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Phenotypic spectrum of patients with mutations in *CHD7*: clinical implications of endocrinological findings

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Abstract

Objective: Heterozygous *CHD7* mutations cause a broad spectrum of clinical phenotypes ranging from typical CHARGE syndrome to self-limited delayed puberty. This study aimed to investigate the clinical characteristics of endocrine dysfunction in patients with *CHD7* mutations.

Methods: The clinical features and endocrine findings from 30 patients with *CHD7* variants were retrospectively reviewed. A diagnosis of CHARGE syndrome was based on the Verloes diagnostic criteria.

Results: Seventeen patients fulfilled the criteria for typical CHARGE syndrome, one patient for partial/incomplete CHARGE, and the remaining eleven patients had atypical CHARGE syndrome. One patient was diagnosed with Kallmann syndrome and unilateral deafness. The most frequently observed features were inner ear anomalies (80.0%), intellectual disability (76.7%), and external ear anomalies (73.3%). The mean height and weight SDSs at diagnosis were −2.6 ± 1.3 and −2.2 ± 1.8, respectively. Short stature was apparent in 18 patients (60%), and 1 patient was diagnosed with growth hormone deficiency. Seventeen males showed genital hypoplasia, including micropenis, cryptorchidism, or both. Seven patients after pubertal age had hypogonadotropic hypogonadism with hyposmia/anosmia and olfactory bulb hypoplasia. Truncating *CHD7* mutations were the most common ($n = 22$), followed by missense variants ($n = 3$), splice-site variants ($n = 2$), and large deletion ($n = 2$).

Conclusions: A diverse phenotypic spectrum was observed in patients with *CHD7* variants, and endocrine defects such as short stature and delayed puberty occurred in most patients. Endocrine evaluation, especially for growth and pubertal impairment, should be performed during diagnosis and follow-up to improve the patient's quality of life.

Introduction

Heterozygous *CHD7* mutations can lead to the development of CHARGE syndrome, characterized by a complex range of congenital malformations including coloboma, heart defects, atresia of choana, retarded growth and development, genital hypoplasia, and ear anomalies. The incidence of CHARGE syndrome is estimated to affect 1 in 10,000–17,000 live births (1). It can be clinically diagnosed according to major and minor criteria by Verloes, which are classified into typical, atypical, and partial/incomplete CHARGE syndrome based on clinical characteristics (2). Genetic analysis of *CHD7* contributes to the diagnosis of CHARGE syndrome and has expanded the

phenotypic spectrum of *CHD7* variants in individuals with lacking the full clinical features of CHARGE syndrome. *CHD7* variants have been identified in normosmic isolated hypogonadotropic hypogonadism (nIHH), Kallmann syndrome (KS), self-limited delayed puberty, as well as CHARGE syndrome (3, 4, 5, 6, 7).

Endocrine dysfunctions are commonly associated with CHARGE syndrome. Approximately 60–80% of patients with CHARGE syndrome present with hypogonadotropic hypogonadism, manifesting as genital hypoplasia such as micropenis or cryptorchidism in males and delayed or absent puberty caused by gonadotropin-releasing hormone (GnRH) deficiency in both sexes (4, 8). Hypoplastic labia has been reported in one-third of female patients, and several cases of reproductive tract anomalies such as uterine and ovarian aplasia have also been reported (9, 10). Growth retardation affects 60–72% of individuals with CHARGE syndrome, becoming apparent from early infancy despite adequate fetal growth (11, 12). This stunted growth can be caused by cleft lip or palate-associated feeding difficulties, cardiac anomalies, and deficiencies in growth hormone (GH) or combined pituitary hormone (8, 13). GH deficiency occurred at a frequency of 10–34% (11, 14, 15, 16), and previous studies reported structural abnormalities of the pituitary gland in two unrelated patients and combined pituitary hormone deficiency in 8% of a Japanese cohort with CHARGE syndrome (16, 17).

Advanced medical and surgical treatment has allowed for life-threatening malformations such as congenital heart defects to be corrected in infancy; however, impaired growth and pubertal development can affect the patient long-term. This study aimed to investigate the phenotypic and mutational spectrum of 30 patients with *CHD7* mutations, with a focus on endocrine findings such as hypogonadotropic hypogonadism and short stature.

