital disorders were variants in *AR* and *SRD5A2* (Figures 2 and 3). Previous study indicated that variants in *SRD5A2* are mostly distributed on exons 1, 4, and 5 (40), and the variant c.680G>A (p.R227Q) is the hotspot site in Chinese 5αRD, which is consistent with our results. The most common genetic change of central hypogonadism is in IHH-related genes, especially *ANOS1*, similar to the results in previous literature (39). The diagnosis of IHH is considered complicated also due to its genetic heterogeneity. Thus, the interpretation of ACMG guidelines for variants is extremely important. However, in China, due to regional differences and department settings, the interpretation of variants is usually performed by clinicians, instead of geneticists. Therefore, the prediction of variants' function may differ among medical centers. Accurate sequencing protocol and proper interpretation methods are required to unify the standard method of gene sequencing.

In addition to common variants, we have also identified novel candidate variants (*GHR, PHIP*) that might be related to 46,XY DSD, As described in the result part, these three subjects presented with specific sexual disorders manifestation and abnormal sexual hormone levels. However, their genetic results showed undefined variants. Variant c.600+1G>C in *PHIP* was classified as likely pathogenic, and variant c.136+1G>A in *GHR* was classified as pathogenic. These two genes are known as causes of different diseases, of which, the common phenotypes do not involve DSD. Differing from our cases, previous cases of *GHR* and *PHIP* variants were reported to have DSD phenotypes such as genital disorders and hypogenitalism (21, 22). Combined with the functional prediction by Alamut software, the splicing variants in *PHIP* and *GHR* are predicted to have impacts on mRNA processing or expression. Thus, although according to current limited information, the pathogenicity of these variants cannot be clarified yet, we still consider it possible that DSD manifestations might be the new phenotypes of these genes. Functional verification *in vivo* and *in vitro* experiments are required to confirm the role of these variants.

After all, we explored the phenotype–genotype correlation in this study. Of variants in *SRD5A2*, most are missense mutations. Mutation sites c.680G>A, p. Arg227Gln and c.607G>A, p. Gly203Ser were frequently detected in Asian patients, especially in the Chinese population (40). Consistent with previous reports (41), in our study, patients carrying homozygous c.680G>A, p. Arg227Gln presented as milder phenotype (isolated micropenis), while those carrying c.607G>A, p. Gly203Ser (including in compound heterozygosity with p. R227Q) presented as partial androgen insensitivity syndrome (PAIS) with more severe phenotypes (ambiguous genitalia and hypospadias). Earlier work, however, suggested that p.R227Q might moderate the functional severity caused by other variants (42). Further relation between genotype and phenotype depends on the impact of genotype caused enzymatic damage.

Of the variants in the *AR* gene, most current reported mutations were located on exons 5–8, coding for the ligand-binding domain (43). Mutation on exon 1 could cause complete androgen insensitivity syndrome (CAIS), PAIS, or mild androgen insensitivity syndrome phenotypes. And it is reported in the earlier literature that most mutations causing CAIS were on exon 1 (44). However, in our cohort, mutations on exons 2–3, coding for the DNA-binding domain, are more likely to cause CAIS. This inconsistency may be due to the limited sample amount in our cohort.

On the other hand, there is still few information on the phenotype–genotype relation of central hypogonadism DSD. Clinical manifestations vary among patients carrying the same variant. Both in our cohort and previous reports, *GLI2* demonstrates an

autosomal dominant inheritance pattern with incomplete penetrance and variable expressivity in hypopituitary patients (45, 46). In IHH cases, two subjects in our cohort carried the same mutation site in *SOX10*, one manifested as isolated hypogonadotropic hypogonadism, while the other presented hypogonadism with obvious intelligence disability. However, certain multi-system phenotypes may help in confirming the pathogenic gene. Congenital unilateral renal agenesis and/or mirror movements of the upper limbs (synkinesis) in IHH patients refer to *ANOS1* mutation (47); Hearing loss refers to *CHD7* mutation (48); Bone anomalies (including syndactyly and dental agenesis) refers to *FGF8/FGFR1* mutations (48).

