

# Variant analysis of the chromodomain helicase DNA-binding protein 7 in pediatric disorders of sex development

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## ABSTRACT

**Importance:** This study investigated the role of the chromodomain helicase DNA-binding protein 7 (*CHD7*) in disorders of sex development (DSD).

**Objective:** We aimed to present the potential pathogenicity of *CHD7* variants in pediatric patients with DSD.

**Methods:** Choosing cases with *CHD7* variants from DSD patients in Beijing Children's Hospital to assess for the study. Prediction software tools were used to predict variant pathogenicity in these subjects.

**Results:** Among the 113 DSD patients, 22 cases had *CHD7* variants. Twenty-four different *CHD7* variants were identified in the 22 DSD patients. Prediction software combined with ClinVar database information and their clinical manifestations revealed that, of the 18 patients with 46, XY DSD, two had CHARGE syndrome and two had Kallmann syndrome. Seven of the variants were highly categorized as "likely to be pathogenic" and seven as "suspected to be pathogenic". Of the four patients with 46, XX DSD, three had ovotesticular DSD (c.305A>G, c.2788G>A, and c.3098G>A) and one had testicular DSD (c.2831G>A).

**Interpretation:** A high frequency of *CHD7* variants was found in the DSD patients, especially those with 46, XY DSD. Thus, the detection of a pathogenic *CHD7* variant could suggest a diagnosis of hypogonadotropic hypogonadism for 46, XY DSD patients, but pre-pubescent patients should be reassessed in adolescence to confirm this diagnosis. This study also suggests that DNA sequencing could help to identify pre-pubescent DSD patients. Further data are required to determine the connection between *CHD7* variants and sex-reversal in patients with 46, XX DSD, and the accumulation of these data is essential and necessary for DSD research.

## KEYWORDS

Disorders of sex development, *CHD7* variants, Genital abnormalities

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## INTRODUCTION

The chromodomain helicase DNA-binding protein 7 (*CHD7*; OMIM #608892) is located on chromosome 8q12.2. The CHD protein family encompasses nine members and is divided into three separate subfamilies; the *CHD7* protein belongs to subfamily III, which also includes *CHD5*, *CHD6*, *CHD8*, and *CHD9*. All CHD proteins share a fairly high level of structural homology, possessing: two N-terminal truncated chromodomains (chromatin organization modifiers); a sucrose non-fermentable-like helicase-ATPase domain involved in transcriptional regulation, mitotic chromosomal stability maintenance, recombination, and DNA repair; and a DNA-binding domain that recognizes AT-rich sequences. *CHD7* also has a 41-amino acid N-terminal BRK domain like those found in the Brahma/BRG1 family of helicases. The domain is thought to play a role in chromatin remodeling but its exact function is unknown.<sup>1</sup>

Because of their relatively high level of homology, CHD proteins overlap in their biological functions. They may act as co-activators or co-repressors to regulate gene expression.<sup>2</sup> CHD proteins with switching-defective protein 3, adaptor 2, nuclear receptor co-repressor, and transcription factor IIIB domains are thought to couple histone binding to enzyme catalysis.<sup>3</sup> Sillibourne et al<sup>4</sup> found that *CHD3/CHD4* proteins function in nucleosome remodeling, histone deacetylation, and chromatin structure regulation during replication, transcription, recombination, repair, embryonic development, and the cell cycle. *CHD7* is thought to have similar functions and is expressed in multiple organs, including the cardiac outflow tract, facio-acoustic preganglion complex, hindbrain, forebrain, otic vesicle, optic stalk/optic vesicle, olfactory pit, and branchial arches,<sup>5,6</sup> which is why *CHD7* variants are likely to cause a wide range of malformations.

A previous study of *CHD7* variants focused on patients with CHARGE syndrome and hypogonadotropic hypogonadism (HH) with or without olfactory abnormalities, all of which are autosomal dominant disorders. CHARGE syndrome is defined as coloboma, heart disease, atresia of the choanae, retarded growth and development, genital hypoplasia, and ear/hearing anomalies, and diagnostic criteria for this condition include a small penis and cryptorchidism. One pathogenic *CHD7* variant was previously found to be the major cause of CHARGE syndrome. This variant can also affect the migration of gonadotrophin-releasing hormone (GnRH) resulting in a micropenis and cryptorchidism as well as hypospadias in some males,<sup>7,8</sup> whereas female patients with this *CHD7* variant can be affected with anomalies in their external genitalia and internal reproductive structures, such as hypoplastic labia majora or minora, hypoplastic clitoris, uterine abnormalities, ovarian agenesis, and persistent amenorrhea.<sup>9,10</sup> However, a

number of overlaps occur between congenital (C) HH and CHARGE syndrome, including hypogonadism, anosmia, and hearing impairment.<sup>11</sup> For the variants pathogenicity, although the variants of CHH patients were predicted to be benign by American College of Medical Genetics and Genomics (ACMG), the patients can be also present the phenotypes of CHARGE syndrome when review the history carefully.<sup>12</sup> It means that it maybe the pathogenicity for CHARGE syndrome, but this requires a large collection of data. This study investigated the clinical features and medical experience of 22 patients with disorders of sex development (DSD) who had *CHD7* variants and evaluated the applied diagnostic methods and the role of *CHD7* gene detection.

