



## A novel CHD7 mutation in a Chinese patient with CHARGE syndrome

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### ABSTRACT

In Genetics Out-patient Department of Shanghai Children's Medical Center, we consulted a 3-year-old boy with multiple anomaly syndrome (congenital heart disease, cryptorchidism, congenital deafness, mental retardation, exophthalmos, laryngeal cartilage dysplasia and high arched palate). We ruled out the possibility of multiple deformities caused by genomic imbalances. The patient was then clinically considered to have CHARGE syndrome, an autosomal dominant multi-system disorder involving defects in multiple organs, and *CHD7* is the only known gene associated with the syndrome. Sequencing analysis of *CHD7* of the proband identified a *de novo* heterogeneous mutation (c.2916\_2917del, p.Gln972HisfsX22), a two-nucleotide deletion causing reading frame shift and resulting in a truncated CHD7 protein. Computational structure analysis suggests that the truncated protein only contains the chromodomains of CHD7, but lacks the SWI2/SNF2-like ATPase/helicase domain and the DNA binding domain, which are indispensable for the proper function of the protein, especially on chromatin remodeling. The patient then received follow up treatment in different clinical departments in a long period. To our best knowledge, this is the first CHARGE syndrome in Chinese patients diagnosed by gene analysis. In summary, the clinical symptoms and the description of treatment in the present case, combined with genetic test and functional prediction of CHD7, are helpful for further understanding and genetic counseling of the CHARGE syndrome.

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## Introduction

CHARGE syndrome is an autosomal dominant multi-system disorder involving coloboma, heart defects, choanal atresia, retarded growth and development, genital hypoplasia, ear anomalies and/or deafness (Jongmans et al., 2006). In 1998, Blake et al. established reformative diagnostic criteria for CHARGE syndrome comprising of major and minor criteria. Major criteria include coloboma, choanal atresia, ear anomalies/deafness and cranial nerve dysfunction; while minor criteria include heart defects, genital hypoplasia, growth deficiency, developmental delay, tracheoesophageal fistula, orofacial cleft, and distinctive facial appearance (Blake and Prasad, 2006).

*CHD7* gene, located at chromosome 8 (8q12) and starting at 61.59 Mb from the p-arm telomere, has a genomic size of 188 kb and consists of 38 exons. It encodes for the chromodomain helicase DNA binding protein, a member of the chromodomain family. In human neural crest cells, *CHD7* forms a protein complex with PBAF (polybromo- and BRG1-associated factor-containing complex) that regulates chromatin structure, gene expression and embryonic development (Bajpai et al., 2010; Hargreaves and Crabtree, 2011). Currently, *CHD7* is the only gene known to be associated with the CHARGE syndrome (Lalani et al., 2006); both heterozygous mutations and deletions of *CHD7* could result in CHARGE syndrome. Up to date, several types of mutations within the *CHD7* coding region have been identified, including nonsense mutations (44%), frame shift causing deletions or insertions (34%), splice sites (11%), missense mutations (8%), larger deletions and duplication (2%), translocations (<1%) and small in-frame deletions (<1%) (Janssen et al., 2012).

In the present study, we consulted and followed up a Chinese boy with multiple deformities and clinically diagnosed with CHARGE syndrome. Molecular genetic analysis identified a new frame-shift-causing deletion of *CHD7*, resulting in a truncated form of *CHD7* protein. The clinical symptoms and the description of treatment in the present case, combined with genetic test and functional prediction of *CHD7*, are helpful for further understanding and genetic counseling of the CHARGE syndrome.

## Materials and methods

### *Clinical information and follow-up*

A 3-year-old little boy was the first child born by non-consanguineous Chinese healthy parents with an unremarkable family history, after uncomplicated pregnancy and delivery. After birth, the infant had been suffering from recurrent respiratory tract infections and thus admitted to hospital frequently. Subsequent examinations revealed multiple malformations such as unique facial features, atrial septal defect (ASD), bilateral ear anomalies, and micropenis. Based on the above observations, he was suspected to have CHARGE syndrome. The clinical features, laboratory results and follow-up treatment of the patient were investigated and recorded in detail.