Finally, when comparing the genetic findings of the two groups using different sequencing methods, we found that the molecular detection rate of group TPS (2016–2018) was 57.61%, while that of group WES (2019–2022) was 40.70%. Before 2018, our patient source was limited to the local population, where prenatal, neonatal, and pubertal screening of reproduction problems received high attention both in hospitals and in the community; After 2018, our center has widened the clinical patient resource and thus complicated the genetic diagnosis. Second, since male infertility has received much more attention in adolescent health, an increasing number of patients with milder manifestations such as isolated micropenis or isolated undeveloped testis seek endocrinology consultation. The pathogenic mechanisms and new candidate genetic variants that might contribute to mild manifestation are still under investigation. The difference between the two technologies can be concluded that TPS covers most of the genes contributing to DSD, which leads to a high efficiency of genetic diagnosis (19). However, WES can filter those new candidate genes or variants that have not been included in the panel. WES technology can also screen small copy number variations for certain diseases (49). In clinical practice, clinicians should select analysis methods according to the patients' clinical performance and economic reasons.

Among the remaining large part of pathogenically undefined subjects, discussions on its etiology revealed that this might be related to oligo-/poly genetic reasons or other single nucleotide variations that could not be detected by currently applied methods (50). DSD disease is considered a monogenic disorder (51); however, some studies have shown that it may be a polygenic or oligogenic disease (52, 53). In a large international DSD cohort, oligogenic inheritance involving two testis development genes, *MAP3K1* (mitogen-activated protein kinase kinase kinase 1) and *ZFPM2* (zinc finger protein, FOG family member 2), was observed in three patients (34). There is an undefined molecular or environmental mechanism that may lead to various phenotypes and genetic heterogeneity. Moreover, recent research has focused on non-coding variants in regulatory elements that alter gene expression and cause disorders in gonadal development (54).

Limitations

First, this was a single-center study with limited patient resources. Second, some VUS and newly discovered DSD pathogenic genes were found, but it still lacks functional experiments to verify the pathogenicity of these variants and their potential molecular mechanisms. Also, additional testing could be performed on WES-negative patients using microarray for CNV, WGS technology, or RNA sequencing methods to identify additional pathogenic mechanisms among 46,XY DSD patients.

Conclusions

Here, we summarized the clinical and genetic characteristics of 178 Chinese children with 46,XY DSD. The total genetic detection rate was 49.44%. Most children manifested as cryptorchidism, micropenis, and hypogonadal hypogonadism. The top classifications of 46,XY DSD detected were 5αRD, IHH, and AIS. The hotspot mutation in the Chinese cohort was p.R227Q on *SRD5A2*. New candidate variants (*GHR, PHIP*) were detected in this cohort.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-23-0029.

Declaration of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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Ethics statement

The studies involving human participants were reviewed and approved by the Research Ethics Committee of Shanghai Children's Medical Center, Affiliated to School of Medicine, Shanghai Jiao Tong University (No. SCMCIRB-K2016013). Written informed consent to participate in this study was provided by the participants' legal guardian or next of kin. Written informed consent was obtained from the individual(s) and minor(s) legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon reasonable request.

Author contribution statement

Conceptualization: Tang Yijun, Chen Yao, Li Xin, Wang Xiumin. Data curation: Tang Yijun, Wang Jiayi, Wang Yirou, Xu Yufei, Wang Jian. Acquisition: Wang Xiumin, Chen Yao. Methodology: Chen Yao, Wang Jian, Wang Xiumin. Investigation: Tang Yijun, Wang Jiayi, Zhang Qianwen. Supervision: Chen Yao, Wang Jian, Wang Xiumin. Writing—original draft: Tang Yijun. Writing—review and editing: Chen Yao, Wang Jiayi, Wang Xiumin.

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References

1 Bennecke E, Thyen U, Grüters A, Lux A & Köhler B. Health-related quality of life and psychological well-being in adults with differences/disorders of sex development. *Clinical Endocrinology* 2017 **86** 634–643. (https://doi.org/10.1111/cen.13296)

2 Hughes IA, Houk C, Ahmed SF, Lee PA, LWPES Consensus Group & ESPE Consensus Group. Consensus statement on management of intersex disorders. *Archives of Disease in Childhood* 2006 **91** 554–563. (https://doi.org/10.1136/adc.2006.098319)