## METHODS

### Clinical cases

Male or female patients aged from 3 months to 14 years, who visited Beijing Children's Hospital were recruited if they met the following inclusion criteria: 1) external genital abnormalities; 2) normal chromosome numbers and karyotype, e.g., 46, XX or 46, XY; and 3) presence of a *CHD7* variant. Exclusion criteria were: 1) *CHD7* variant negative; and 2) abnormal chromosomes.

### Research methods

Clinical information, including symptoms, signs, laboratory test results, and clinical diagnoses were collected from all patients.

The pathogenicity of *CHD7* variants was analyzed using three online prediction software tools selected from a report of bioinformatics software performance<sup>13</sup>: SIFT, Polyphen-2, and MutationTaster. Reference scores were as follows: SIFT software, < 0.05 (affect protein function), > 0.05 (tolerated); Polyphen-2 software, 0.957–1 (probably damaging), 0.453–0.956 (possibly damaging), and 0–0.452 (benign); MutationTaster software, A: disease-causing-automatic, D: disease-causing, N: polymorphism, P: polymorphism-automatic. A minor allele frequency of < 5% was selected as a reference index based on ClinVar archives, dbSNP, DiseaseDX database and previous study.<sup>14</sup> No software or past studies about the pathogenicity of intronic *CHD7* variants were identified, but because the clinical data confirmed these findings, we considered them to be pathogenic.

Whole-exome sequencing, whole-genome sequencing, or sequencing using DSD gene panels was performed to detect the *CHD7* variants. Peripheral blood samples were collected from patients and sent to domestic qualified company for commercial sequencing (Beijing Kangso Medical Laboratory Zhongguancun Huakang Gene Institute; Chigene Translational Medicine Research Center Co., Ltd). After receiving the results, we audited them in

combination with the corresponding clinical phenotypes as well as multiple data banks.

### Treatment plan and follow-up

The social ability and intelligence of the patients were assessed by the Gesell and Wechsler Intelligence Scale for Children Chinese Revised. Adolescents with a bone age of  $\geq 12$  years but negative results from a Human Luteinizing Hormone-Releasing Hormone (LHRH) stimulation test<sup>15</sup> were offered further treatment to induce puberty. Young patients whose bone age was  $< 12$  years in our study were followed up.

## RESULTS

Of the 113 DSD patients who were tested, 22 (18%) were found to have *CHD7* variants. These 22 patients were enrolled and then divided into two groups. Group 1 contained 18 46, XY DSD patients, including two with CHARGE syndrome, two with Kallmann syndrome (KS), and 14 with suspected CHH whose puberty will be followed-up later because they were too young to be studied. Group 2 contained four 46, XX DSD patients, including three with ovotesticular DSD and one with testicular DSD.

Due to the limitation of age and the use range of ACMG, we applied a pathogenicity prediction based on *CHD7* variants to divide group 1 into three subgroups: group 1A, group 1B, and group 1C. The clinical features of the 46, XY subjects are shown in Table 1, and the corresponding analysis of variants is shown in Table 2. In group 1, the frequency of *CHD7* variants combined with other gene variants was about 47%, while that of single *CHD7* gene variants was about 53%.

Group 1A contained four 46, XY DSD patients with a clear clinical diagnosis of CHH. Consistent with the diagnostic criteria of CHARGE syndrome,<sup>16</sup> patient 1 who was 5.0 years old, showed the following symptoms: cryptorchidism; hypophrenia; coloboma (hyperopia and amblyopia); deafness; typical facial features including arched eyebrows, low hairline, flat nose, and high palatal arch; nasal mucosa hypertrophy; rhinostenosis; and tricuspid incompetence. He was found to have a maternal origin *CHD7* variant (c.2831G>A) (p.944R>H) that has been previously reported in the Human Gene Variant Database (HGMD). Both his mother and aunt experienced their first menarche at 16–17 years of age. Patient 2, who was 2.5 years, also displayed symptoms concordant with CHARGE syndrome; he showed external genitalia along with other CHARGE syndrome characteristics, such as deafness, patent ductus arteriosus, mental retardation, low hairline, prosopoplegia, and typical facial features including epicanthus, blue sclera, and temporal depression. He was identified as having a de novo *CHD7* variant (c.3301T>C) (p.662A>V), which was absent from all