The study was approved by the Ethnic Committee of Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine. Informed consents were obtained from the parents.

### *Genetic analysis*

Genomic DNA was extracted from peripheral blood of the patient and his parents using QIAamp DNA Blood Mini Kit (Qiagen GMBH, Hilden, Germany). Chromosomal Microarray Analysis was applied to detect large structural variants (SVs) using CytoScanHD chip (Affymetrix, Santa Clara, CA).

Then all of the 38 exons and exon-intron boundaries of the *CHD7* (GenBank accession number NM\_017780.2) were amplified by polymerase chain reaction (PCR) using 76 primers. The primer sequences, PCR mixture components and cycling conditions were available upon request. The amplified products were purified by QIAquick Gel Extraction Kit (Qiagen GMBH, Hilden, Germany) and then sequenced via ABI3730XL sequencer (Applied Biosystems, Foster City, CA).

### Structure modeling

The structures of wild type human CHD7 and truncated proteins were simulated by SWISS-MODEL (<http://swissmodel.expasy.org/>), and the figure for modeled structure was prepared by PyMOL ([Hhttp://www.pymol.org/H](http://www.pymol.org/H)).

## Results

### Clinical information and follow-up

Upon the first admission to the hospital, the patient presented unique facial features, congenital heart disease history, severe dysaudia, genital hypoplasia and retarded growth/development. Typical and atypical symptoms were shown in [Table 1](#).

### Coloboma

Upon admission, the patient presented with exophthalmos. After a specialist consultation and funduscopic examination, a right-sided iris coloboma was identified, while both visual acuity and visual field were not affected.

### Heart defects

Congenital heart disease was diagnosed and treated at the Department of Cardiology in our hospital about 6 months before admission this time. Ultrasound cardiogram showed ASD, bicuspid aortic valve, right aortic arch, right atrial and ventricular enlargement. ASD was closed by an occlusion surgery at twenty months of age.

### Choanal atresia

Nasopharyngoscopy showed choanal atresia caused by abnormal soft tissue, with half of the choanae being obstructed by nasopharyngeal adenoids hypertrophy. It is noteworthy that laryngoscope demonstrated tracheal stenosis and laryngeal cartilage dysplasia in this case.

### Genital hypoplasia

External genital examination showed micropenis (about 2–3 cm), and ultrasound revealed bilateral inguinal cryptorchidism with right spermatic cord hydrocele.

### Ear anomalies

Auditory brainstem response test showed bilateral hearing loss with ABR thresholds > 100 dB. Brainstem auditory evoked potential described bilateral severe BAEP abnormalities, suggesting distal acoustic nerve or cochlear damage. Temporal computed tomography (CT) showed bilateral inner ear

**Table 1**

Typical and atypical symptoms of the patient in this case.

	Diagnostic criteria of CHARGE	Symptoms of the patient in this case
Major	Ocular coloboma or microphthalmia	+
	Choanal atresia or stenosis	+
	Characteristic external ear anomaly, or middle ear malformations or mixed deafness	+
Minor	Cranial nerve dysfunction	+
	Congenital cardiovascular malformations	+
	Tracheoesophageal defect	+
	Genital hypoplasia/delayed pubertal development	+
	Cleft lip and/or palate	–
	Developmental delay	+
	Growth retardation	+
	Characteristic face	+
Atypical symptoms	Laryngeal cartilage dysplasia; exophthalmos	