3 Lux A, Kropf S, Kleinemeier E, Jürgensen M, Thyen U & DSD Network Working Group. Clinical evaluation study of the German network of disorders of sex development (DSD)/intersexuality: study design, description of the study population, and data quality. *BMC Public Health* 2009 **9** 110. (https://doi.org/10.1186/1471-2458-9-110)

4 Mao Y, Chen S, Wang R, Wang X, Qin D & Tang Y. Evaluation and treatment for ovotesticular disorder of sex development (OT-DSD) - experience based on a Chinese series. *BMC Urology* 2017 **17** 21. (https://doi.org/10.1186/s12894-017-0212-8)

5 Yu BQ, Liu ZX, Gao YJ, Wang X, Mao JF, Nie M & Wu XY. Prevalence of gene mutations in a Chinese 46,XY disorders of sex development cohort detected by targeted next-generation sequencing. *Asian Journal of Andrology* 2021 **23** 69–73. (https://doi.org/10.4103/aja.aja_36_20)

6 Hiort O, Birnbaum W, Marshall L, Wünsch L, Werner R, Schröder T, Döhnert U & Holterhus PM. Management of disorders of sex development. *Nature Reviews. Endocrinology* 2014 **10** 520–529. (https://doi.org/10.1038/nrendo.2014.108)

7 Ono M & Harley VR. Disorders of sex development: new genes, new concepts. *Nature Reviews. Endocrinology* 2013 **9** 79–91. (https://doi.org/10.1038/nrendo.2012.235)

8 León NY, Reyes AP & Harley VR. A clinical algorithm to diagnose differences of sex development. *Lancet. Diabetes and Endocrinology* 2019 **7** 560–574. (https://doi.org/10.1016/S2213-8587(18)30339-5)

9 Chen Y, Chen J, Tang Y, Zhang Q, Wang Y, Li Q, Li X, Weng Z, Huang J, Wang X, *et al.* Difference of precocious puberty between before and during the COVID-19 pandemic: a cross-sectional study among shanghai school-aged girls. *Frontiers in Endocrinology* 2022 **13** 839895. (https://doi.org/10.3389/fendo.2022.839895)

10 Chen Y, Wang Y, Ding G, Tian Y, Zhou Z, Wang X, Shen L & Huang H. Association between bisphenol A exposure and idiopathic central precocious puberty (ICPP) among school-aged girls in Shanghai, China. *Environment International* 2018 **115** 410–416. (https://doi.org/10.1016/j.envint.2018.02.041)

11 Cameron N. The methods of axiological anthropometry. In *Human Growth*, 2nd ed. F Fanlkner & JM Tanner, Eds. London: Plenum Press 1978.

12 Marshall WA & Tanner JM. Variations in pattern of pubertal changes in girls. *Archives of Disease in Childhood* 1969 **44** 291–303. (https://doi.org/10.1136/adc.44.235.291)

13 Greulich WW & Pyle SI. *Radiographic Atlas of Skeletal Development of the Hand and Wrist*, 2nd ed., pp. 518–520 Stanford: Stanford University Press 1959.

14 Lee SH, Joo EY, Lee JE, Jun YH & Kim MY. The diagnostic value of pelvic ultrasound in girls with central precocious puberty. *Chonnam Medical Journal* 2016 **52** 70–74. (https://doi.org/10.4068/cmj.2016.52.1.70)

15 Feng G, Zhuang Y, Yao F, Ye Y, Wan Q & Zhou W. Development of the Chinese smell identification test. *Chemical Senses* 2019 **44** 189–195. (https://doi.org/10.1093/chemse/bjz006)

16 Wu X, Geng Z, Zhou S, Bai T, Wei L, Ji GJ, Zhu W, Yu Y, Tian Y & Wang K. Brain structural correlates of odor identification in mild cognitive impairment and Alzheimer's disease revealed by magnetic resonance imaging and a Chinese olfactory identification test. *Frontiers in Neuroscience* 2019 **13** 842. (https://doi.org/10.3389/fnins.2019.00842)

17 Zhang Q, Ding Y, Feng B, Tang Y, Chen Y, Wang Y, Chang G, Liu S, Wang J, Li Q, *et al.* Molecular and phenotypic expansion of Alström syndrome in Chinese patients. *Frontiers in Genetics* 2022 **13** 808919. (https://doi.org/10.3389/fgene.2022.808919)