databases. Patients 3 and 4, whose ages were 11.0 and 1.4 years, respectively, had normal karyotypes, and both had microphallus and cryptorchidism without hypospadias and with normal basal sex hormones. Magnetic resonance imaging (MRI) revealed an abnormal olfactory bulb and olfactory tract in both these patients. Therefore, they were diagnosed with KS. These four patients were all followed-up based on their ages.

Group 1B contained seven 46, XY DSD patients aged from 3 months to 10 years, all of whom had a clinical diagnosis of suspected CHH. These patients presented with microphallus, cryptorchidism, and hypospadias. Because of their young ages, none had an MRI of the olfactory bulb performed, so we made a diagnosis of suspected CHH and predicted that their puberty would be incomplete or that they would not enter puberty. Patient 5 carried the same *CHD7* variant as patient 1. Patient 6 harbored two heterozygous *CHD7* variants, which were both predicted to be strongly pathogenic, and this predication was supported by the patient's clinical manifestations. Patients 7 and 8 each carried the same novel *CHD7* variation, but patient 7 also carried the *PROKR2* variant c.533G>C, which supports a diagnosis of HH.<sup>9</sup> Patient 9 harbored a truncated *CHD7* variant causing a premature termination codon that affects the structure and function of the protein and is predicted to be pathogenic. Patients 10 and 11 each carried *CHD7* variants (c.2678G>T and c.4516G>A) that were probably pathogenic. Based on their diagnoses, puberty should be induced in these patients when they reach peri-adolescence age. This subgroup of patients had genetic finding that support a diagnosis of CHH.

Group 1C contained seven 46, XY DSD patients who carried *CHD7* variants with unclear pathogenicity. The external genitalia of six out of the seven patients resembled that of females. Several of the patients' *CHD7* variants originated from maternal genes, and this, combined with clinical evidence, suggests that they are causative of disease. Patient 12 had complex heterozygous *CHD7* variants (c.409T>G and c.1697C>T), which had a parental origin. Although, the pathogenicity prediction results for each of these two *CHD7* variants was benign, due to the potential cumulative effect in these variants, we could not exclude *CHD7* as the main causative factor of disease in patient 12. Patient 13 presented with microphallus, cryptorchidism, and hypospadias, and he carried an intronic *CHD7* variant with an unclear significance that was inherited maternally. Combined with the patient's clinical data and findings from a previous study about another pathogenic intronic variant (*FGFR1* c.1664-9T>G),<sup>17</sup> the variant (c.1665+8G>A) was considered to be causative of disease. Patient 14 harbored *CHD7* variant c.2830C>T, which leads to amino acid change p.944R>C. Variant prediction results for c.2830C>T were benign, but patient 14 also carried *BMP15* variant c.788\_789 insert TCT, which might be pathogenic. This case suggests that

