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Digenic *CHD7* and *SMCHD1* inheritance Unveils phenotypic variability in a family mainly presenting with hypogonadotropic hypogonadism

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ABSTRACT

Objectives: CHARGE syndrome is a congenital hereditary condition involving multiple systems. Patients are easily misdiagnosed with idiopathic hypogonadotropic hypogonadism (IHH) due to the overlap of clinical manifestations. An accurate clinical diagnosis remains challenging when the predominant clinical manifestation resembles hypogonadotropic hypogonadism.

Methods: This original research is conducted based on the genetic finding and analysis of clinical cases. Whole-exome sequencing (WES) and in-silico analyse were performed on two sisters to investigate the pathogenesis in this family. Homology modelling was conducted to evaluate structural changes in the variants.

Results: WES and Sanger sequencing revealed two siblings carrying a nonsense mutation (NM_017780.4: c.115C > T) in exon 2 of *CHD7* inherited from a mildly affected mother and a missense mutation (NM_015295.3: c.2582T > C) in exon 20 of *SMCHD1* inherited from an asymptomatic father. The nonsense mutation in *CHD7* was predicted to generate nonsense-mediated decay, whereas the missense mutation in *SMCHD1* decreased protein stability.

Conclusions: We identified digenic *CHD7* and *SMCHD1* mutations in IHH-associated diseases for the first time and verified the synergistic role of oligogenic inheritance. It was also determined that WES is an effective tool for distinguishing diseases with overlapping features and establishing a molecular diagnosis for cases with digenic or oligogenic hereditary disorders, which is beneficial for timely treatment, and family genetic counseling.

Abrrevations: ACMG, American College of Medical Genetics and Genomics; ACTH, Adrenocorticotropic hormone; BBS, Bardet-Biedl syndrome; CHD7, chromodomain helicase DNA binding protein 7; FSH, follicle-stimulating hormone; LH, luteinizing hormone; GnRH, gonadotropin-releasing hormone; HPG, axis hypothalamic–pituitary–gonadal axis; IBS, Illustrator for Biological Sequences; IHH, idiopathic hypogonadotropic hypogonadism; KS, Kallmann syndrome; MPHD, Multiple Pituitary Hormone Deficiency; nIHH, normosmic idiopathic hypogonadotropic hypogonadism; SMCHD1, structural maintenance of chromosomes flexible hinge domain containing protein 1; WES, Whole-exome sequencing; WS, Waardenburg syndrome.

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1. Introduction

CHARGE syndrome is a complex congenital hereditary condition involving multiple systems, including the ocular, cardiac, aural, reproductive, and endocrine systems. The manifestations of CHARGE syndrome are vary and include colobomas, heart defects, choanal atresia, developmental delay, genital hypoplasia, ear anomalies and deafness [1]. Hypogonadotropic hypogonadism, is characterized by the insufficient synthesis and secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) synthesis and secretion, due to disturbances or dysfunction of the hypothalamic–pituitary–gonadal (HPG) axis [2,3]. Hypogonadotropic hypogonadism is a prominent clinical feature of CHARGE syndrome, that may result in delayed sexual maturation, failure to develop secondary sexual characteristics, and abnormal genitalia. Additionally, it may result in infertility, resulting enormous psychological and physiological challenges for affected individuals and their families [4]. Hypogonadotropic hypogonadism presents in several different genetic disorders, such as idiopathic hypogonadotropic hypogonadism (IHH), Waardenburg syndrome (WS), Bardet-Biedl syndrome (BBS) and Turner Syndrome [1].

IHH is a group of rare reproduction-related genetic disorders attributed to the in-sufficiency of gonadotropin-releasing hormone (GnRH). The major clinical features of IHH include absent or delayed puberty, underdeveloped secondary sexual characteristics, and variable non-reproductive features [5]. The non-reproductive features of the IHH include cleft lip/palate, conductive deafness, ear anomalies, congenital heart disease, renal anomalies, dental agenesis, visual impairment and intellectual disabilities, and so on [5,6]. There are two subtypes of IHH: Kallmann syndrome (KS) with anosmia or hyposmia and normosmic idiopathic hypogonadotropic hypogonadism (nIHH) with a normal sense of smell [7].

In addition to the overlapping characteristics, CHARGE syndrome and IHH also share the causal genes, *CHD7*. *CHD7* gene encodes an integral membrane protein. It belongs to the chromatin remodeling family, which manipulates chromatin structure to activate and repress transcription [8]. The *CHD7* mutations have been reported in more than 90 % of patients with CHARGE syndrome [9] and 3–10 % of patients with IHH [10–12]. In addition to *CHD7* gene, more than 100 genes [13] known to involving in various developmental processes, such as migration, secretion, fate specification and olfactory axon guidance [5,10], have been reported to be associated with IHH.

Given the overlapbetween CHARGE syndrome and IHH, as well as the variable penetrance and expressivity of the pathogenic gene [14], it is challenging to deter-mine which specific genetic disorder it belongs to, especially when hypogonadotropic hypogonadism is a leading symptom of the patient. Hencs, genetic testing is a valuable tool to distinguish diseases with overlapping features. Moreover, some additional features have been discovered in patients with IHH by following careful examination of their genetic results. Therefore, whole-exome sequencing (WES) may help improve therapeutic and management strategies for affected individuals and families [5,15].

SMCHD1 belongs to the SMC gene superfamily, a family of proteins that involved in chromosome condensation and cohesion, genome maintenance and gene regulation. SMCHD1 has been implicated in X staining inactivation and epigenetic gene silencing through CpG island methylation modification. Researchers have found that SMCHD1 could play important role in the development of autosomal dominant inherited diseases such as facioscapulohumeral muscular dystrophy [16]. Herein, we report a Chinese family comprised of three patients who manifested variable features among the family members, mainly exhibiting hypogonadotropic hypogonadism with novel digenic disease-causing mutations in *CHD7* and *SMCHD1* by WES.

2. Materials and methods

Ethical approval

This study was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (TJ-IRB20211016). Written informed consent was obtained from all participants.

2.1. Whole-exome sequencing and Bioinformatics analysis

The proband and her older sister underwent WES. DNA was extracted from the peripheral blood of the patients using Qiagen DNA Blood Midi/Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. Then, 50 ng genomic DNA was interrupted to generate around 200 bp fragments, which were then subjected to end-repair, deoxyadenosine addition at the 3′ends, and adaptors ligation. Subsequently, DNA fragments were hybridized and captured using Berry's NanoWES Human Exome V1.0 (Berry Genomics, Beijing, China) following the manufacturer's instructions. The amplified and qualified library was sequenced on the Novaseq 6000 platform (Illumina, San Diego, USA) with an average depth of 100-fold coverage, and raw sequence reads were generated as 150bp paired end. Sequencing reads were aligned to the human genome assembly hg38 (GRCh38) using the Burrows–Wheeler Aligner (BWA) tool after removing adaptor sequences. Variant calling was performed with GATK (https://software.broadinstitute.org/gatk/) and Verita Trekker® Variants Detection System by Berry Genomics. Furthermore, variant annotation was performed using ANNOVAR [13] and the Enliven® Variants Annotation Interpretation System (Berry Genomics). The rare variants with minor allele frequencies (MAF) < 1 % in protein-coding regions in population databases (dbSNP, gnomAD, 1000 Genomes, and Shenzhou Genome Database of the Berry Genomics) were taken for further inter-pretation. In total, four prediction tools (SIFT, Polyphen2, MutationTaster, CADD) were used to assess variant pathogenicity, and the conservation scores were evaluated using GERP and PhyloP. The interpretation pathogenicity of variants was classified based on the guideline of American College of Medical Genetics

and Genomics (ACMG).