Patients and methods

Patients

This study included 30 patients (20 males and 10 females) with *CHD7* mutations who had been diagnosed between March 1994 and May 2021 at the Department of Pediatrics, Asan Medical Center Children's Hospital, Seoul, Korea. Patients were diagnosed with CHARGE syndrome according to the Verloes criteria (2). Those who did not carry *CHD7* mutations were excluded. Clinical and endocrinological findings such as absent or incomplete puberty were used to diagnose KS and nIHH as previously described (18).

The study was approved by the Institutional Review Board at Asan Medical Center (IRB No. 2019-0773). DNA analysis was performed after obtaining written informed consent from patients or their guardians.

Clinical and endocrinological evaluation

Height and weight SDS was calculated based on the age- and sex-matched references from the Korean National Growth Charts (19). Pubertal stage was evaluated according to the Marshall and Tanner criteria (20), and testicular volume was measured using a Prader orchidometer. Short stature was defined as the height SDS below −2.0 SDS, and delayed puberty was defined as incomplete or absent puberty in girls and boys older than 13 and 14 years, respectively (18).

Serum thyroid-stimulating hormone (TSH) and free T4 levels were measured using an IRMA (IRMA, TSH CTK-3®, DiaSorin, Saluggia, Italy) and RIA (FT4 RIA Kit®, Beckman Coulter, Prague, Czech Republic), respectively. Serum insulin-like growth factor 1 (IGF-1) and IGF-binding protein 3 (IGFBP-3) levels were determined by IRMA (Immunotech, Marseilles, France) and expressed as an SDS based on age- and sex-matched normative data from Korean references (21). Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were measured with IRMA (Byk-Sangtec Diagnostica, Hessen, Germany), while estradiol and testosterone levels were measured by RIA (Coat-A-Count, Diagnostic Products, Los Angeles, CA, USA). Two different GH stimulation tests were performed using L-dopa and arginine or regular insulin (22). Indications for evaluation of the GH stimulation test include individuals with short stature and an abnormal growth rate or low IGF-1 levels. GH deficiency was defined as peak GH less than 10 ng/mL in both stimulation tests. The GnRH stimulation test was conducted using an i.v. bolus infusion of a standard dose of 60 $\mu g/m^2$ GnRH (Relefact®, Sanofi-Aventis, Frankfurt, Germany) in patients with delayed puberty ($n = 7$). Serum LH and FSH levels were measured immediately before the injection of GnRH and at 30, 45, 60, and 90 min after injection.

Molecular analysis of the *CHD7* gene

Genomic DNA was extracted from peripheral blood leukocytes using a Puregene DNA isolation kit (Qiagen). All coding exons (2–38) and exon–intron boundaries of *CHD7* were amplified by PCR using specific oligonucleotide primers. PCR products were directly sequenced using an ABI3130x1 Genetic Analyzer (Applied Biosystems). Multiplex ligation-dependent probe amplification (MLPA)

analysis was conducted in two patients without sequence variants in *CHD7* to detect exon deletions or duplications using the SALSA MLPA P201 CHARGE probemix (MRC-Holland, Amsterdam, Netherlands) according to the manufacturer's instructions.

Targeted panel sequencing or whole-exome sequencing (WES) was performed in five patients: three with atypical CHARGE syndrome, one with partial/incomplete CHARGE syndrome, and one with KS. The SureSelect Target Enrichment system kit and SureSelect Human All Exon V5 (Agilent Technologies) were used for targeted panel sequencing and WES, respectively. Sequencing was performed using the NextSeq500 platform (Illumina Inc., San Diego, CA, USA), and sequenced reads were aligned to the human reference genome (hg19) using the Burrow–Wheeler Alignment program (BWA version 0.7.12). SAMtools 0.1.19 and Genome Analysis Toolkits (GATK version 3.5) were used to detect single nucleotide variants, and GATK version 3.5, FreeBayes 0.9.2.1, and Scalpel-0.5.3 for insertion–deletion variant calling. Annotation was performed with a Variant Effect Predictor (23). Rare sequence variants found in targeted panel sequencing or WES were validated by Sanger sequencing. Pathogenicity was determined in novel sequence variants according to the standards and guidelines of the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (24).