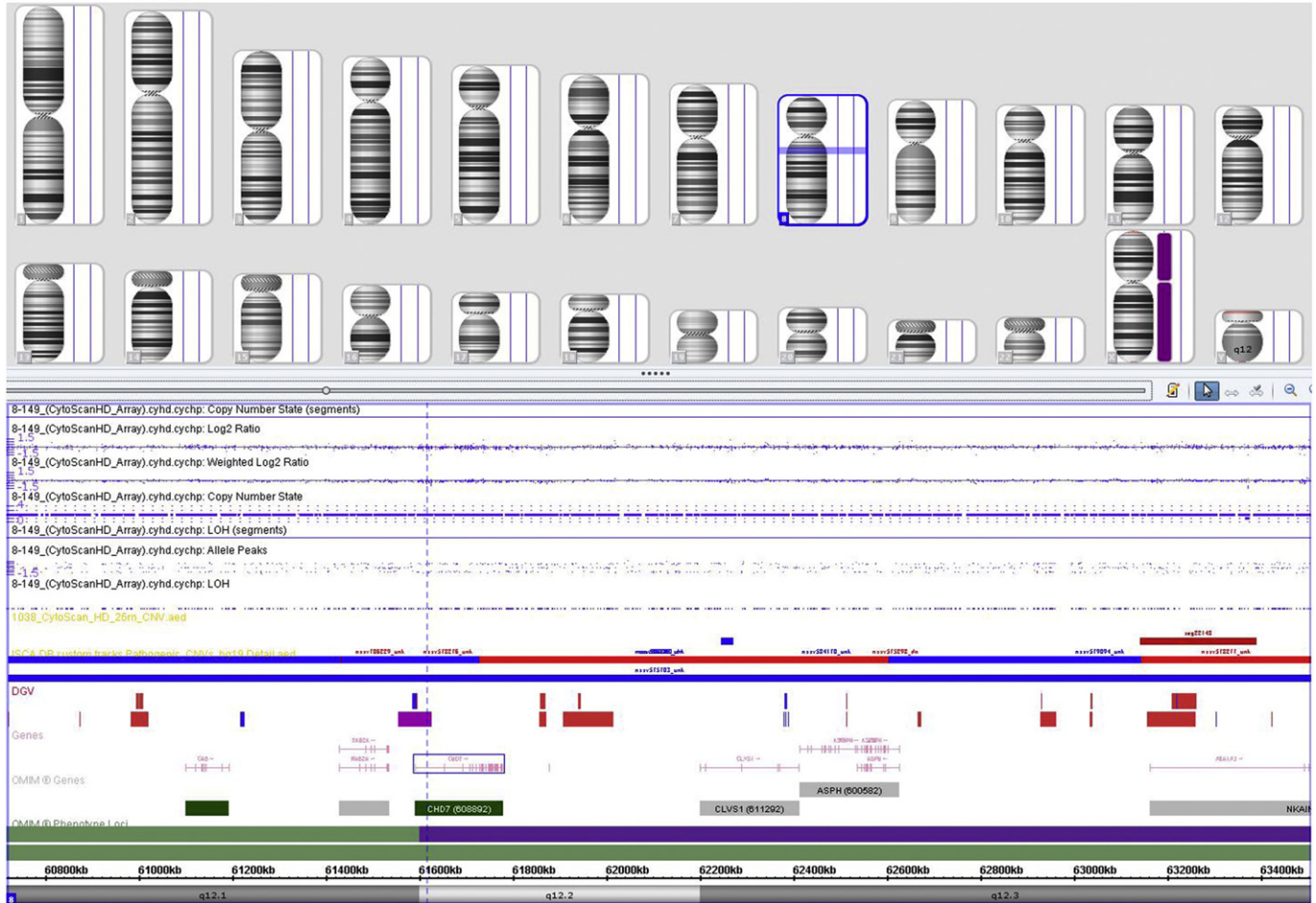
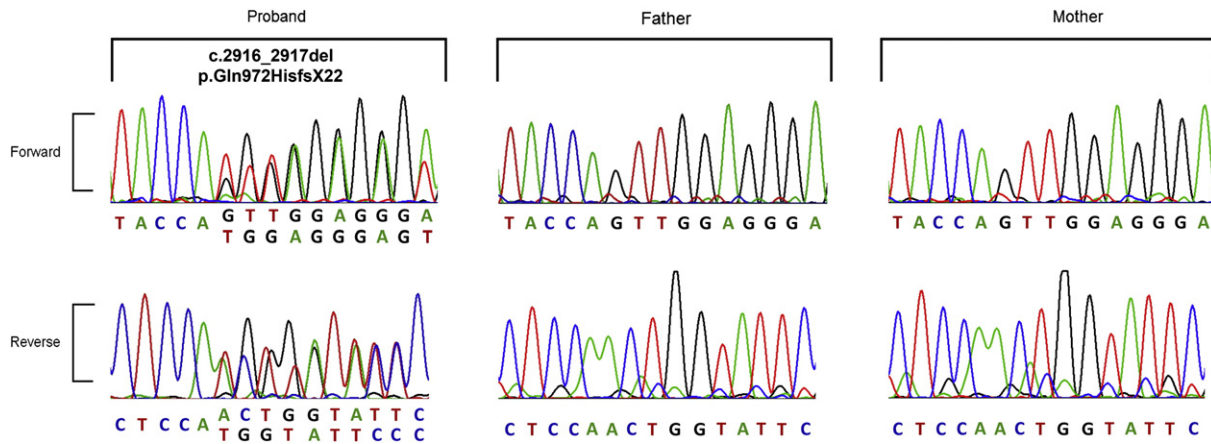