2.2. Sanger sequencing

The two candidate variants identified by WES were verified in the two siblings and their parents using Sanger sequencing. The primers were designed using Primer3 and the products were analyzed using an ABI 3730XL DNA Analyzer.

2.3. Pathogenicity analysis

Protein domains of the *CHD7* were generated by Illustrator for Biological Sequences (IBS) software [17]. Conservative predictions of *CHD7* and *SMCHD1* were conducted using UGENE software [18]. The three-dimensional (3D) structure of *SMCHD1* was analyzed by PyMOL (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC), using AF-A6NHR9-F1-model_v4 as the template, which was generated by Al-phaFold (https://alphafold.ebi.ac.uk). Prediction of protein stability was carried out with DUET (http://biosig.unimelb.edu.au/duet/stability) and I-Mutant (http://gpcr.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi), respectively. The potential phosphorylation site was predicted with NetPhos-3.1 (http://www.cbs.dtu.dk/services/NetPhos/).

2.4. Literature review of the oligogenic patterns associated with CHD7

We searched the PubMed to investigate the cases of oligogenic inheritance together with *CHD7* gene. The key words are as follow: *CHD7*, Kallmann syndrome, hypogonadotropic hypogonadism, digenic and oligogenic. The chromosomal locations of the partner genes with *CHD7* were determined using tools on the NCBI (http://www.ncbi.nlm.nih.gov/genome/tools/gdp). The protein-protein interactions were predicted with Genemania tool (https://genemania).

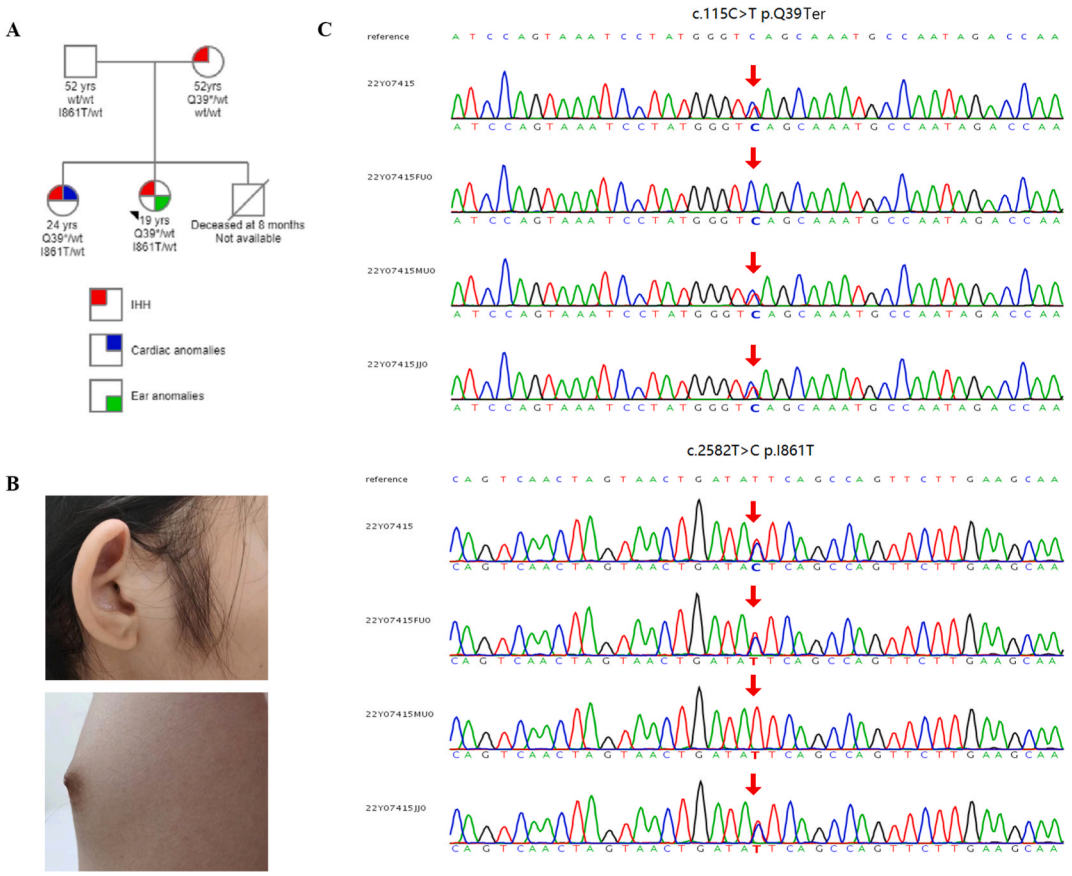

Fig. 1. Pedigrees, clinical characteristics, and detection of variants in the family with IHH-associated diseases. (A) Pedigree of the affected family. Arrows indicate the probands. (B): Ear and breast of the proband. (C) Sanger sequencing of two variants in the affected family (upper row: c.115C > T (p.Gln39Ter); lower row: c.2582T > C (p.Ile861Thr)).22Y07415: proband; 22Y07415FU0: father; 22Y07415MU0: mother; 22Y07415JJ0: sister.

3. Results

3.1. The clinical characteristics

3.1.1. Case 1

A 15-year-old Chinese girl was referred to gynecologic department of state hospital in October 2018 with primary amenorrhea and the developmental retardation of secondary sexual characteristics. She was born in April 2003 by an uneventful spontaneous delivery from a non-consanguineous family (Fig. 1A). The patient presented with delayed puberty, hyposmia, short stature (height: 150 cm), protruding ears and mild mental retardation (Fig. 1B) (Table 1). She self-reported the congenital deafness and underwent cochlear implantation at a young age. An olfactory test revealed that she was unable to identify alcohol, vinegar or water. The clinical characteristics of the proband were indicative of KS.

The patient's breast development had not initiated, and a swab could be inserted into the vagina up to approximately 8 cm. Subsequently, an endocrine test revealed inappropriately low levels of follicle stimulating hormone (FSH: 0.34 IU/L) and luteinizing hormone (LH: <0.1 IU/L) (Table 2). However, the patient's karyotype was normal (46, XX). Therefore, the patient was continuously treated with oral estradiol valerate for 21 days and progesterone for 10 days per cycle starting in November 2018. Following the administration of the hormone therapy, the patient experienced withdrawal bleeding. Regular reviews of liver and kidney function indicated continuously elevated alkaline phosphatase levels and treatment for liver protection was administered simultaneously. Nine months after initial treatment, she was switched to oral estradiol tablets/estradiol and dydrogesterone tablets due to side effects on the liver.