18 Özgen İT, Torun E, Bayraktar-Tanyeri B, Durmaz E, Kılıç E & Cesur Y. The relation of urinary bisphenol A with kisspeptin in girls diagnosed with central precocious puberty and premature thelarche. *Journal of Pediatric Endocrinology and Metabolism* 2016 **29** 337–341. (https://doi.org/10.1515/jpem-2015-0235)

19 Xu Y, Wang Y, Li N, Yao R, Li G, Li J, Ding Y, Chen Y, Huang X, Chen Y, *et al.* New insights from unbiased panel and whole-exome sequencing in a large Chinese cohort with disorders of sex development. *European Journal of Endocrinology* 2019 **181** 311–323. (https://doi.org/10.1530/EJE-19-0111)

20 Sasaki G, Ogata T, Ishii T, Hasegawa T, Sato S & Matsuo N. Novel mutation of TBX3 in a Japanese family with ulnar-mammary syndrome: implication for impaired sex development. *American Journal of Medical Genetics* 2002 **110** 365–369. (https://doi.org/10.1002/ajmg.10447)

21 Kaur H & Panigrahi I. Chung-Jansen Syndrome with obesity. *Obesity Research and Clinical Practice* 2021 **15** 303–305. (https://doi.org/10.1016/j.orcp.2021.03.015)

22 Laron Z & Werner H. Laron syndrome: a historical perspective. *Reviews in Endocrine and Metabolic Disorders* 2021 **22** 31–41. (https://doi.org/10.1007/s11154-020-09595-0)

23 Dupé C, Guey S, Biard L, Dieng S, Lebenberg J, Grosset L, Alili N, Hervé D, Tournier-Lasserve E, Jouvent E, *et al.* Phenotypic variability in 446 CADASIL patients: impact of notch3 gene mutation location in addition to the effects of age, sex and vascular risk factors. *Journal of Cerebral Blood Flow and Metabolism* 2023 **43** 153–166. (https://doi.org/10.1177/0271678X221126280)

24 Alkhzouz C, Bucerzan S, Miclaus M, Mirea AM & Miclea D. 46,XX DSD: developmental, clinical and genetic aspects. *Diagnostics*