Fig. 1. Results of the microarray analysis.



**Fig. 2.** Sequencing result of the patient's *CHD7* gene. The chromatogram showed a two-base (GT) deletion in exon 11 of the patient's *CHD7* gene. This mutation introduced a frame-shift resulting in a truncated protein with a premature stop codon 21 positions downstream (p.Q972HfsX22).

	950	960	970	980	990	999
<b>Patient</b>						
	ADDWKKSESSREYKNNNKLREYHGGSKLATFQLYQHAKLHFSR*-----					
<i>H.sapiens</i>	ADDWKKSESSREYKNNNKLREYQLEGVNWLLFNWYNMRNCILADEMGLGK					
<i>M.musculus</i>	ADDWKKSESSREYKNNNKLREYQLEGVNWLLFNWYNMRNCILADEMGLGK					
<i>C.lupus</i>	ADDWKKSESSREYKNNNKLREYQLEGVNWLLFNWYNMRNCILADEMGLGK					
<i>B.taurus</i>	ADDWKKSESSREYKNNNKLREYQLEGVNWLLFNWYNMRNCILADEMGLGK					
<i>M.fascicularis</i>	ADDWKKSESSREYKNNNKLREYQLEGVNWLLFNWYNMRNCILADEMGLGK					
<i>G.gorilla</i>	ADDWKKSESSREYKNNNKLREYQLEGVNWLLFNWYNMRNCILADEMGLGK					
<i>R.norvegicus</i>	ADDWKKSESSREYKNNNKLREYQLEGVNWLLFNWYNMRNCILADEMGLGK					
<i>G.Gallus</i>	ADDWKKSESSREYKNNNKLREYQLEGVNWLLFNWYNTRNCILADEMGLGK					