Results

Clinical characteristics of patients with sequence variants in *CHD7*

Seventeen patients fulfilled the criteria for typical CHARGE syndrome, one for partial/incomplete CHARGE syndrome, eleven patients had atypical CHARGE syndrome, and one patient was diagnosed with KS and deafness at the age of 16 years (Table 1). Additionally, three patients with atypical CHARGE syndrome were initially misdiagnosed with nIHH or KS because of delayed puberty. The mean age at presentation was 5.0 ± 6.4 years (range, 1 month–26.5 years). The clinical characteristics of each patient are summarized in Fig. 1.

Eighteen patients (60%) had coloboma. Seven patients (23.3%) had choanal atresia, which was found only in patients with typical CHARGE syndrome. CT revealed inner ear anomalies such as aplasia or dysplasia of the semicircular canal, absent or hypoplastic cochlea, or Mondini dysplasia in 24 patients (80%), all of whom had hearing loss.

Table 1 Clinical and endocrine characteristics of patients with sequence variants in CHD7.

Characteristics	Total (*n* = 30)	Typical (*n* = 17)	Atypical and partial (*n* = 12)	Kallmann syndrome (*n* = 1)
Age at evaluation (years)	5 ± 6.4	4.3 ± 5.5	5.9 ± 7.5	16
Sex, no. (%)				
Female	10 (33.3%)	8 (47.1%)	2 (16.7%)	0
Male	20 (66.7%)	9 (52.9%)	10 (83.3%)	1
Gestational age, weeks	38 + 5 (±9.7 days)	38 + 4 (±11 days)	39 + 0 (±6.6 days)	40
Birth weight, grams	2948 ± 357	2931 ± 334	3023 ± 420	3100
SGA, no. (%)	5 (16.7%)	1 (5.9%)	4 (33.3%)	0
AGA, no. (%)	25 (83.8%)	16 (94.1%)	8 (66.7%)	1
Anthropometric parameters				
Height SDS	−2.6 ± 1.3	−2.43 ± 1.36	−2.6 ± 1.49	−1.87
Weight SDS	−2.2 ± 1.8	−2.27 ± 1.52	−2.4 ± 2.22	0.29
BMI SDS	−0.8 ± 1.4	−1.22 ± 1.1	−0.5 ± 1.64	1.36
Growth, no. (%)				
Short stature	18 (60%)	11 (64.7%)	7 (63.6%)	0
GH stimulation test	5 (16.7%)	4 (23.5%)	1 (8.3%)	0
GHD	1 (3.3%)	1 (5.9%)	0	0
Cryptorchidism	12/20 (60%)	5/9 (55.6%)	7/10 (70%)	0
Micropenis	14/20 (70%)	4/9 (44.4%)	10/10 (100%)	0
Hyposmia/anosmia	7 (23.3%)	2 (11.8%)	4 (33.3%)	1
Patients with pubertal age	7	4	2	1
HH	6	4	2	1
SRT	6	4	2	1[†]

[†]Reversible Kallmann syndrome.
AGA, appropriate for gestational age; GH, growth hormone; GHD, growth hormone deficiency; GnRH, gonadotropin-releasing hormone; HH, hypogonadotropic hypogonadism; SGA, small for gestational age; SRT, sex hormone replacement therapy.
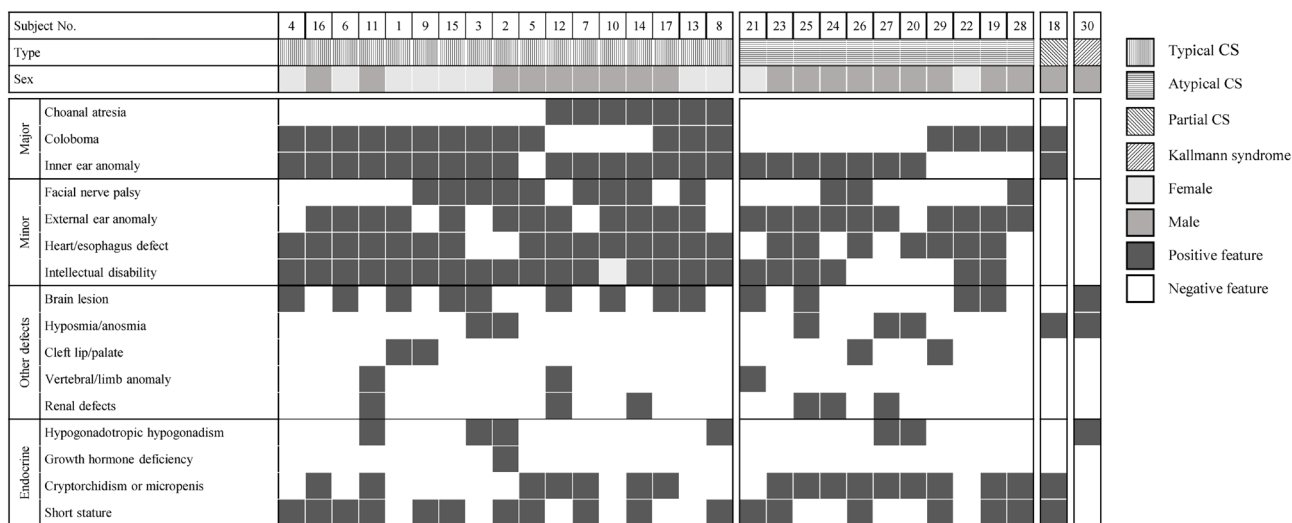
Figure 1
Phenotypic characteristics of 30 patients with CHARGE syndrome and Kallmann syndrome. Patients are represented by columns and clinical features by rows. Clinical features are indicated as positive (dark gray) or negative (white). CS, CHARGE syndrome; No, number.