**TABLE 1** Clinical phenotype and genotype of the 46, XY DSD patients carrying *CHD7* variants

Group Patient	Phenotype					<i>CHD7</i> gene					Other gene							
	Sex	Age (years)	Micro-phallus	Cryptor-chidism	Hypos-padias	Anosmia/Hyposmia	MRI (abnormal olfactory bulb/olfactory tract)	Family history	Clinical diagnosis	Type	Nucleotide change	Amino acid change	Source of variant	Combined gene	Type	Nucleotide change	Amino acid change	Source of variant
1A	1	M	5.0	+	-	u	-	-	Charge	<i>Hetero</i>	c.2831G>A (exon 10)	p.944, R>H	m	<i>EP300</i>	<i>Hetero</i>	c.1985C>T	p.662, A>V	f
	2	M	2.5	+	-	+	+	-	Charge	<i>Hetero</i>	c.3301T>C (exon 13)	p.1101, C>R	de novo	/	/	/	/	/
	3	M	11.0	+	-	+	+	-	KS	<i>Hetero</i>	c.4687A>G (exon 21)	p.1563, R>G	de novo	/	/	/	/	/
	4	M	1.4	+	-	u	+	-	KS	<i>Hetero</i>	c.2613+5G>A	-	m	/	/	/	/	/
1B	5	M	10.0	+	-	-	-	-	HH	<i>Hetero</i>	c.2831G>A (exon 10)	p.944, R>H	f	/	/	/	/	/
	6	M	1.3	+	+	u	-	-	HH	<i>Hetero</i>	c.8194G>A (exon 38)	p.2732, A>T	m	/	/	/	/	/
	7	M	0.9	+	-	-	-	-	HH	<i>Hetero</i>	c.8597C>T (exon 38)	p.2866, A>V	f	/	/	/	/	/
	8	M	1.3	+	-	-	-	-	HH	<i>Hetero</i>	c.6955C>T (exon 33)	p.2319, R>C	de novo	<i>PROKR2</i>	<i>Hetero</i>	c.533G>C	p.178, W>S	f
	9	M	0.2	+	-	-	-	-	HH	<i>Hetero</i>	c.1501-c.1502:insertAT	p.501, H>Hfs64	de novo	/	/	/	/	/
	10	M	0.5	-	+	-	-	-	HH	<i>Hetero</i>	c.2678G>T (exon 9)	p.893, S>I	f	<i>LHCGR</i>	<i>Hetero</i>	c.880C>A	p.294, H>N	f
	11	M	1.8	+	+	-	-	-	HH	<i>Hetero</i>	c.4516G>A (exon 19)	p.1506, G>S	m	<i>SRD5A2</i>	<i>Compound Hetero</i>	c.679G>A	p.227, E>K	f
1C	12	M	0.5	+	+	u	-	-	HH	<i>Compound Hetero</i>	c.4091>G (exon 2)	p.137, S>A	f	/	/	/	/	/
	13	F	0.5	+	+	-	-	-	HH	<i>Hetero</i>	c.1697C>T (exon 3)	p.566, P>L	m	/	/	/	/	/
	14	F	14.2	+	+	-	-	-	HH	<i>Hetero</i>	c.1665+8G>A	-	m	/	/	/	/	/
	15	F	0.4	+	+	-	-	+	HH and AIS	<i>Hetero</i>	c.2830C>T (exon 10)	p.944, R>C	u	<i>BMP15</i>	<i>Hemi</i>	c.788_789 insTCT	p.262_263 insLeu	u
	16	F	6.1	+	+	-	-	-	HH	<i>Hetero</i>	c.8250T>G (exon 38)	p.2750, F>L	m	<i>FLRT3</i>	<i>Hetero</i>	c.1129G>A	p.377, A>T	m
	17	F	2.7	+	+	u	-	-	HH	<i>Hetero</i>	c.2182G>A (exon 4)	p.728, D>N	m	<i>HS6ST1</i>	<i>Hetero</i>	c.1132C>A	p.378, R>S	m
	18	M	8.0	+	-	-	-	+	HH	<i>Hetero</i>	c.425G>C (exon 2)	p.142, S>T	f	<i>AR</i>	<i>Hemi</i>	c.1094A>G	p.365, Y>C	f
										<i>Hetero</i>	c.521C>T (exon 2)	p.174, P>L	f	<i>DMXL2</i>	<i>Hetero</i>	c.9023G>A	p.3008, R>Q	f
										<i>Hetero</i>	c.373G>T (exon 2)	p.125, V>F	m	<i>TSPYL1</i>	<i>Hetero</i>	c.373G>T	p.125, V>F	m

DSD, disorders of sex development; *CHD7*, chromodomain helicase DNA-binding protein 7; M, male; F, female; m, mother; f, father; u, unclear; KS, Kallmann syndrome; HH, hypogonadotropic hypogonadism; AIS, androgen insensitivity syndrome; +, positive phenotype; -, negative phenotype; +<sup>‡</sup>, mother and aunt were 16–17 years old at the time of menarche; +<sup>‡</sup>, aunt has poor olfactory sensation; +<sup>‡</sup>, great-uncle had hypospadias and no children, and uncle with cryptorchidism; /, undetected.