	1000	1010	1020	1030	1040	1050
<b>Patient</b>						
	-----					
<i>H.sapiens</i>	TIQSITFLYEIYLKGIHGPFLVIAPLSTIPNWEREFRTWTELNVVVYHGSQ					
<i>M.musculus</i>	TIQSITFLYEIYLKGIHGPFLVIAPLSTIPNWEREFRTWTELNVVVYHGSQ					
<i>C.lupus</i>	TIQSITFLYEIYLKGIHGPFLVIAPLSTIPNWEREFRTWTELNVVVYHGSQ					
<i>B.taurus</i>	TIQSITFLYEIYLKGIHGPFLVIAPLSTIPNWEREFRTWTELNVVVYHGSQ					
<i>M.fascicularis</i>	TIQSITFLYEIYLKGIHGPFLVIAPLSTIPNWEREFRTWTELNVVVYHGSQ					
<i>G.gorilla</i>	TIQSITFLYEIYLKGIHGPFLVIAPLSTIPNWEREFRTWTELNVVVYHGSQ					
<i>R.norvegicus</i>	TIQSITFLYEIYLKGIHGPFLVIAPLSTIPNWEREFRTWTELNVVVYHGSQ					
<i>G.Gallus</i>	TIQSITFLYEIYLKGIHGPFLVIAPLSTIPNWEREFRTWTELNVVVYHGSQ					

**Fig. 3.** Sequence alignment of CHD7 protein in 8 species. CHD7 sequences used for phylogenetic analysis were obtained from the NCBI conserved domain database.

dysplasia, including cochlear and vestibular dysplasia, horizontal and superior semicircular canal aplasia, short posterior semicircular canal, and internal auditory canal stenosis. MRI for inner ear further indicated bilateral vestibular cochlear nerve hypoplasia. Hearing loss was significantly improved after a cochlear implant surgery.

#### Retarded growth and development

The patient cannot sit stably yet when admitted at 3 year old. Language delay and cerebral dysplasia were also observed in this patient. Cranial MRI showed bilateral lateral ventricle and subarachnoid space enlargement, and myelination of the brain white matter was slower than full-term normal infants.

#### Others

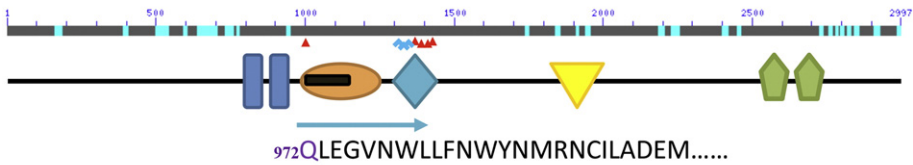
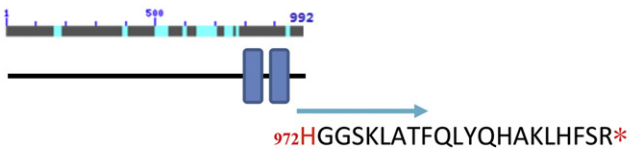
A series of biochemical analysis also helped to exclude metabolic diseases. All of the biochemical results were normal. GS/MS metabolic disorder test was also negative, with no evidence of metabolic diseases.

According to the above clinical observations and laboratorial results, the patient was finally diagnosed as CHARGE syndrome.

#### Genetic analysis

Results of the genomic imbalance analysis were negative in the case (Fig. 1), with no evidence of copy number variation (including deletions and duplications).

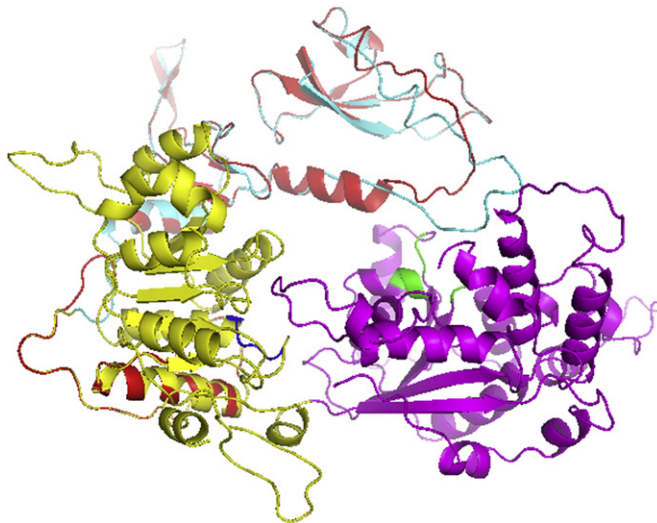
A new heterozygous, two-base deletion of *CHD7* located in exon11 was identified in this case (Fig. 2). The c.2916\_2917del mutation causes a reading frame shift starting from residue Q972, while the new reading frame ends with a premature stop codon 21 positions downstream (p.Q972HfsX22). Sequence alignment of CHD7 in 8 species shows that the region containing the mutation is extremely conserved, suggesting the indispensability of these residues and importance of CHD7 in vertebrates (Fig. 3).