Regular reviews of basal pituitary hormone levels and gynecological transrectal ultrasonography results are shown in Table 2. At the age of 18, physical examination indicated a loss of breast development, sparse pubic or axillary hair, naive vulva, and a height of 144 cm in March 2021. Additionally, bone's age (15 years old) lagged behind her chronological age (18 years old). After another 4 months (Jul 2021), her breast development had progressed (Tanner stage 3), pubic or axillary hair was visible, and height had increased to 148 cm. In addition, FSH and LH levels were both lower than 0.1 IU/L at the subsequent follow-ups (Table 2).

The patient was later evaluated at the age of 19. Her height and weight were 150 cm and 38 kg, respectively. Moreover, FSH and LH were still less than 0.1 IU/L. Notably, pubertal development corresponded to Tanner stage 3. Computed tomography (CT) of the temporal bone revealed normal semicircular canals. Additionally, tests of eyes, teeth, extremities and joints showed normal results. Furthermore, ultrasound of thyroid, liver, gallbladder, spleen, pancreas, kidney and heart revealed no significant abnormalities. Finally, adrenocorticotropic hormone (ACTH) and growth hormone levels were <1.00 (normal range: 7.2–63.3 pg/ml) and 0.53 ng/ml, respectively.

3.1.2. Case 2

Patient 2 was the older sister of the proband. She exhibited persistent cyanosis of the lips and distal extremities at birth (Aug 1997). At the age of 4 years, she underwent cardiac surgery as the ultrasound results indicated tetralogy of fallot and atrial septal defects. She presented with delayed puberty and was diagnosed with hypogonadotropic amenorrhea at 18 years of age (Table 1). Subsequently, the patient received hormone replacement therapy and induced menstrual cycles. Her breasts developed quickly, and her height increased significantly one month after the therapy. In addition, she had normal senses of smell and exhibited no other clinical deformities. The sub-sequent follow-ups of the basal pituitary hormones are listed in Table 2.

Table 1
Clinical manifestations of three affected individuals in the family.

Diagnostic criteria[a]	Proband	Sister	Mother	Incidence in CS patients Incidence in CS patients [19]
Age (years)	19	24	52	
Major[a]				
Coloboma	Absent	Absent	Absent	66 %
Choanal atresia or cleft lip or palate	Absent	Absent	Absent	47 %
Abnormal external, middle, or inner ears, including hypoplastic semicircular canals	Protruding ears	Absent	Absent	94 %
Pathogenic CHD7 variant	✓	✓	✓	
Minor[a]				
Cranial nerve dysfunction including hearing loss	Anosmia, hearing loss	Absent	Anosmia	69–90 %
Dysphagia/feeding difficulties	Absent	Absent	Absent	82 %
Structural brain anomalies	Absent	Absent	Absent	50 %
Developmental delay/intellectual disabilities/ autism	Mild intellectual disabilities	Absent	Absent	88 %
Hypothalamo-hypophyseal dysfunction and genital anomalies	Primary amenorrhea, delayed puberty	Primary amenorrhea, delayed puberty	Spaniomenorrhea	68 %
Cardiac anomalies	Absent	Tetralogy of fallot, atrial septal defects	Absent	78 %
Renal anomaliesSkeletal/limb anomalies	Absent	Absent	Absent	46–80 %

[a] According to diagnostic criteria by Hale [19]. CHARGE syndrome (CS): 2 majors + any number of minor criteria.

Table 2
Basal pituitary hormones' assessment and gynecological transrectal ultrasonography results of siblings.

Measures	Proband						Sister	
	2018.10	2019.05	2020.08	2021.02	2022.01	2023.03	2017.05	2020.09
FSH(IU/L)	0.34	/	<0.10	<0.10	/	<0.05	<0.1	<0.1
LH(IU/L)	<0.10	/	<0.10	<0.10	/	0.03	<0.1	<0.1
E2(pg/mL)	5.12	/	16.79	11.15	/	204.84	7.88	7.24
PRL(ng/mL)	12.14	/	9.85	8.96	/	11.98	8.87	11.40
T(ng/mL)	<0.025	/	0.111	0.041	/	0.250	0.045	0.063
P(ng/mL)	/	/	<0.05	0.224	/	0.10	0.435	<0.05
Size of uterine (cm)	2.2*1.9*1.2	1.9*1.7*	3.3*2.8*2.1	/	/	3.9*3.4*2.6	/	/
Anteroposterior diameter of cervix(cm)	1.3	1.1	/	/	1.7	2.5	/	/
Endometrial thickness(cm)	0.29	0.19	/	/	0. 6	0.9	/	/
Size of left ovary(cm)	unclear	1.4*0.8	/	/	1.8*0.8	/	/	/
Size of right ovary(cm)	2.2*1.4	1.3*0.9	/	/	1.5*0.8	/	/	/
Antral follicle count	/	/	/	/	/	/	/	/

"/" indicates not detected.

3.1.3. Other family members

The proband's mother was 52 years old (height: 150 cm, menopause at 48 years). She had a history of spaniomenorrhea with menstrual cycle of 3–4 months and experienced menopause at 48 years of age. She also exhibited olfactory dysfunction (Table 1).

Among the family members, the third sibling had a normal pregnancy history. However, the patient developed cerebral palsy and osteomalacia. He died at the age of approximately 8 months. Notably, the paternal grandparents exhibited bilateral congenital blindness, whilethe father (height: 170 cm) and other family members had no significant history of delayed puberty, anosmia, hearing impairment, congenital blindness, cardiac anomalies, or other anomalies.

