

Cole–Carpenter syndrome-1 with a de novo heterozygous deletion in the *P4HB* gene in a Chinese girl

A case report

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Abstract

Rationale: Cole–Carpenter syndrome-1 (CLCRP1) is an independent osteogenesis imperfect (OI)-like disorder that manifests as bone fragility, craniosynostosis, ocular proptosis, hydrocephalus, and distinctive facial features. Only 2 types of mutation sites in the *P4HB* and *CRTAP* genes have been reported.

Patient concerns: A 14-month-old Chinese girl presented with prominent ocular proptosis, frontal bossing, craniosynostosis, plump anterior fontanel, growth retardation, osteopenia, and distinctive facial features that were strikingly similar to those in the original 2 cases.

Diagnoses: Whole-exome sequencing revealed a novel deletion variation in exons 5 to 8 of the *P4HB* gene, which was found to be heterozygous using fluorogenic quantitative-polymerase chain reaction.

Lessons: This de novo deletion mutation in exons 5 to 8 of the *P4HB* gene advances our understanding of CLCRP1, expands the mutation spectrum of *P4HB*, and diversifies the cases reported for this condition.

Abbreviations: CLCRP1 = Cole–Carpenter syndrome-1, FQ-PCR = fluorogenic quantitative-polymerase chain reaction, WES = whole-exome sequencing.

Keywords: de novo deletion mutation, growth deviation, ocular proptosis, *P4HB* gene, skeletal system abnormality

1. Introduction

Cole–Carpenter syndrome-1 (CLCRP1, online Mendelian inheritance in man [OMIM]:112240) was first reported in 1987 by Cole and Carpenter, who described 2 unrelated infants with notably similar clinical manifestations: bone fragility, craniosynostosis, ocular proptosis, hydrocephalus, and distinctive facial features. This newly recognized type of osteogenesis imperfect (OI)^[1] now bears their names (ie, Cole–Carpenter syndrome). Improvements in our understanding of OI have led clinicians to identify Cole–Carpenter syndrome as an independent OI-like disorder rather than an atypical form of OI.^[2–5] CLCRP1 is a rare disorder, with only a few cases have been reported between 1987

and 2017. The features of some of these cases differ from those of true clinical CLCRP1. Meanwhile, only 2 types of mutations in the *P4HB* and *CRTAP* genes have been discovered in relation to this disorder.^[2,6]

In the present study, we used diagnostic whole-exome sequencing (WES) to identify the pathogenesis of illness in a Chinese girl whose clinical manifestation was strikingly similar to that reported in the original 2 cases. WES revealed a significant loss of heterozygosity mutation in the *P4HB* gene that has not yet been reported. Herein, we report the 1st case of such a disorder in a Chinese girl and describe the clinical features and deletion mutation underpinning the condition of the patient.

2. Informed consent

The patient's parents provided written informed consent to conduct the genetic tests and further study.

3. Presenting concerns

A Chinese girl presented with ocular proptosis, which was first observed at birth. An ultrasound and computerized tomography (CT) 3-dimensional reconstruction of the orbit showed no organic lesions. She is the 2nd, nonconsanguineous child of healthy parents. Her elder sister exhibits a healthy phenotype and is apparently unaffected.

4. Clinical findings

A girl of 1 year and 2 months of age exhibited a head circumference of 42.8 cm (–2SD to –1SD, WHO), distinct ocular

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Figure 1. Facial dysmorphism, including prominent eyes with a proptotic appearance, micrognathia, and distinctive facial features.



Figure 2. Facial dysmorphism, including frontal bossing.

proptosis (Figs. 1 and 2), frontal bossing, craniostylosis, a plump anterior fontanel, growth retardation, osteopenia, and distinctive facial features. Meanwhile, her cognitive development was normal for her age, and she had never experienced a fracture. Notably, her paternal grandfather's brother exhibited ocular proptosis in 1 eye. Because he passed away 2 years prior to her presentation, we could not perform any examinations. Nevertheless, her parents and older sister exhibited a normal phenotype. She was delivered via a cesarean section after 39⁺¹ weeks of gestation following a normal pregnancy. Her birth length was 48 cm (−1SD–median, WHO) and birth weight was 2600 g (−2SD to −1SD, WHO). A routine ultrasound at 37⁺⁶ weeks indicated that her femur length was short for her gestational age. She did not develop perinatal asphyxia or other diseases until 6 months of age, at which time she experienced laryngeal obstruction due to inflammation. Growth retardation was apparent since birth (Figs. 3 and 4), and she was first admitted to our clinic at 11 months of age. At that time, she was 69.5 cm long and weighed 6630 g (data not shown). Before 6 months of age, her gross motor development was normal, as indicated by her ability to raise her head at 3 months and sit at 6 months. However, since that time, she was unable to crawl or walk independently until visiting our clinic at 11 months of age. Her fine motor and cognitive ability developed well, and she was able to speak several words and understand instructions from her parents. Progressive ocular proptosis and frontal bossing developed along with her growth. A work-up by an ophthalmologist returned no diagnosis and an ultrasound and CT

3-dimensional reconstruction of the orbit showed no organic lesions.