External ear anomalies were detected in 22 patients (73.3%). Additionally, we noted facial palsy (seventh cranial nerve) in 12 patients (40%) and swallowing or feeding difficulties in 15 patients (50%). Brain MRI revealed anomalies in 14 of 17 patients, including cerebellar vermis hypoplasia ($n = 6$), ventriculomegaly ($n = 6$), olfactory bulb aplasia ($n = 3$), dysgenesis of the corpus callosum ($n = 2$), and brainstem hypoplasia ($n = 1$). Moreover, three patients had multiple brain anomalies. There were no structural abnormalities in the pituitary gland. Twenty-three patients (76.7%) had intellectual disabilities, and non-lesional focal epilepsy was observed in one patient (3.3%).

Twenty-two patients (73.3%) had congenital heart defects. Conotruncal and outflow tract anomalies were most commonly found, including coarctation of the aorta ($n = 3$, 10%), tetralogy of Fallot ($n = 2$, 6.7%), interrupted aortic arch ($n = 2$, 6.7%), pulmonary atresia with intact ventricular septum ($n = 1$, 3.3%), and transposition of the great arteries ($n = 1$, 3.3%). Isolated patent ductus arteriosus (PDA), ventricular septal defect (VSD), and atrial septal defect (ASD) were detected in 10% ($n = 3$), 6.7% ($n = 2$), and 3.3% ($n = 1$) of patients, respectively, and ASD with PDA and VSD with PDA was shown in 13.3% ($n = 4$) and 6.7% ($n = 2$), respectively. Vascular ring and PDA was evident in one patient (3.3%), and tracheoesophageal fistula and esophageal stenosis were present in one patient.

Six patients (20%) showed renal defects including solitary kidney ($n = 1$), hydronephrosis ($n = 2$), bilateral renal hypoplasia ($n = 1$), horseshoe kidney ($n = 1$), and vesicoureteral reflux ($n = 1$).

Growth impairment and lack of pubertal development

The mean birth weight of all patients was 2.95 ± 0.36 kg, all were born at full term except for two born preterm at 35 weeks and 35 + 6 weeks of gestation, respectively. Five patients (16.7%) were born small for gestational age (SGA) (birth weight < third percentile for gestational age). Growth impairment was evident during the postnatal period, and the mean height and weight SDSs at 5.0 ± 6.4 years of age were −2.6 ± 1.3 (range −5.21 to −0.2) and −2.2 ± 1.8 (range −5.71 to 0.74), respectively. Of these, short stature was apparent in 18 patients (60%).

The GH stimulation test was conducted in five patients with short stature and decreased growth velocity or low serum IGF-1 levels. Among them, one male (patient 2) was confirmed to have GH deficiency at 8 years of age. However, the patient refused recombinant human GH (rhGH) treatment and was therefore treated with testosterone enanthate from 14.8 years of age. The mean change in height (ΔHt SDS) was +0.5 (from −2.84 to −2.34) over 3.5 years of testosterone treatment. rhGH was administered to four patients with short stature, including a patient with SGA, and the mean ΔHt SDS was +0.84 ± 0.60 during the treatment period of 3.9 ± 3.1 years. Three of them achieved a good response to GH treatment (ΔHt SDS +1.3 ± 0.04 for 3.7 ± 2.5 years), while one patient showed a poor response (ΔHt SDS +0.22 for 4.8 years).