**TABLE 2** Pathogenicity analysis of *CHD7* variants in 22 DSD patients

Patient	Site	Amino acid	RS number	Location	Prediction software tools				MAF value			Clinical evidence	Significance
					Mutation/Taster	Polyphen-2 (Value)	SIFT (Value)	gnomAD	1000Genomes	Chinese	ExAC (East Asian)		
1	c.2831 G>A	p.944 R>H	rs117506164	Chromo 2	disease causing	Benign (0.015)	Tolerated (0.57)	0.0006	0.0008	0.0082	0.01	Strong	P
2	c.3301 T>C	p.1101 C>R	-	Helicase ATP-binding	disease causing	Probably damaging (0.968)	Affect protein function (0.02)	-	-	-	-	Strong	P
3	c.4687 A>G	p.1563 R>G	-	-	disease causing	Possible damaging (0.594)	Affect protein function (0.01)	-	-	-	-	Strong	P
4	c.2613+5 G>A	-	-	-	-	-	-	-	-	-	-	-	P
5	c.2831 G>A	p.944 R>H	rs117506164	Chromo 2	disease causing	Benign (0.015)	Tolerated (0.57)	0.0006	0.0008	0.0082	0.01	Strong	LP
6	c.8194 G>A	p.2732 A>T	-	-	disease causing	Possible damaging (0.695)	Tolerated 0.23	-	-	-	-	Moderate	LP
7	c.6955 C>T	p.2319 R>C	rs121434341	-	disease causing	Probably damaging (0.99)	Affect protein function (0.00)	-	-	-	-	Moderate	LP
8	c.6955 C>T	p.2319 R>C	rs121434341	-	disease causing	Probably damaging (0.99)	Affect protein function (0.01)	-	-	-	-	Moderate	LP
9	c.1501-1502 insAT	p.501 H>His64	-	-	disease causing	-	-	-	-	-	-	Moderate	LP
10	c.2678 G>T	p.893 S>L	-	Chromo 2	disease causing	Possible damaging (0.474)	Affect protein function (0.03)	-	-	-	-	Moderate	LP
11	c.4516 G>A	p.1506 G>S	-	-	disease causing	Probably damaging (0.926)	Tolerated (0.13)	-	-	-	0.0009	Moderate	LP
12	c.409 T>G	p.137 S>A	-	-	disease causing	Benign (0.004)	Tolerated (0.17)	-	-	-	0.0016	Uncertain	VUS
	c.1697 C>T	p.566 P>L	-	-	disease causing	Benign (0.006)	Tolerated (0.33)	-	-	-	-	Uncertain	VUS
13	c.1665+8 G>A	-	-	-	-	-	-	-	-	-	-	Uncertain	VUS
14	c.2830 C>T	p.944 R>C	rs587783435	Chromo 2	disease causing	Benign (0.045)	Affect protein function (0.00)	0.000004	-	0	0	Uncertain	VUS
15	c.8250 T>G	p.2750 F>L	rs3750308	-	disease causing	Benign (0.021)	Tolerated (1.00)	0.00021	0.0008	0.0041	0.0033	Uncertain	VUS
16	c.2182 G>A	p.728 D>N	rs756365280	-	disease causing	Benign (0.043)	Affect protein function (0.00)	-	-	-	0.0001	Uncertain	VUS
17	c.425 G>C	p.142 S>T	-	-	polymorphism	Benign (0.00)	Tolerated (0.27)	-	-	-	0	Uncertain	VUS
18	c.521 C>T	p.174 P>L	-	-	disease causing	Benign (0.073)	Tolerated (0.12)	-	-	-	0	Uncertain	VUS
19	c.305 A>G	p.102 H>R	-	-	disease causing	Probably damaging (0.950)	Affect protein function (0.02)	-	-	-	-	Uncertain	VUS
20	c.2788 G>A	p.930 E>K	rs377330239	Chromo 2	disease causing	Benign (0.274)	Tolerated (0.13)	0.00007	0.0002	-	0	Uncertain	VUS
21	c.3098 G>A	p.1033 R>K	-	Helicase ATP-binding	disease causing	Possible damaging (0.842)	Affect protein function (0.01)	-	-	-	-	Uncertain	VUS
22	c.2831 G>A	p.944 R>H	rs117506164	Chromo 2	disease causing	Benign (0.015)	Tolerated 0.57	0.0006	0.0008	0.0082	0.01	Uncertain	VUS

*CHD7*, chromodomain helicase DNA-binding protein 7; DSD, disorders of sex development; P, pathogenicity; LP, likely pathogenicity; VUS, uncertain significance; -, undetected.