When the girl visited our outpatient department, ocular proptosis was readily notable. Based on previous findings, we arranged for her to undergo a laboratory test, radioscopy, and WES. Her serum levels of calcium, phosphorus, alkaline phosphatase, parathyroid hormone, insulin-like growth factor-1 binding protein 3, free thyroxine, and free triiodothyronine were normal. However, her insulin-like growth factor-1 level was 45.2 ng/mL and her thyroid stimulating hormone level was 0.600 mIU/L, both of which were lower than the reference range. Radiographic findings indicated deformities in the cortex of the long bone (which had become thinner), frontal bone (which had sharpened), physiological bending of the thoracolumbar spine (which had straightened), and cranial bones (which became irregular and with inconsistent density). Portions of her brain parenchyma were nonhomogeneous and the anterior fontanel was plump and protrusive (Figs. 5 and 6).

5. Genetics analyses and assessments

A heterozygous deletion of exons 5 to 8 in the *P4HB* gene was identified using diagnostic WES, which included deep sequencing of more than 20,000 genes at a depth of at least 100. Previous reports of this condition and our WES results indicate that a mutation in *P4HB* is highly correlated with Cole–Carpenter syndrome-1. Notably, this is the first reported case of such a mutation in the literature. Her parent's genotypes were normal,

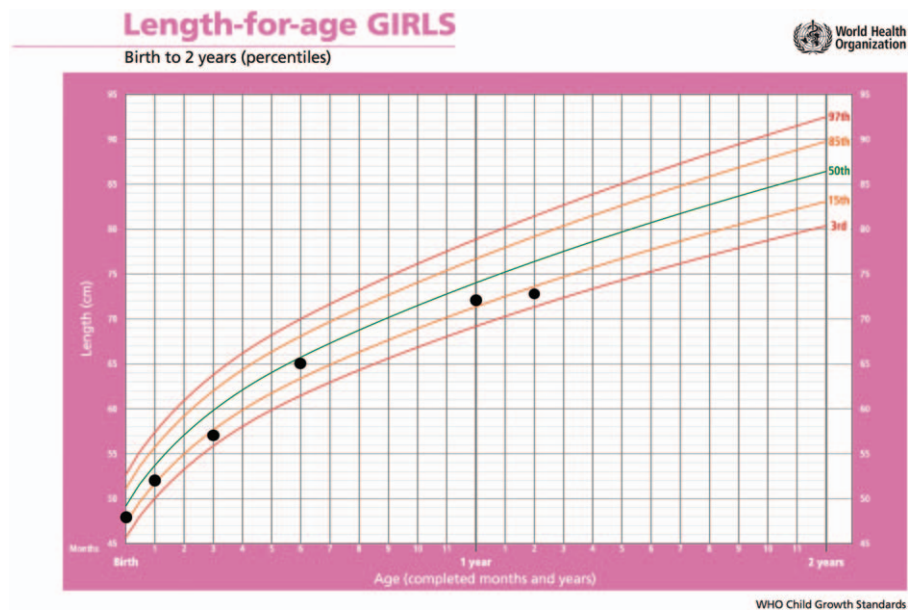


Figure 3. Growth curve (height).

indicating that this mutation was de novo. Fluorogenic quantitative-polymerase chain reaction (FQ-PCR) was used to examine the proband, whereas samples from her parents and a normal control confirmed the WES results. On checking the copy number (CN) of exons 5 to 8 in the *P4HB* gene using the *ALB* gene as a reference, we found that the CN ratio of the proband and normal reference was 0.5, whereas that of the parents was 1.0. This indicated heterozygous deletions in the proband, but no abnormalities were detected in her parents (Fig. 7). After validation with FQ-PCR, the clinical manifestation and genetic

tests results confirmed the diagnosis of Cole–Carpenter syndrome-1.

6. Discussion and conclusion

This case of a Chinese girl of 1 year and 2 months of age exhibited the same phenotype of Cole–Carpenter syndrome, including craniosynostosis, ocular proptosis, and distinctive facial features. However, certain features differed from the syndrome, such as the presence of fractures and hydrocephalus. This is the first