(Basel, Switzerland) 2021 **11** 1379. (https://doi.org/10.3390/diagnostics11081379)
25 Shamma RA, Atef S & Arafa N. Etiological classification and clinical spectrum of Egyptian pediatric patients with disorder of sex development, single center experience. *Endokrynologia Polska* 2021 **72** 558–565. (https://doi.org/10.5603/EP.a2021.0045)
26 Cools M, Nordenström A, Robeva R, Hall J, Westerveld P, Flück C, Köhler B, Berra M, Springer A, Schweizer K, *et al.* Caring for individuals with a difference of sex development (DSD): a Consensus Statement. *Nature Reviews. Endocrinology* 2018 **14** 415–429. (https://doi.org/10.1038/s41574-018-0010-8)
27 Barroso PS, Jorge AAL, Lerario AM, Montenegro LR, Vasques GA, Lima Amato LG, Gontijo Silveira LF, Mendonca BB & Latronico AC. Clinical and genetic characterization of a constitutional delay of growth and puberty cohort. *Neuroendocrinology* 2020 **110** 959–966. (https://doi.org/10.1159/000504783)
28 Ahmadifard M, Kajbafzadeh A, Panjeh-Shahi S, Vand-Rajabpour F, Ahmadi-Beni R, Arshadi H, Setoodeh A, Rostami P, Tavakkoly-Bazzaz J & Tabrizi M. Molecular investigation of mutations in androgen receptor and 5-alpha-reductase-2 genes in 46,XY Disorders of Sex Development with normal testicular development. *Andrologia* 2019 **51** e13250. (https://doi.org/10.1111/and.13250)
29 Hatabu N, Amano N, Mori J, Hasegawa Y, Matsuura H, Sumitomo N, Nishizawa K, Suzuki M, Katakura S, Kanamoto N, *et al.* Pubertal development and pregnancy outcomes in 46,XX patients with nonclassic lipoid congenital adrenal hyperplasia. *Journal of Clinical Endocrinology and Metabolism* 2019 **104** 1866–1870. (https://doi.org/10.1210/jc.2018-01752)
30 Bennecke E, Bernstein S, Lee P, van de Grift TC, Nordenskjöld A, Rapp M, Simmonds M, Streuli JC, Thyen U, Wiesemann C, *et al.* Early genital surgery in disorders/differences of sex development: patients' perspectives. *Archives of Sexual Behavior* 2021 **50** 913–923. (https://doi.org/10.1007/s10508-021-01953-6)
31 Sandberg DE & Gardner M. Differences/disorders of sex development: medical conditions at the intersection of sex and gender. *Annual Review of Clinical Psychology* 2022 **18** 201–231. (https://doi.org/10.1146/annurev-clinpsy-081219-101412)
32 Gomes NL, Lerário AM, Machado AZ, Moraes DR, Silva TED, Arnhold IJP, Batista RL, Faria Júnior JAD, Costa EF, Nishi MY, *et al.* Long-term outcomes and molecular analysis of a large cohort of patients with 46,XY disorder of sex development due to partial gonadal dysgenesis. *Clinical Endocrinology* 2018 **89** 164–177. (https://doi.org/10.1111/cen.13717)
33 Akcay T, Fernandez-Cancio M, Turan S, Güran T, Audi L & Bereket A. AR and SRD5A2 gene mutations in a series of 51 Turkish 46,XY DSD children with a clinical diagnosis of androgen insensitivity. *Andrology* 2014 **2** 572–578. (https://doi.org/10.1111/j.2047-2927.2014.00215.x)
34 Eggers S, Sadedin S, van den Bergen JA, Robevska G, Ohnesorg T, Hewitt J, Lambeth L, Bouty A, Knarston IM, Tan TY, *et al.* Disorders of sex development: insights from targeted gene sequencing of a large international patient cohort. *Genome Biology* 2016 **17** 243. (https://doi.org/10.1186/s13059-016-1105-y)
35 Ea V, Bergougnoux A, Philibert P, Servant-Fauconnet N, Faure A, Breaud J, Gaspari L, Sultan C, Paris F & Kalfa N. How far should we explore hypospadias? Next-generation sequencing applied to a large cohort of hypospadiac patients. *European Urology* 2021 **79** 507–515. (https://doi.org/10.1016/j.eururo.2020.12.036)
36 Miclea D, Alkhzouz C, Bucerzan S, Grigorescu-Sido P, Popp RA, Pascanu IM, Cret V, Ghervan C, Blaga L & Zaharie G. Molecular and cytogenetic analysis of Romanian patients with differences in sex development. *Diagnostics (Basel, Switzerland)* 2021 **11** 2107. (https://doi.org/10.3390/diagnostics11112107)
37 Wang H, Zhang L, Wang N, Zhu H, Han B, Sun F, Yao H, Zhang Q, Zhu W, Cheng T, *et al.* Next-generation sequencing reveals genetic landscape in 46, XY disorders of sexual development patients with variable phenotypes. *Human Genetics* 2018 **137** 265–277. (https://doi.org/10.1007/s00439-018-1879-y)
38 Ata A, Özen S, Onay H, Uzun S, Gökşen D, Özkınay F, Özbaran NB, Ulman İ & Darcan Ş. A large cohort of disorders of sex development and their genetic characteristics: 6 novel mutations in known genes. *European Journal of Medical Genetics* 2021 **64** 104154. (https://doi.org/10.1016/j.ejmg.2021.104154)