the *CHD7* variant c.2830C>T may make a synergistic contribution to pathogenesis. Patient 15 was only 0.4 years old and had a more complex family history, with infertility in the matrilineal line and cryptorchidism in the patrilineal line. This patient also carried two extra variants of genes associated with HH: *FLRT3* and *HS6ST1*. Furthermore, patient 15 was found to have two variants in *AR* inherited maternally that were predicted to be probably damaging. Although the presence of microphallus and cryptorchidism combined with the variant predictions strongly suggested a diagnosis of HH, the clinical manifestations of the patient (female appearance; basic testosterone: 186 ng/dL; luteinizing hormone: 2 IU/L; follicle-stimulating hormone: 1.25 IU/L; post human chorionic gonadotropin testosterone: 1126 ng/dL) were consistent with partial androgen insensitivity syndrome. We propose that the *CHD7* variant in this patient was synergistic with other HH-associated gene variants. Although *FLRT3* variants usually associate with variants in other HH-associated genes, such as *FGFR1*, *HS6ST1*, and *FGF17*, to cause CHH, they may have synergized with the *CHD7* variant in this patient.<sup>18</sup> The *FGFR1* and *HS6ST1* variants are also likely to have a pathogenic determinant in the patient's pubertal development. However, we will have to follow this patient until adolescence to confirm that this combination of gene variants is pathogenic. Patients 16 and 18 with single *CHD7* gene variants, present the phenotypes of HH, which suggest that the variants relative to the cause of HH. Patient 17 with three gene variants, but *DMXL2* gene and *TSPYL1* gene are not relative to HH from OMIM database (<http://www.omim.org/>), so we consider that *CHD7* gene is the reason of HH. Because this subgroup is composed of patients with several different gene variants in the subgroup, it showed the phenomenon of a high frequency of gonads relative to carried *CHD7* gene variants.

All the above-mentioned patients are expected to have developmental disorders in puberty. At present, because of their young age, they are receiving symptomatic treatment, and their pubertal development will be closely observed.

Group 2 contained four 46, XX DSD cases (Tables 2 and 3), all with genital abnormalities. Three of the patients (patients 19, 20, and 21) presented as male with microphallus and cryptorchidism, while the fourth (patient 22) presented as female with clitoral hypertrophy. Imaging and histopathological examination showed that the three gender male patients were 46, XX, ovotesticular DSD, while the fourth was 46, XX, testicular DSD. All four patients carried heterozygous *CHD7* variants, of which three were maternally inherited and one was de novo (the variant in case 21). Little is known about 46, XX DSD, so the clinical relevance of *CHD7* variants in this condition is uncertain. Even though all the patients in this group carried *CHD7* gene variants, the pathogenicity of these *CHD7* variants in the sex reverse remains uncertain. Here, we present the gene results for these patients; further analyses, like those previously described for *NR5A1* variants,<sup>19</sup> will need to wait for additional evidence.

## DISCUSSION

DSD is a complex disease in children. Symptoms include abnormal external genitalia, such as microphallus, hypospadias, or cryptorchidism in males and hypoplastic labia majora and minora, uterine abnormalities, and ovarian agenesis in females, as well as sexual deformity and sexual reversal; some patients also have facial or limbs deformities. The etiology of DSD is varied, ranging from abnormal embryo development to the abnormal secretion or function of hormones. Therefore, its diagnosis and differential diagnosis are difficult. It is particularly difficult to evaluate the hypothalamic-pituitary-gonadal

**TABLE 3** Clinical phenotype and genotype of the 46, XX DSD patients carrying *CHD7* variants

Patient	Phenotype					<i>CHD7</i> gene					Other gene			
	Age (years)	Sex	Surgery/post-operative sex	Vulva phenotype after postnatal	Clinical diagnosis	Type	Nucleotide change	Amino acid change	Source of variant	Combined gene	Type	Nucleotide change	Amino acid change	Source of variant
19	2.8	M	Oophorectomy/M	Microphallus and hypospadias	Ovotesticular DSD	<i>Hetero</i>	c.305A>G (exon 2)	p.102, H>R	m	/				
20	4.0	M	Hypospadias repair/M	Microphallus and hypospadias	Ovotesticular DSD	<i>Hetero</i>	c.2788G>A (exon 10)	p.930, E>K	m	/				
21	2.0	M	-	Microphallus and hypospadias	Ovotesticular DSD	<i>Hetero</i>	c.3098G>A (exon 12)	p.1033, R>K	u	/				
22	1.6	F	-	Clitoral hypertrophy	Ovotesticular DSD	<i>Hetero</i>	c.2831G>A (exon 10)	p.944, R>H	m	<i>HOXD13</i>	<i>Hetero</i>	c.296C>G	p.99, P>R	
										<i>KALI</i>	<i>Hemi</i>	c.463A>G	p.155, N>D	f

DSD, disorders of sex development; *CHD7*, chromodomain helicase DNA-binding protein 7; M, male; F, female; m, mother; f, father; u, unclear; -, without surgery; /, undetected.

(H-P-G) axis in childhood. In this work, we studied patients with microphallus, with or without cryptorchidism and hypospadias. We found that data from genetic analyses helped to support the diagnoses of HH in these patients.