Seventeen males showed genital hypoplasia, including micropenis (20%, 4 of 20 males), cryptorchidism (15%,

3 of 20 males), or both (50%, 10 of 20 males). Seven patients (five males and two females) reached pubertal age, and all patients manifested absent puberty and were diagnosed with hypogonadotropic hypogonadism after receiving sex hormone replacement therapy. Spontaneous recovery of hypogonadotropic hypogonadism was observed in one patient (*CHD7* c.118C>T, p.Q40*) at age 25.5 years after 9.4 years of testosterone enanthate therapy.

Mutation spectrum of the *CHD7* gene

The sequence variants in *CHD7* were distributed throughout the gene without hotspots. Most sequence variants were truncated including 11 nonsense (37.9%) and 11 frameshift (37.9%), followed by three missense variants (10.3%), two splice-site variants (6.9%), and two large deletions (6.9%; Fig. 2). Of these, 14 were novel and 15 variants had been previously reported as pathogenic or likely pathogenic. Most sequence variants in *CHD7* were found only in a single family; however, one known

pathogenic nonsense mutation (p.R2627*) was identified in two unrelated patients with typical and atypical CHARGE syndrome, respectively.

Nonsense and frameshift mutations were evenly distributed across patients with typical (70.6%, *n* = 12) and atypical CHARGE syndrome (81.8%, *n* = 9). Moreover, two splice-site variants were found in each patient with typical or atypical CHARGE syndrome. The novel c.2836-2A>G variant in patient 10 with typical CHARGE syndrome occurred *de novo* through parental testing. In contrast, the c.5405-7G>A variant in patient 20 with atypical CHARGE syndrome had been previously reported (25), although family testing had not been available. Two patients with typical CHARGE syndrome harbored whole gene deletion (patient 16) and deletion of exon 3–38 (patient 2), respectively. One patient with KS had a possible mosaic variant of *CHD7* p.Q40*, of which the variant was visually inspected by Integrative Genomics Viewer version 2.8.9 (https://software.broadinstitute.org/software/igv/) and was validated by Sanger sequencing (Supplementary Fig. 1,
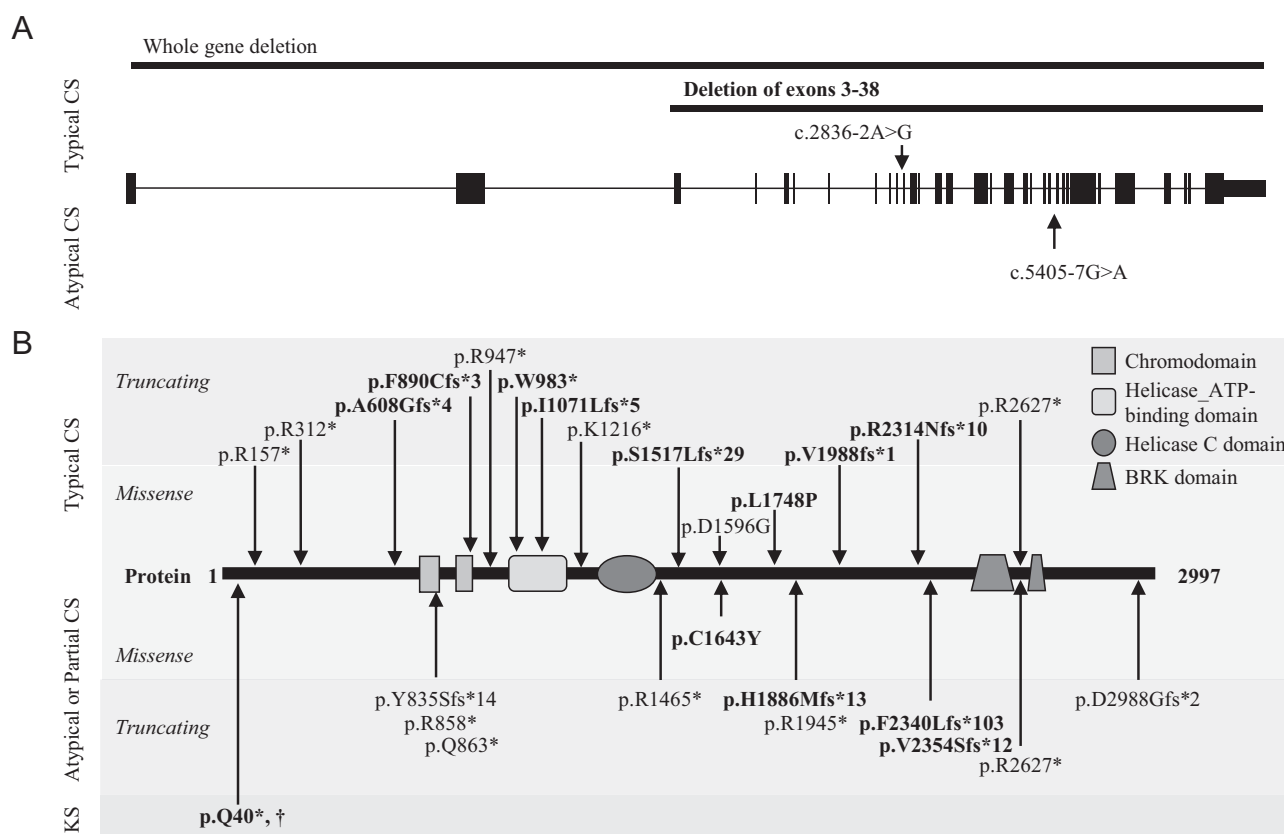