39 Wang Y, Qin M, Fan L & Gong C. Correlation analysis of genotypes and phenotypes in Chinese male pediatric patients with congenital hypogonadotropic hypogonadism. *Frontiers in Endocrinology* 2022 **13** 846801. (https://doi.org/10.3389/fendo.2022.846801)
40 Samtani R, Bajpai M, Ghosh PK & Saraswathy KN. SRD5A2 gene mutations--a population-based review. *Pediatric Endocrinology Reviews* 2010 **8** 34–40.
41 Avendaño A, Paradisi I, Cammarata-Scalisi F & Callea M. 5-α-reductase type 2 deficiency: is there a genotype-phenotype correlation? A review. *Hormones (Athens, Greece)* 2018 **17** 197–204. (https://doi.org/10.1007/s42000-018-0013-9)
42 Fan L, Song Y, Polak M, Li L, Ren X, Zhang B, Wu D & Gong C. Clinical characteristics and genotype-phenotype correlations of 130 Chinese children in a high-homogeneity single-center cohort with 5α-reductase 2 deficiency. *Molecular Genetics and Genomic Medicine* 2020 **8** e1431. (https://doi.org/10.1002/mgg3.1431)
43 Eisermann K, Wang D, Jing Y, Pascal LE & Wang Z. Androgen receptor gene mutation, rearrangement, polymorphism. *Translational Andrology and Urology* 2013 **2** 137–147. (https://doi.org/10.3978/j.issn.2223-4683.2013.09.15)
44 Gulía C, Baldassarra S, Zangari A, Briganti V, Gigli S, Gaffi M, Signore F, Vallone C, Nucciotti R, Costantini FM, *et al.* Androgen insensitivity syndrome. *European Review for Medical and Pharmacological Sciences* 2018 **22** 3873–3887. (https://doi.org/10.26355/eurrev_201806_15272)
45 Elward C, Berg J, Oberlin JM & Rohena L. A case series of a mother and two daughters with a GLI2 gene deletion demonstrating variable expressivity and incomplete penetrance. *Clinical Case Reports* 2020 **8** 2138–2144. (https://doi.org/10.1002/ccr3.3085)
46 Arnhold IJ, França MM, Carvalho LR, Mendonca BB & Jorge AA. Role of GLI2 in hypopituitarism phenotype. *Journal of Molecular Endocrinology* 2015 **54** R141–R150. (https://doi.org/10.1530/JME-15-0009)
47 Meczekalski B, Podfigurna-Stopa A, Smolarczyk R, Katulski K & Genazzani AR. Kallmann syndrome in women: from genes to diagnosis and treatment. *Gynecological Endocrinology* 2013 **29** 296–300. (https://doi.org/10.3109/09513590.2012.752459)
48 Topaloğlu AK. Update on the genetics of idiopathic hypogonadotropic hypogonadism. *Journal of Clinical Research in Pediatric Endocrinology* 2017 **9**(Supplement 2) 113–122. (https://doi.org/10.4274/jcrpe.2017.S010)
49 Li L, Gao F, Fan L, Su C, Liang X & Gong C. Disorders of sex development in individuals harbouring MAMLD1 variants: WES and interactome evidence of oligogenic inheritance. *Frontiers in Endocrinology* 2020 **11** 582516. (https://doi.org/10.3389/fendo.2020.582516)
50 Mazen I, Abdel-Hamid M, Mekkawy M, Bignon-Topalovic J, Boudjenah R, El Gammal M, Essawi M, Bashamboo A & McElreavey K. Identification of NR5A1 mutations and possible digenic inheritance in 46,XY gonadal dysgenesis. *Sexual Development* 2016 **10** 147–151. (https://doi.org/10.1159/000445983)
51 Houston BJ, Riera-Escamilla A, Wyrwoll MJ, Salas-Huetos A, Xavier MJ, Nagirnaja L, Friedrich C, Conrad DF, Aston KI, Krausz C, *et al.* A systematic review of the validated monogenic causes of human male infertility: 2020 update and a discussion of emerging gene-disease relationships. *Human Reproduction Update* 2021 **28** 15–29. (https://doi.org/10.1093/humupd/dmab030)

52 Camats N, Flück CE & Audí L. Oligogenic origin of differences of sex development in humans. *International Journal of Molecular Sciences* 2020 **21** 1809. (https://doi.org/10.3390/ijms21051809)
53 Zidoune H, Ladjouze A, Chellat-Rezgoune D, Boukri A, Dib SA, Nouri N, Tebibel M, Sifi K, Abadi N, Satta D, *et al.* Novel genomic variants, atypical phenotypes and evidence of a digenic/oligogenic contribution to disorders/differences of sex development in a large North African cohort. *Frontiers in Genetics* 2022 **13** 900574. (https://doi.org/10.3389/fgene.2022.900574)
54 Baetens D, Mendonça BB, Verdin H, Cools M & De Baere E. Non-coding variation in disorders of sex development. *Clinical Genetics* 2017 **91** 163–172. (https://doi.org/10.1111/cge.12911)

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