Here, we studied the clinical status of *CHD7* variants in 22 children with DSD (18%) from a pool of 113 suspected CHH (DSD) patients. The data revealed that the frequency of *CHD7* variants in isolation or of *CHD7* variants accompanied by variants in other HH-associated genes was higher than those of other HH-associated genes in the DSD patients with suspected CHH. This result suggests that *CHD7* may synergistically be causative of HH. There was a higher frequency of hypospadias in patients with a *CHD7* variant, especially when it was combined with other gene variants. Thus, DSD symptoms may be more severe when pathogenic variants of associated genes are combined.

Patient 22 carried both *CHD7* and *KALI* variants; both *CHD7* and *KALI* can affect the development and function of gonadotropin neurons, which control pituitary function as well as the development of gonads and external genitalia.<sup>20</sup> While additional evidence is needed to support this conclusion, these gene variants could be considered the main cause of a patient's CHH when they are found in combination with the patient's clinical manifestations, positive family history, and appropriate mode of genetic effect (*CHD7* variants cause autosomal dominant diseases, whereas *KALI* variants cause X-linked recessive diseases). Notably, caution is needed when analyzing multiple mutations.<sup>21</sup> This study also highlights the varied nature of the clinical manifestations in patients with DSD, which range from mild to severe, and demonstrates that patients in the same family may have different symptoms.<sup>22</sup> For example, patient 1 carried the same variant as his mother. However, the boy presented with multiple organ abnormalities that can be diagnosed as CHARGE syndrome, whereas his mother had only delayed menarche. Furthermore, cases 5 and 22, who both also carried the same *CHD7* variant as case 1, each had different clinical manifestation compared with case 1. For the pathogenicity of this variant, it was benign from the result of ClinVar database, while it presented three times in our cases. So it may have two reasons to analyze the condition: one is that it exists with benign in patients with CHARGE syndrome, CHH or healthy person. Another reason is that when only *CHD7* variant in patients, other proteins may make up for the function of it, but when accompanying other gene variants, the person may present some phenotypes, like case 1 and 22. But it needs functional experiment verification, or a large amount of data to analyze.

Classic phenotypes of 46, XX DSD with typical *CHD7* variants include hypoplasia of the labia majora and minora, uterus, and ovaries. *CHD7* variants are mainly thought to affect the function of GnRH neurons, reducing

GnRH levels in adolescence and leading to the abnormal development of gonads; they rarely influence the differentiation of the uterus and ovaries from the genital ridge, so there is no evidence for their involvement in sex reversal. Therefore, even if the *CHD7* variants in 46, XX ovotesticular DSD and 46, XX testicular DSD patients are predicted to be pathogenic, their clinical significance remains uncertain. Because *CHD7* is expressed in multiple organs, it is conceivable that the *CHD7* protein impacts gonadal development during the embryonic stage, resulting in the dual form of male CHH. However, there are several reports about CHARGE syndrome caused by *CHD7* variants in females representing dysplasia of the external genitalia but without sex reversal. Therefore, additional cases need to be examined to confirm this. In the study of DSD caused by *CHD7* variants, we found that the co-existence of pathogenic genes is common, so it is worth investigating the mechanism of the synergistic pathogenicity of the oligomeric gene. Gonadal diseases are non-fatal and non-disabling diseases with a relatively high prevalence rate; we propose that the mechanism of cooperative pathogenesis (oligogenicity) should be studied further as it may play an important role in DSD.

In summary, the diagnosis of CHH in young patients by genetic testing is helpful because it enables prospective puberty to be monitored during follow-up. Due to the small subject pool available for investigating *CHD7* gene association with the development of the testis in 46, XX DSD, we were unable to establish a direct relationship between the *CHD7* variants and the diseases. However, our findings suggest that *CHD7* may potentially serve as a synergistic gene in causing the DSD phenotype. To reach clinically meaningful conclusions, additional clinical observations are required for these patients, and more 46, XX DSD patients should be assessed to better interpret the clinical process. This research is just an objective present the gene results for DSD patients. We perspective more result from different researchers.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

## REFERENCES

1. Allen MD, Religa TL, Freund SM. Solution structure of the BRK domains from CHD7. *J Mol Biol.* 2007;371:1135-1140.
2. Hall JA, Georgel PT. CHD Proteins: a diverse family with strong ties. *Biochem Cell Biol.* 2007;85:463-473.
3. Marfella CG, Imbalzano AN. The Chd family of chromatin remodelers. *Mutat Res.* 2007;618:30-40.
4. Sillibourne CG, Delaval B, Redick S, Sinha M, Doxsey SJ. Chromatin remodeling proteins interact with pericentromere to regulate centrosome integrity. *Mol Biol Cell.* 2007;18:50-57.
5. Lalani SR, Safiullah AM, Fernbach SD, Harutyunyan KG, Thaller C, Peterson LE, et al. Spectrum of *CHD7* mutations in 110 individuals with CHARGE syndrome and genotype-