Figure 2
The schematic diagram of CHD7 protein domains and positions of identified *CHD7* variants in our cohort. (A) The structure of the *CHD7* gene. Coding exons are indicated in black bars, while introns are indicated by black lines. Large deletions and splice-site variants are described schematically. (B) The structure of CHD7 protein and mutations identified in the study cohort. Protein domains are labeled, and arrows indicate mutations. CS, CHARGE syndrome; KS, Kallmann syndrome; Bold, novel mutation; †, germline mosaicism.

see section on supplementary materials given at the end of this article). Missense variants were found in two typical and one atypical CHARGE patients with two novel variants (p.C1643Y and p.L1748P); however, family testing was not available. *In silico* prediction programs determined that the variants were deleterious (Supplementary Table 1) and were not found in the Genome Aggregation Database (https://gnomad.broadinstitute.org/). The p.C1643Y and p.L1748P variants were classified as likely pathogenic and variant of uncertain significance, respectively (Supplementary Table 1). Collectively, mutational loci and types were similarly distributed across typical and atypical CHARGE syndrome in our cohort.

Discussion

This study demonstrated that most patients with *CHD7* variants have associated features of endocrine dysfunction such as short stature and hypogonadotropic hypogonadism. Patients with typical and atypical CHARGE syndrome exhibited a wide phenotypic spectrum with similar distribution of variants along the protein. Most patients harbored truncating mutations, and only three patients had missense variants; thus, our cohort could not determine the genotype–phenotype correlation. However, a patient with KS had possible mosaic *CHD7* variants, showed reversible KS, which is assumed that mosaicism can result in milder, attenuated phenotypes depending on the distribution and frequency in different tissues (26). Ultradeep sequencing in multiple tissues is required to verify pathogenicity of mosaic variants.

In our cohort, inner and external ear anomalies accompanied by hearing loss were observed in most patients with typical and atypical CHARGE syndrome. However, choanal atresia presented in 23.3% of patients, exclusively in those with typical CHARGE syndrome, and coloboma and complex heart disease were more frequently found in typical CHARGE syndrome compared with atypical patients (76.5% vs 45.5%, respectively; 64.7% vs 45.5%, respectively). Various brain abnormalities were detected mainly in the midbrain, rhombencephalon, and the olfactory bulbs of 17 patients who underwent brain MRI, in line with previous studies (27, 28, 29). The clinical relevance of brainstem or cerebellar vermian hypoplasia is unclear, but previous studies suggest that it may be associated with cognitive deficit and behavioral problems (30, 31). There are no guidelines for brain imaging; however, a recent study has recommended that a comprehensive review of brain malformation in CHARGE syndrome can lead to the provision of optimal patient care. Improving awareness, such as of difficult airway for general anesthesia and enabling further research, can improve the understanding of diverse brain anomalies (32).