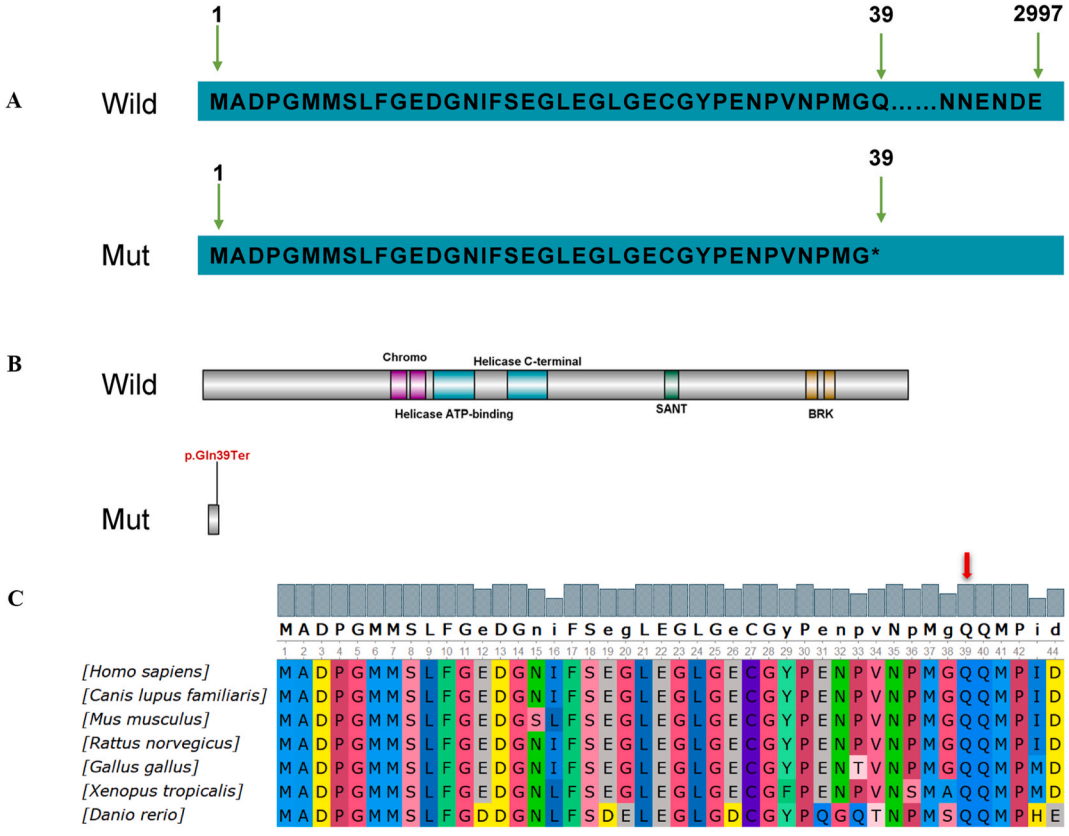

Fig. 2. Structure and functional analysis of c.115C > T; p.Gln39Ter mutation in *CHD7*.. (A) The premature termination codon arise from c.115C > T (p.Gln39Ter). (B) Truncated protein of *CHD7* arise from c.115C > T(p.Gln39Ter). (C) Conservation of the Gln39 across various species.

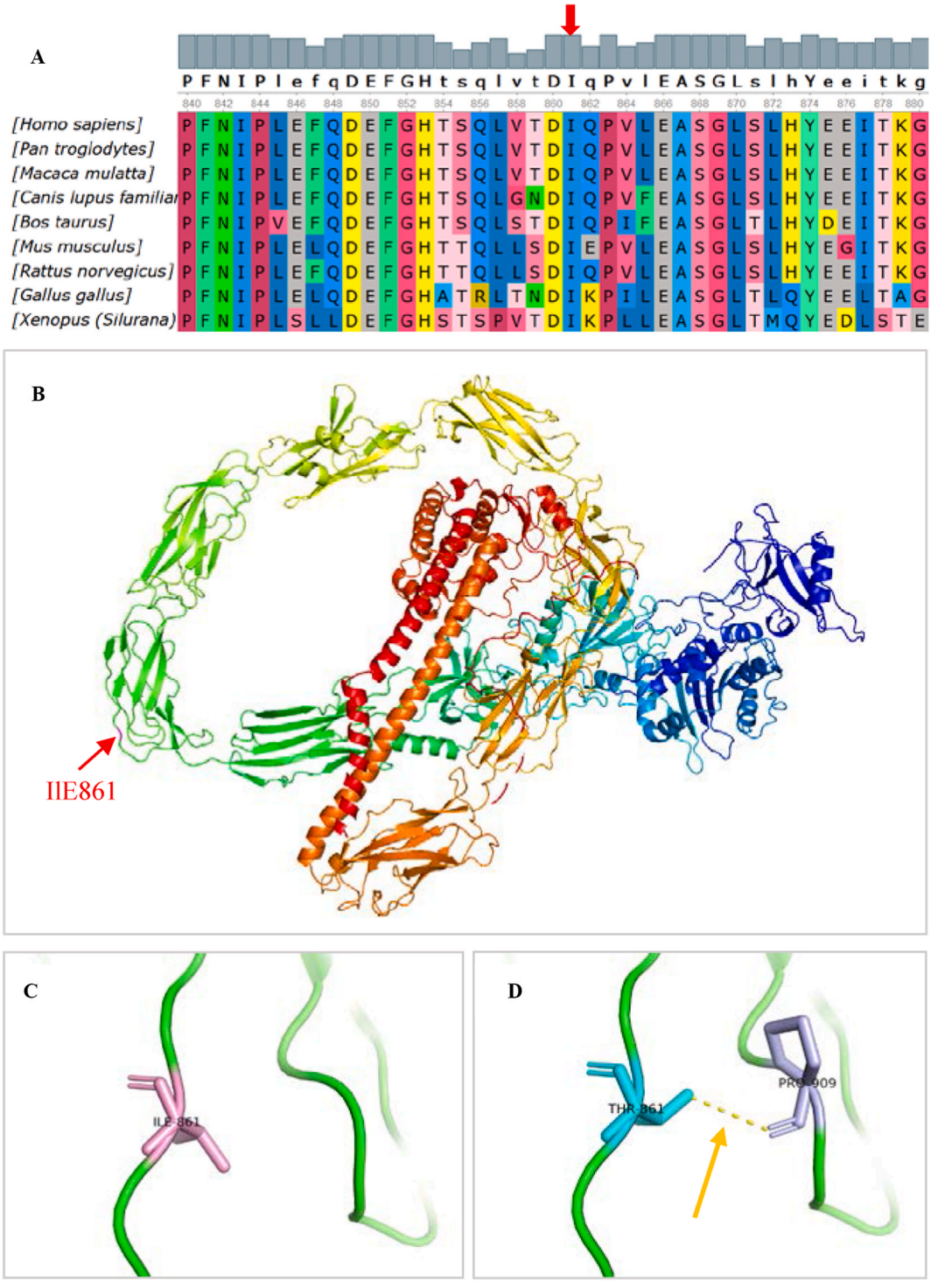

Fig. 3. Schematic representation of c.2582T > C; p.Ile861Thr mutation in *SMCHD1* protein models. (A) Conservation of the ILE861 across various species. (B) The red arrow points to the position of ILE861 in the *SMCHD1* protein model. (C) Stick models shows the amino acids around ILE861. (D) A new hydrogen bond between Thr861 and Pro909 (orange arrow) was created in mutant type. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Overview of the coexistence of *CHD7* and other known IHH-associated gene in digenic or oligogenic mode with IHH-related diseases in previous literature.