- phenotype correlation. *Am J Hum Genet.* 2006;78:303-314.
6. Aramaki M, Kimura T, Udaka T, Kosaki R, Mitsuhashi T, Okada Y, et al. Embryonic expression profile of chicken *Chd7*, the ortholog of the causative gene for CHARGE syndrome. *Birth Defects Res A Clin Mol Teratol.* 2007;79:50-57.
  7. Vissers LE. Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. *Nat Genet.* 2004;36:955-957.
  8. Wang Y, Gong C, Qin M, Liu Y, Tian Y. Clinical and genetic features of 64 young male paediatric patients with congenital hypogonadotropic hypogonadism. *Clin Endocrinol (Oxf).* 2017;87:757-766.
  9. Ayers KL, Bouty A, Robevska G, van den Bergen JA, Juniarto AZ, Listyasari NA, et al. Variants in congenital hypogonadotropic hypogonadism genes identified in an Indonesian cohort of 46,XY under-virilised boys. *Hum Genomics.* 2017;11:1.
  10. Reynaert N, Zegher FD, Francois I, Devriendt K, Beckers D, Casteels K. Expanding the CHARGE geno-phenotype: A girl with novel *CHD7* deletion, hypogonadotropic hypogonadism, and agenesis of uterus and ovaries. *Horm Res Paediatr.* 2016;85:288-290.
  11. Jongmans MC, van Ravenswaaij-Arts CM, Pitteloud N, Ogata T, Sato N, Claahsen-van der Grinten HL, et al. *CHD7* mutations in patients initially diagnosed with Kallmann syndrome – the clinical overlap with CHARGE syndrome. *Clin Genet.* 2009;75:65-71.
  12. Xu C, Cassatella D, van der Sloot AM, Quinton R, Hauschild M, De Geyter C, et al. Evaluating CHARGE syndrome in congenital hypogonadotropic hypogonadism patients harboring *CHD7* variants. *Genet Med.* 2018;20:872-881.
  13. Chen Q, Dai C, Zhang Q, Du J, Li W. Evaluation of five bioinformatics software for missense mutation prediction performance. *Chin J Med Genet.* 2016;33:625-28. (In Chinese)
  14. The International HapMap Consortium. A haplotype map of the human genome. *Nature.* 2005;437:1299-1320.
  15. Chinese Medical Association endocrine credits gonadology group. The diagnosis and treatment of Idiopathic Hypogonadotropic Hypogonadism in expert consensus. *Chin J Inter Med.* 2016;54:739-744. (In Chinese)
  16. Verloes A. Updated diagnostic criteria for CHARGE syndrome: a proposal. *Am J Med Genet.* 2005;133A:306-308.
  17. Käsäkoski J, Vaaralahti K, Raivio T. New intronic Fibroblast Growth Factor Receptor 1 (*FGFR1*) mutation leading to disrupted splicing and Kallmann syndrome. *Hum Report.* 2018;33:328-330.
  18. Miraoui H, Dwyer AA, Sykiotis GP, Plummer L, Chung W, Feng B, et al. Mutations in *FGF17*, *IL17RD*, *DUSP6*, *SPRY4*, and *FLRT3* are identified in individuals with congenital hypogonadotropic hypogonadism. *Am J Hum Genet.* 2013;92:725-743.
  19. Swartz JM, Ciarlo R, Guo MH, Abrha A, Weaver B, Diamond DA, et al. A 46, XX Ovotesticular Disorder of Sex Development Likely Caused by a Steroidogenic Factor-1 (*NR5A1*) Variant. *Horm Res Paediatr.* 2017;83:191-195.
  20. Qin M, Gong CX, Qi Z, Wu D, Liu M, Gu Y, et al. Clinical characteristics of *KAL1* and *FGFR1* gene mutation in idiopathic hypogonadotropin hypogonadism. *Chin J Pediatr.* 2014;52:942-947. (In Chinese)
  21. Maione L, Dwyer AA, Francou B, Guiochon-Mantel A, Binart N, Bouligand J, et al. Genetics in endocrinology: Genetic counseling for congenital hypogonadotropic hypogonadism and Kallmann syndrome: new challenges in the era of oligogenism and next-generation sequencing. *Eur J Endocrinol.* 2018;178:R55-R80.
  22. Hughes SS, Welsh HI, Safina NP, Bejaoui K, Ardinger HH. Family history and clefting as major criteria for CHARGE syndrome. *Am J Med Genet A.* 2014;164A:48-53.

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