Three patients (patients 20, 25, and 28) were initially misdiagnosed with isolated KS or micropenis/cryptorchisim; however, after confirming the *CHD7* mutations through targeted gene panel sequencing or WES, they were re-classified as atypical CHARGE syndrome by Verloes criteria. Patient 20 with postpubertal age manifested hypogonadotropic hypogonadism with anosmia. However, the other two patients with cryptorchidism/micropenis or anosmia could not be diagnosed with KS because they were prepubertal age. The young patients with cryptorchidism/micropenis and anosmia should be followed up for pubertal progression. In addition, we recommend screening for clinical criteria of CHARGE syndrome in patients diagnosed with KS/nIHH or with multiple anomalies, particularly with inner or external ear anomalies.

Over 60–90% of patients with clinical CHARGE syndrome carry a heterozygous mutation in *CHD7* (11, 25). These mutations have also been found in approximately 6% of KS cases, and the patients present with minor features of CHARGE syndrome (3, 4, 33). Over 498 variants in *CHD7* have been reported to be pathogenic or likely pathogenic according to the ACMG standards and guidelines (https://www.ncbi.nlm.nih.gov/clinvar/). Most *CHD7* mutations in CHARGE syndrome are truncating across all coding exons, whereas missense mutations are enriched in patients with KS or nIHH (3, 34). In our study, two patients carrying the same mutation (p.R2627*) exhibited typical and atypical CHARGE syndrome. The variable phenotypic spectra and penetrance suggest the genetic modifier can modulate the expression of the CHD7; further research is therefore needed to understand these clinical implications (3). The recent application of WES or whole-genome sequencing technologies in rare Mendelian disorders has uncovered rare sequence variants that are previously difficult to detect by Sanger sequencing and copy number variations or structural variations in retrotransposon (35, 36, 37). Undetermined cases with CHARGE syndrome or IHH should therefore undergo further genome-scale sequencing.

Hypogonadotropic hypogonadism is the finding most commonly revealed in CHARGE syndrome, present in more than 80% of patients (11). Genital hypoplasia in males and absence or incomplete puberty in both sexes are

common. Defects in *CHD7* cause improper development or migration of olfactory and GnRH neurons, leading to various forms of hypogonadism. Therefore, the olfactory phenotype has been proposed as a predictor of hypogonadotropic hypogonadism in patients with CHARGE syndrome (38). Similarly, seven patients with CHARGE syndrome in our cohorts who reached pubertal age manifested hypogonadism and olfactory dysfunction. However, most of our patients (76.7%) were of prepubertal age; long-term follow-up is therefore required to evaluate pubertal development.

GH deficiency has been reported in 12–34% of cases across several studies (11, 16). GH deficiency in our patients was relatively uncommon; however, GH stimulation tests were not performed for all cases (11). Although no randomized controlled study was conducted, rhGH treatment showed benefit in patients with CHARGE syndrome with GH deficiency (39). In our study, four short-stature patients without rhGH deficiency underwent rhGH therapy and three showed a good response (40, 41). Although rhGH replacement was beneficial, the height SDS of three patients ranged from –1.8 to –2.3 according to age- and sex- matched references (19). A multidisciplinary approach, such as nutritional support and endocrinological management, will be necessary to optimize growth. Combined pituitary hormone deficiency is not commonly observed in patients with CHARGE syndrome (17). Moreover, central hypothyroidism has a reported prevalence of 6–8% (16, 42), and although primary hypothyroidism has also been reported, determining an association with this disease has been difficult (14, 17). Although the incidences of combined pituitary hormone deficiency and thyroid defect are relatively low, these deficits can exacerbate growth and developmental impairment in patients with CHARGE syndrome.

This study is limited by its retrospective, single-center study design and the inclusion of a small number of patients of pubertal age. As previously described, demonstrating a relationship between genotype and phenotype is challenging, given its rarity.

In conclusion, *CHD7* heterozygous defects lead to a diverse phenotype, including endocrine manifestations, ocular and ear anomalies, and brain anomalies in the midbrain and rhombencephalon. Endocrinological evaluation for anterior pituitary dysfunction, particularly GH secretion and hypogonadism, is necessary in patients with these mutations. Appropriate hormone replacement therapy can increase adult height and induce pubertal development, thereby preventing comorbidities and improving patients' quality of life.

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