No.	Sex	Disease	Race	Gene	c.DNA and amino acid change	State	Resource	ACMG	Reference
1	M	KS	Chinese	CHD7	c.2824A > G p.Thr942Ala	Het	NA	LB	[20]
				SEMA3E	c.760G > C p.Glu254Gln	Het	NA	NA	
				NSMF	c.188C > T p.Pro63Leu	Het	NA	NA	
2	M	KS	Chinese	CHD7	c.8250T > G p.Phe2750Leu	Het	NA	LB	
				FGFR1	c.736C > T p.Arg246Trp	Het	NA	NA	
3	M	KS	Chinese	CHD7	c.3247A > G p.Thr1083Ala	Het	NA	NA	
				CHD7	c.6379G > A p.Ala2127Thr	Het	NA	VUS	
				HS6ST1	c.1177G > A p.Asp393Asn	Het	NA	NA	
4	M	KS	Chinese	CHD7	c.7912A > G p.Ile2638Val	Het	NA	NA	
				FGF17	c.580C > G p.Gln194Glu	Het	NA	NA	
5	M	nIHH	Chinese	CHD7	c.6955C > T p.Arg2319Cys	Het	NA	NA	
				PROKR2	c.533G > C p.Trp178Ser	Het	NA	NA	
6	M	nIHH	Chinese	CHD7	c.2656C > T p.Arg886Trp	Het	NA	NA	
				SOX2	c.6955C > A p.Thr 232Asn	Het	NA	NA	
7	M	KS	Caucasian	CHD7	c. 2840G > A p.Arg947Gln	Het	Paternal	VUS	[21]
				SEMA3A	c. 618_619delCC Ile206MetfsTer5	Het	Maternal	LP	
8	M	CS	Chinese	CHD7	c.4033C > T p.Arg1345Cys	Het	NA	VUS	[22]
				SEMA3A	c.196C > T p.Arg66Trp	Het	NA	VUS	
9	M	CS	Chinese	CHD7	c.2189C > T p.Thr730Ile	Het	NA	VUS	
				NSMF	c.533C > A p.Thr178Asn	Het	NA	VUS	
10	M	CS	Chinese	CHD7	c.4549_4563del p.S1517_T1521del	Het	NA	VUS	
				SEMA3E	c.760G > C p.Glu254Gln	Het	NA	VUS	
11	M	KS	Caucasian	CHD7	c.3088A > C p.Asn1030His	Het	NA	P	[23]
				FGFR1	c.853C > T p.Arg285Trp	Het	NA	LP	
12	M	KS	/	CHD7	c.2548A > C p.Lys850Gln	Het	NA	LP	
				LHX4	c.913G > T p.Gly305Trp	Het	NA	LP	
13	M	KS	Caucasian	CHD7	c.2053_2058dupGCAAAA p.Lys683_Thr684insAlaLys	Het	NA	VUS	
				GNRHR	c.416G > A p.Arg139His	Het	NA	P	
				GNRHR	c.30_31delinsAA p.Asn10_Gln11delinsLysLys	Het	NA	LP	
14	M	KS	Chinese	CHD7	c.7235A > T p.Glu2412Val	Het	Maternal	NA	[13]
				PROKR2	c.533G > C p.Trp178Ser	Het	Maternal	NA	
15	M	Anosmia	Chinese	CHD7	c.5980T > G p.Trp1994Gly	Het	Paternal	NA	
				CDON	c.2905G > A p.Val969Ile	Het	Maternal	NA	
16	Ms	KS	Chinese	CHD7	c.5980T > G p.Trp1994Gly	Het	Paternal	NA	
				CDON	c.2905G > A p.Val969Ile	Het	Maternal	NA	
17	M	KS	Chinese	CHD7	c.8773G > A p.Ala2925Thr	Het	Maternal	VUS	[24]
				PROKR2	c.337T > C p.Tyr113His	Het	Maternal	P	
				FEZF1	c.748C > G p.Arg250Gly	Het	Maternal	VUS	
18	M	KS	/	CHD7	c.6107C > T p.Pro2036Leu	Het	NA	NA	[25]
				PCSK1	c.239G > A p.Arg80Gln	Het	NA	NA	
19	F	nIHH	Caucasian	CHD7	c.1565G > T p.Gly522Val	Hom	NA	NA	[26]
				MCM9	c.911A > G p.Asn304Ser	Hom	NA	NA	
20	M	KS	Chinese	CHD7	c.3311T > C p.Ile1104Thr	Het	NA	NA	[27]
				RELN	c.4441A > C p.Lys1481Gln	Het	NA	NA	
21	M	KS	Chinese	CHD7	c.3568C > G p.Leu1190Val	Het	NA	NA	
				RELN	c.4441A > C p.Lys1481Gln	Het	NA	NA	
22	/	nIHH	Chinese	CHD7	c.4516G > A p.Gly1506Ser	Het	NA	NA	
				NSMF	c.877A > C p.Thr293Pro	Het	NA	NA	
23	M	KS	Chinese	CHD7	c.4516G > A p.Gly1506Ser	Het	NA	NA	
				EGF	c.1813G > C p.Val605Leu	Het	NA	NA	
24	M	nIHH	Chinese	CHD7	c.2656C > T p.Arg886Trp	Het	NA	NA	
				PLXNA1	c.2803G > A p.Ala935Thr	Het	NA	NA	
25	M	nIHH	Chinese	CHD7	c.2189C > T p.Thr730Ile	Het	NA	NA	
				DLX5	c.593A > C p.Asn198Thr	Het	NA	NA	
26	M	KS	Chinese	CHD7	c.4516G > A p.Gly1506Ser	Het	NA	NA	
				ERBB4	c.1972A > T p.Ile658Phe	Het	NA	NA	
27	M	KS	Chinese	CHD7	c.30del p.Phe10LeufsTer25	Het	NA	NA	
				NRP2	c.1333A > C p.Ile445Leu	Het	NA	NA	
				PLXNB1	c.4740C > A p.His1580Gln	Het	NA	NA	
28	M	KS	Chinese	CHD7	c.1727C > T p.Pro576Leu	Het	NA	VUS	
				RELN	c.4441A > C p.Lys1481Gln	Het	NA	NA	
				MTOR	c.889G > A p.Asp297Asn	Het	NA	NA	
29	F	KS	Finnish	CHD7	c.8188G > A p.Ala2730Thr	Het	NA	VUS	[28]
				FGFR1	p.Arg365LysfsTer5	Het	NA	NA	
30	F	KS	Finnish	CHD7	c.4847A > G p.Tyr1616Cys	Het	NA	VUS	
				SEMA3A	c.196C > T p.Arg66Trp	Het	NA	NA	
31	M	nIHH	Finnish	CHD7	c.2613+5G > A	Het	NA	VUS	

(*continued on next page*)

Table 3 (*continued*)

No.	Sex	Disease	Race	Gene	c.DNA and amino acid change	State	Resource	ACMG	Reference
				FGFR1	p.Ser436PhefsTer3	Het	NA	NA	
32	M	KS	Chinese	CHD7	c.6955C > T p.Arg2319Cys	Het	NA	P	[29]
				PROKR2	c.533G > C p.Trp178Ser	Het	NA	NA	
33	M	nIHH	Caucasian	CHD7	c.3245C > T p.Thr1082Ile	Het	Paternal	NA	[30]
				FGFR1	c.12G > T p.Trp4Cys	Het	Paternal	NA	
				PROKR2	c.802C > T p.Arg268Cys	Het	Maternal	NA	
34	M	KS	/	CHD7	c.3056T > G p.Phe1019Cys	Het	NA	NA	[31]
				SEMA3E	c.1855C > T p.Arg619Cys	Het	NA	NA	
35	F	KS	/	CHD7	c.2440C > T p.Gln814Ter	Het	NA	P	[32]
				KAL1	c.1627G > A p.Val543Ile	Het	NA	NA	
36	F	CS	Chinese	CHD7	c.115C > T p.Gln39Ter	Het	Maternal	P	This study
				SMCHD1	c.2582T > C p.Ile861Thr	Het	Paternal	VUS	

F: Female; M: Male; KS: Kallmann syndrome; nIHH: nIHH; CS: CHARGE syndrome; LB: NA: Not available.