**Normal CHD7 protein****Truncated CHD7 protein**

**Fig. 4.** A schematic representation of the wild type and mutant CHD7 protein in the present case. Domains are depicted approximately to scale. Adapted from human reference sequence (NP\_060250.2) and (Janssen et al., 2012).

**Structure modeling**

According to the analysis on the primary sequence of CHD7, wild type CHD7 protein is comprised of a pair of chromo (chromatin organization) domains, a helicase domain [ATPase domain, containing DEXDc and Helicase C (HELICc) domains], a SANT (SWI3, ADA2, N-CoR and TFIIIB) domain and a pair of BRK (BRN and KIS) domains (Janssen et al., 2012) (Fig. 4). The c.2916\_2917del mutation identified in the patient causes an amino acid change at residue 972 (Q → H), which is located in the helicase domain (containing



**Fig. 5.** The 3D structure model of human CHD7 protein. Wild type CHD7: amino acids 800aa–1600aa; truncated CHD7: amino acids 800aa–992aa. The chromodomains, DEXDc and HELICc are colored cyan, yellow and magenta, respectively. The ATP-binding site and nucleotide binding region are colored blue and green, respectively. The truncated protein is colored red.

the ATP-binding region). Furthermore, the consequent frame shift due to the small deletion led to a premature stop codon 21 position downstream Q972 (at residue 993), resulting in a truncated protein with nearly two thirds of the critical functional domains being lost, including the ATP-binding region, the nucleotide binding region, the putative ion binding site, the phosphorylation site, and the BRK domain. The predicted three-dimensional structure of wild type and truncated CHD7 protein (including the chromo- and helicase domains, amino acids from 800aa–1600aa) were depicted in Fig. 5, indicating that the mutant CHD7 induced by c.2916\_2917del in the patient only maintains the chromodomains but lacks all the other functional domains.

## Discussion

The reported incidence of CHARGE syndrome is 0.1–1.2/10,000 depending on professional recognition, and most of the cases are sporadic (Blake and Prasad, 2006). Clinical phenotype of CHARGE syndrome is highly variable. Standard diagnostic criteria contain 4 major criteria and 7 minor criteria. Herein, we reported a patient representing both typical and atypical symptoms of CHARGE (Table 1) and clinically diagnosed as CHARGE syndrome. Typical symptoms in this case included choanal atresia, coloboma, characteristic ear anomalies, cardiovascular malformations, genital hypoplasia, growth deficiency and developmental delay, while atypical symptoms included exophthalmos, tracheal stenosis and laryngeal cartilage dysplasia. Targeted treatments were introduced to the patient according to the findings above. Remarkable achievements were observed after accurately treatments: hearing loss was significantly improved after a cochlear implant surgery, and recurrent respiratory tract infection was controlled after an ASD occlusion surgery. The mother was advised to do gene diagnosis during her 2nd pregnancy, which was proved to be useful. No mutation has been identified in the *CHD7* gene of the fetus, and after an uncomplicated full term pregnancy, a healthy baby was delivered safely.

The difficulty for clinical diagnosis of CHARGE syndrome is how to differentiate from the 22q deletion syndrome, Kabuki syndrome, Velo-cardio-facial syndrome (VCFS), Cat Eye syndrome, retinoic acid embryopathy, VACTERL association and PAX2 abnormalities, which have many similar behavioral and clinical features with CHARGE. Therefore, gene analysis is the most accurate and definitive method for differential diagnosis (Blake and Prasad, 2006).