3.2. Genetic findings

The proband and her older sister were subjected to WES. The coverage at a depth of 20X as 98.50 % and 98.27 % for the proband and their sister, respectively.

Two heterozygous variants were revealed and shared by two siblings, which were inherited from the mildly affected mother and asymptomatic father respectively (Fig. 1C). A maternally inherited variant at the start of exon 2 in *CHD7* (NM_017780.4: c.115C > T; p.Gln39Ter) generated a substitution of glutamine with a stop codon at position 39. As a result, the truncated *CHD7* lost all functional domains (Fig. 2A and B). Furthermore, multiple functional con-sequence annotation algorithms predicted that this mutation would be deleterious (Table S1). The wild Gln39 in this sequence was evolutionarily conserved across various species (Fig. 2C). This variant was neither recorded by any human public database, nor described in previous literature. Accordingly, it was classified as pathogenic based on the following criteria: null variant with very strong evidence of pathogenicity (PVS1), absence in control population databases (PM2), and co-segregation with the disease in three affected members (PP1).

The other identified variant in the patients was a paternally inherited variant (NM_015295.3: c.2582T > C; p.Ile861Thr) that induced a novel missense substitution in exon 20 of the *SMCHD1* gene. This variant has been found at an extremely low frequency in the eastern populations within gnomAD (0.0005823) and ExAC (0.0001) databases. Moreover, the p.Ile861Thr mutation in *SMCHD1* was predicted to be damaging, possibly damaging, disease-causing, tolerable, conserved and conserved, using SIFT, Polyphen2, Mutation Taster, CADD, GERP and phyloP tools respectively (Table S1). Multiple sequence alignment of *SMCHD1* amino acid sequences with UGENE demonstrated the c.2582T > C (p.Ile861Thr) variant was in a conserved region among species (Fig. 3A). Homology modeling of *SMCHD1* protein was performed using AlphaFold and visualized using PyMOL (Fig. 3B). The results indicated the mutant Thr861 formed a new hydrogen bond with adjacent Pro909, unlike Ile861 (Fig. 3C and D), which could create a significant conformational alteration and potentially destabilize the protein structure.

We predicted the stability of the mutation protein using DUET and the result indicated decreased protein stability (ΔΔG value = −1.285 kcal/mol). The other prediction tool, I-Mutant also supported the decreased stability of the protein (ΔΔG value = −1.63 kcal/mol). NetPhos-3.1 (http://www.cbs.dtu.dk/services/NetPhos/) suggested the mutation p.Ile861Thr introduced a potential Thrphosphorylation site which phosphorylated by protein kinase DNAPK and cdc2. Collectively, this variant was listed as having un-known significance (VUS) (PM2, PP2) based on ACMG criteria.

3.3. Digenic or oligogenic patterns

We compiled the literature and found that *CHD7* gene has been associated with CS or KS/nIHH in a digenetic or oligogenic manner with other known 24 IHH-causing genes in 36 cases (including the current study) (Table 3). Notably, The majority of the cases were diagnosed as KS or nIHH. Almost 66.67 % (24/36) of the participants were Chinese in origin. The most frequent co-existence partner genes were *PROKR2* and *FGFR1*, followed by *NSMF*, *RELN*, *SEMA3E* and *SEMA3A*. The chromosomal localizations of each gene are shown in Fig. 4A. The PPI networks with 45 nodes and 902 links based on GeneMANIA illustrated strong connections among these 25 IHH genes (Fig. 4B). The functions of these genes were significantly enriched in signaling pathways of neuron and axon guidance, growth factor receptor regulation and cell migration. Co-expression represented the largest percentage of the interactions (71.12 %), followed by predicted (14.21 %) and physical interactions (9.95 %). Additionally, *CHD7* and *SMCHD1* showed co-expression relationships which were recorded in GSE17920 and GSE70461. Finally, the PPI networks of *CHD7* and *SMCHD1* demonstrated that *CHD8* exhibited the most significant association with these genes (Fig. 4C).

4. Discussion

In the present study, we describe a family who were initially diagnosed with KS. The proband was reclassified as having CHARGE syndrome after genetic testing revealed *CHD7* nonsense mutations that met the criteria by Hale [19]. However, the affected sister was diagnosed with nIHH, as their condition did not fulfill any of the diagnostic criteria for CHARGE syndrome recommended by Blake

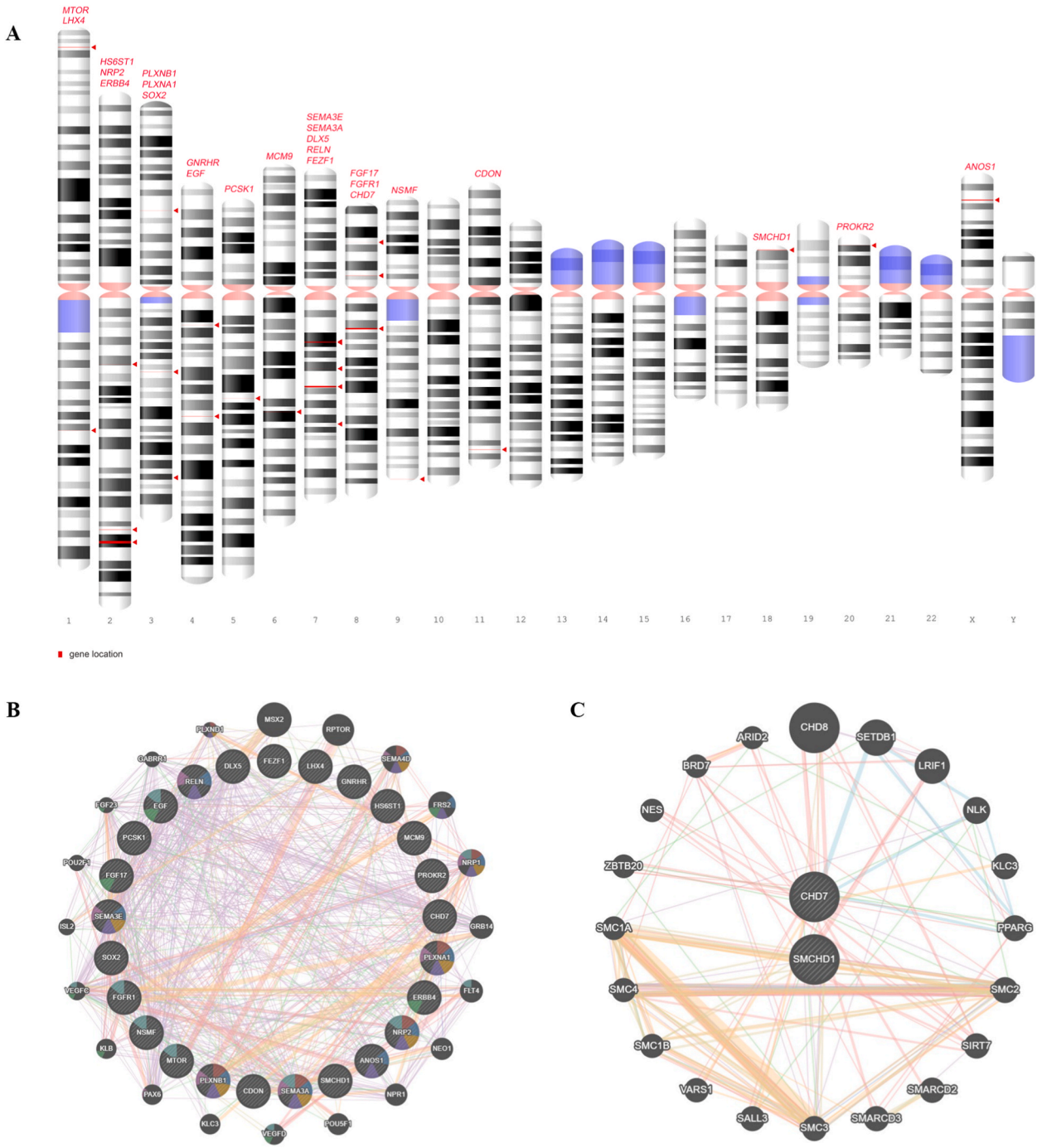

Fig. 4. Genes linked to oligogenic inheritance with *CHD7* gene in IHH individuals. (A) The chromosomal location of the partner genes; (B) Protein-protein interactions of the partner genes; (C) *CHD7* and *SMCHD1* protein interaction.

[33], Verloes [34], and Hale [19].

CHD7 gene encodes an integral membrane protein. It belongs to the chromatin remodeling family, which manipulates chromatin structure to activate and repress transcription [8]. The *CHD7* gene is located on 8q12 and consists of 38 exons encoding chromodomain helicase DNA binding (CHD)protein 7 [35,36]. *CHD7* protein is a highly conserved gene widely expressed in various tissues, containing 2997 amino acids and locating in the nucleoplasm and nucleolus. As a member of the chromosomal domain helicase family, *CHD7* is an ATP-dependent chromatin remodeling enzyme that regulates DNA accessibility in a tissue-specific manner. The *CHD7* protein belongs to a member of *CHD7* family which contains four major domains: two chromodomains, an SNF2 family N-terminal domain, a helicase

ATP binding domain, and two BRK do-mains [37]. *CHD7* has been shown to play a critical role in chromatin remodeling, apoptosis, cell cycle regulation, transcription, and embryonic stem cell differentiation [38,39]. Pathogenic CHD7 mutations are often de novo nonsense or frameshift mutations, which may lead to the loss of function of protein products due to translational termination, resulting in CHD7 haploid deficiency [40]. Of note, deleterious loss-of function CHD7 mutations interrupt the regulation of gene expression and result in disordered neural crest development [41]. *CHD7* gene mutations are considered to be the genetic cause of over 90 % of patients with typical CHARGE syndrome [42–44]. Therefore, identifying each novel pathogenic mutation of CHD7 gene, expanding the mutational spectrum, and incorporating it into the database is beneficial for the diagnosis of CHARGE syndrome and estimating the prevalence of the disease [45,46].

In this study, we identified a nonsense variant p. Gln39Ter in exon 2 of *CHD7* gene. Exon 2, which is the first and largest coding exon of the *CHD7* gene, encodes 555 amino acid. All the nonsense variants identified in this exon abolish all functional domains of the protein or are predicted to be degraded by nonsense-mediated decay (NMD), leading to haploinsufficiency. More than 74 nonsense mutations have been recorded in the HGMD, Clinvar and *CHD7* databases. Among these, 62 mutations were associated with CHARGE syndrome, 2 with KS, and 8 lacked clinical records (Table S2). Among prior cases, two patients with whole *CHD7* gene deletion and deletion of exon 3–38, exhibited typical CHARGE syndrome [20]. Among all these nonsense mutations, ap-proximately 73 % of them involve the mutations in the amino acid Gln (glutamine) to a premature termination codon in the exon 2 of *CHD7* gene. The nonsense mutation p.Gln39Ter was considered deleterious and potentially caused the phenotype observed in our three patients, consistent with haploinsufficiency due to NMD. Collectively, we speculated that variant p.Gln39Ter is deleterious and may be a causative factor in the observed phenotype in our three patients.

Clinical symptoms of *CHD7* mutations present a wide spectrum, from severe CHARGE syndrome to mild self-limited delayed puberty [47]. The *CHD7* mutations are reported in more than 90 % of patients with CHARGE syndrome [9] and 3–10 % of patients with KS/nIHH [10–12]. CHARGE syndrome is a well-known genetic disorder with multisystem congenital malformations, characterized mainly by eye coloboma, heart deformities, choanal atresia, retardation of growth, genital hypoplasia, and ear defects) [48]. Notably, CHARGE syndrome and KS/nIHH share some phenotypic similarities, such as genital hypoplasia, hearing loss, cleft lip and palate, and renal abnormalities [12].

In CHARGE syndrome patients, truncating *CHD7* mutations are predominantly observed, whereas missense variants are frequently observed in IHH patients [49,50]. Additionally, *CHD7* mutations have also been reported in affected individuals initially diagnosed with KS/nIHH and then re-evaluated for CHARGE syndrome [12,47]. Similarly, the proband in our study was re-diagnosed with CHARGE syndrome after identifying the *CHD7* nonsense mutation. The result further supported the notion that KS/nIHH are the milder allelic variant of CHARGE syndrome [1,11]. The term "*CHD7*-related disorder" was proposed to encompass the overall clinical spectrum of deleterious *CHD7* mutations [19]. These findings highlight the importance of a com-prehensive clinical assessment by a specialized multidisciplinary team for patients with KS/nIHH with null *CHD7* mutation, to distinguish between CHARGE syndrome and KS/nIHH [51].

The majority of CHARGE syndrome cases are sporadic and familiar CHARGE syndrome cases are relatively uncommon [52]. A previous review article indicated that 17 known CHARGE syndrome families exhibit significant intrafamilial clinical variability. These families included parent-child, siblings, and monozygotic twin pairs. Notably, the parent-child pair showed that the parents of a severely affected patient were usually mildly affected, and this became apparent only after the detection of the *CHD7* mutation [9]. Meanwhile, in a Finnish family, the mildly affected father transmitted p.Gln1599* mutation in *CHD7* to his two more severely affected sons. In agreement with the previous observations, the moderately affected mother of the proband in our study presented with spaniomenorrheaand mild olfactory dysfunction, without other CHARGE-associated symptoms. However, the symptoms of two siblings were more severe than those of their mothers.