The only pathogenic gene involved in CHARGE syndrome reported to date is *CHD7*. Heterozygous *CHD7* mutations existed in more than 90% of the patients with typical CHARGE syndrome based on the clinical diagnostic criteria (Corsten-Janssen et al., 2013). We documented a *de novo* heterozygous mutation on *CHD7* gene (c.2916\_2917del) in the present case, which was a novel small deletion causing a frame-shift in *CHD7*. The novel variant was expected to lead to a truncated protein and considered to be pathogenic because it is very likely to result in haploinsufficiency.

*CHD7* belongs to the chromosome helicase DNA binding (CHD) family which evolutionarily conserved in eukaryotes. Studies on the protein structure of *CHD7* indicate that it composes of at least four major domains: N-terminal tandem chromodomains, central helicase domain, DNA binding/SANT domain and two C-terminal BRK domains (de la Cruz et al., 2005; Zentner et al., 2010). It was demonstrated that the chromodomain of *CHD7* was able to bind methylated H3K4, therefore creating an open chromatin state at promoters of certain genes (Schnetz et al., 2009). The helicase domain is thought to facilitate ATP-dependent translocation of proteins along DNA, and generate force for movement or destabilization of nucleosomes (Cairns, 2007). Daniel et al. showed that DNA-binding domain is required for *Saccharomyces cerevisiae* Chd1 to bind and remodel nucleosomes (Ryan et al., 2011). The conserved role of DNA-binding domain in CHD proteins suggests the same function of this domain in *CHD7*. Although the function of BRK domains is currently unknown, it is interesting to note that mutation that introduces a stop codon in the middle of the second BRK domain causes CHARGE syndrome, suggesting the importance of this region (Lalani et al., 2006). Feng et al. reported that *CHD7* stimulates the expression of *Sox4* and *Sox11* genes in neuron stem cells via remodeling their promoters to an open chromatin state, thus regulating stem cell differentiation (Feng et al., 2013). *CHD7* loss of function impaired neuronal differentiation and dendritic development of newborn neurons, providing a possible mechanism of the learning and intellectual disability in 75% of the CHARGE patients (Bergman et al., 2011). The truncated protein in this patient only contains chromodomains of *CHD7*, which is clearly insufficient for protein function. Although chromodomain itself may still recognize methylated H3K4 and preserve the ability to bind modified nucleosome, the activity of chromatin remodeling is abolished (Fig. 5).



To the best of our knowledge, this is the first CHARGE syndrome in Chinese patients diagnosed by gene analysis, in which a de novo truncated CHD7 mutation was identified. Most of the patients with CHARGE syndrome reported to date, which were identified by genetic diagnosis, were from the western countries, and there are also several cases reported in Korea (Cho et al., 2013) and Japan (Aramaki et al., 2006). However, without genetic diagnosis, CHARGE syndrome in China was difficult to diagnose and often considered as an incurable disease. Before our study, only two cases probably suspected as CHARGE syndrome according to the clinical symptoms were reported, but without further verification (Qu et al., 2010). Therefore, the clinical symptoms and pertinent treatment in the present case, combined with genetic analysis and functional prediction of CHD7, fill the gap of genetic diagnosis of CHARGE syndrome in Chinese patients, and suggests that identifying the underlying genetic defects in patients with CHARGE syndrome is helpful for accurate diagnosis, appropriate treatment, and genetic counseling.

### Authors' contributions

XM and YGY critically revised the manuscript, contributed to the study design, analysis, and supervised the research. YGY coordinated clinical diagnosis and collected phenotype information. LBL and TTY drafted and critically revised the manuscript, performed the statistical analysis, and contributed to the interpretation of the data. LLW performed the blood collection, DNA extraction, microarray and data analysis. All authors read and approved the final manuscript.

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