The striking clinical interfamilial variability of *CHD7* mutation indicates that additional pathogenic mutations may also be present in the affected family in digenetic or oligogenic inheritance. For instance, the siblings in our study carried an additional heterozygous *SMCHD1* mutation (p.Ile861Thr) which inherited from the unaffected father. The *SMCHD1* protein contains two domains: a GHKL-type ATPase domain, a SMC hinge domain. The variant c.2582T > C (p.Ile861Thr) is located at the junction between the GHKL-type ATPase and SMC hinge domain. Heterozygous loss-of-function *SMCHD1* mutations are known to involve in facioscapulohumeral muscular dystrophy type 2. Heterozygous gain-of-function missense *SMCHD1* mutations are associated with Bosma arhinia microphthalmia syndrome (BAMS), which is characterized with absence of the nose, microphthalmia, and IHH [53]. This suggests that there issignificant phenotypic overlap between the *SMCHD1*-related diseases and IHH, such as hypoplasia of corpus callosum, hearing impairment, HH, and ocular abnormalities.

SMCHD1 is an epigenetic repressor which is overexpressed in the human olfactory epithelium and 97 % of the patients had hypogonadal features in 41 cases [54]. *SMCHD1* has also been observed in some patients with IHH without nasal abnormalities [55]. The mutation p.Ile861Thr was predicted to be deleterious and enhanced contactwith adjacent amino acids 908 and 909. It also generated a new potential Thrphosphorylation site, which is phosphorylated by protein kinases DNAPK and cdc2. Collectively, we speculated p.Ile861Thr mutation increased *SMCHD1*'s activity and resulted in IHH through the gain-of-function mechanism.

The multigenerational families harboring *SMCHD1* mutations were found with broad clinical features, presenting with only mild dysmorphism or anosmia. Thus, the authors speculated that coexistence of oligogenic genes with *SMCHD1* gene influences phenotypic variability. We hypothesized that *SMCHD1* p.I861 mutation interacts as a modifier gene to underlie the phenotypic heterogeneity in the family harboring the *CHD7* pathogenic mutation. However, the pathogenicity of *SMCHD1* p.Ile861Thr requires further analysis through in vivo and in vitro experimental studies.

CHD7 gene has been described to contribute to CHARGE syndrome or KS/nIHH in a digenetic or oligogenic manners with other 24 known IHH-causing genes in 36 cases. The majority of the cases were diagnosed as KS ornIHH. The most frequent co-existence partner

gene with *CHD7* was *PROKR2*, which reported in four KS/nIHH cases with disrupted GnRH neuron development [13,20,24,29]. In our analysis, the PPI networks demonstrated significant interactions between these genes. In addition, *CHD7* and *SMCHD1* were found to be co-expressed and shared connections with *CHD8*, providing further evidence of synergistic pathogenic role of *CHD7* and *SMCHD1*. However, most inherited oligogenic partner genes are typically discovered within families with limited sample sizes. Therefore, additional functional validation is re-quired to clarify the molecular mechanisms underlying the oligogenic genetic contribution to pathogenesis of IHH, given the complexity of disease symptoms.

Since most patients with CHARGE syndrome exhibit features of hypogonadotropic hypogonadism, which is typical characteristics of IHH, it is crucial to consider the possibility of CHARGE syndrome when clinically diagnosing IHH. In addition, Multiple Pituitary Hormones Deficiency (MPHD) also needs to be distinguished during the diagnosis. In this context, WES, as an un-biased genomic approach, could provide a more precise and comprehensive analysis of the molecular causes of diseases [56]. In the realm of IHH, most patients require life-long sex steroid replacement and fertility induction with GnRH [5]. Furthermore, particularly in case of CHARGE syndrome, attention should bepaid to the treatment of other potentially affected organs, in addition to gonads.

When *CHD7* mutations are detected, a thorough clinical history and physical examination of both the patient and family members are essential for optimize medical management. This is particularly relevant for girls carrying *CHD7* mutations, as they may not initially present with distinctive clinical features; nevertheless, they could exhibit developmental abnormalities later during adolescence. In this situation, timely intervention plays a crucial role in promoting the development of secondary sexual characteristics. For individuals with the hypogonadotropic hypogonadism phenotype, fertility can often be restored through GnRH therapy. However, disease-causing mutations may potentially transmit into future generations and may exhibit more severe symptoms in off-spring [9]. The prenatal diagnosis of CHARGE syndrome is quite difficult. Prenatal ultrasound has certain limitations in the diagnosis of this syndrome. The main clinical defects of this syndrome, such as eye defects, posterior nostril occlusion, and hearing impairment, are difficult to detect on prenatal ultrasound. Once the *CHD7* pathogenic variant has been identified in an affected family member, prenatal and preimplantation genetic testing are possible. Therefore, it is crucial to emphasize the risk of transmitting the disease to all IHH-related family members and consider prenatal diagnosis or preimplantation genetic diagnosis [57]. Genetic detection is helpful for the timely intervention in promoting the development of secondary sexual characteristics and beneficial for guiding the treatment recommendations of pregnancy in CHARGE syndrome. Gene targeted therapy may be a new trend in the future development of CHARGE syndrome. In addition, WES increases the diagnostic yield of disorders with genetic or oligogenic inheritance, further reinforcing the importance of WES in elucidating complex inheritance mechanisms [13].

However, there are still some limitations in this study. First, we did not find the genetic cause of the third child in this family who died after birth because no biological samples were retained. Second, the possible molecular mechanisms underlying the oligogenic genetic contribution to pathogenesis of IHH need to be further explored in future work. In addition, our study involves only three patients, the existing results still need to be further validated in a larger population in the future.

5. Conclusions

We identified digenic *CHD7* and *SMCHD1* mutations in IHH-associated diseases for the first time and verified the synergistic role of oligogenic inheritance. Our findings indicate the significance of a thorough clinical assessment and follow-up for CHARGE features, as well as individualized genetic counseling for patients with *CHD7* mutation. These results could broad the evidence of oligogenic inheritance in IHH-related diseases to some extent. Additionally, we report that WES serves as an effective tool for genetic testing for IHH-associated diseases, which can aid in the diagnosis, prognosis, and genetic counseling of patients.

Declarations

5.1. Ethics statement

The study was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (TJ-IRB20211016).

5.1.1. Consent statement

Written informed consent was obtained from all the participants.

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Data availability statement

The data that has been used is confidential.

CRediT authorship contribution statement

Tian Wang: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing, Data curation. **Wu Ren:** Formal analysis, Methodology. **Fangfang Fu:** Methodology, Validation. **Hairong Wang:** Formal analysis, Methodology. **Yan Li:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. **Jie Duan:** Conceptualization, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23272.

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