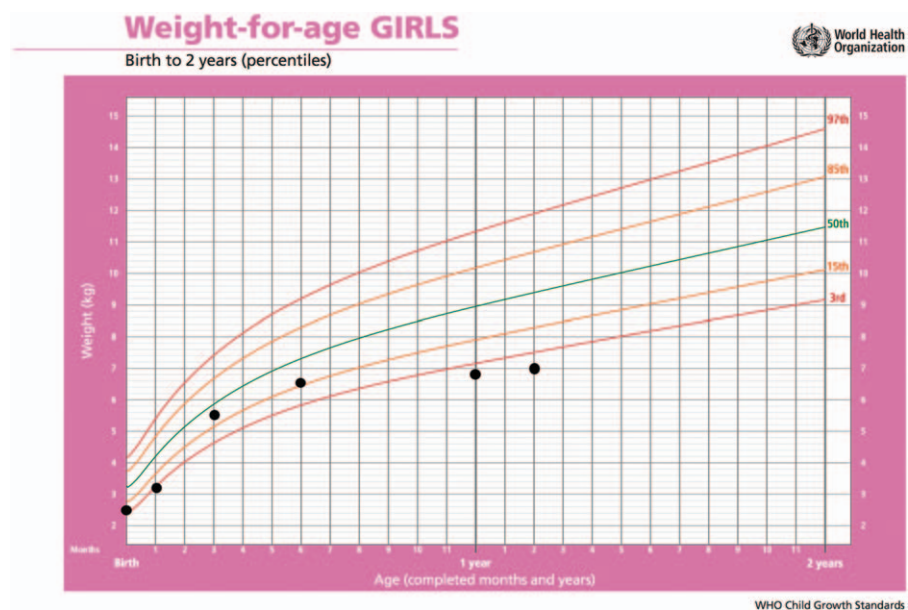


Figure 4. Growth curve (weight).

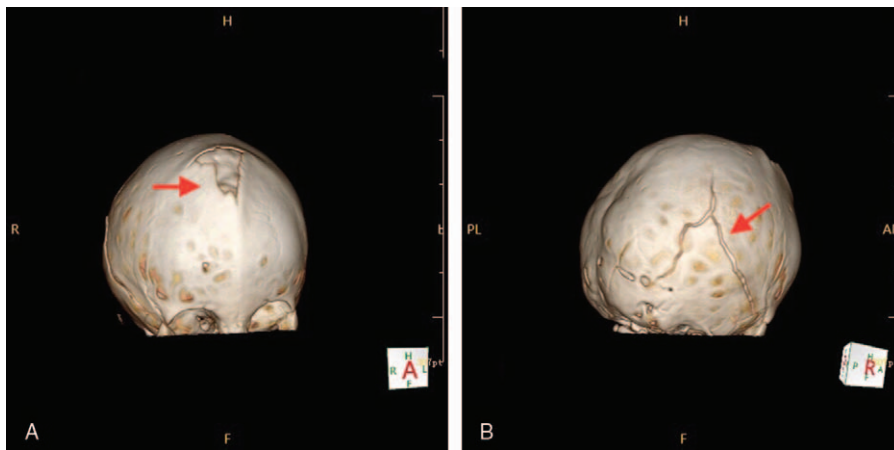


Figure 5. Three-dimensional computerized tomography (3-D CT) reconstruction of the skull vault (A, B), demonstrating irregular cranial bones, a nonuniform bone density, craniosynostosis of all sutures, a plump and protrusive anterior fontanel, and sharpening of the frontal bone.



Figure 6. X-ray examination of vertebrae (A) demonstrating physiological bending that had straightened, legs (B–D) showing osteopenia, a thinner cortex, and an irregularly widened metaphysis.

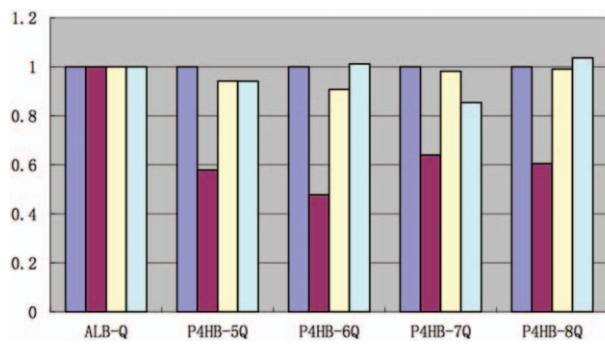


Figure 7. Fluorogenic quantitative-polymerase chain reaction (FQ-PCR) for repeatable validation. Purple represents a normal reference, red represents the proband, yellow represents the father of the proband, and green represents the mother of the proband. The copy number ratio of exons 5 to 8 in the *P4HB* gene between the proband and normal reference was 0.5, whereas that of the parents were both 1.0. This indicated a heterozygous deletion in the proband, but no abnormalities in her parents.

report of case with a heterozygous deletion of exons 5 to 8 in the *P4HB* gene and the first report of the syndrome in a Chinese individual.

Several cases have reported that fractures are the main symptom of this syndrome, which present following the patient's birth. Moreover, patients suffer from frequent fractures.^[2,6,7] However, this was not observed in our patient, who had experienced no fractures. However, an X-ray of all bones reflected a remarkably thinner cortex and heterogeneous bone density. This indicated that the patient had experienced osteopenia and bone fragility. In this way, it is possible that the patient will experience a bone fracture in the future if serious attention is not paid. WES and FQ-PCR illustrated that there was a significant loss of function mutation in the *P4HB* gene, which encodes disulfide isomerase (PDI), a key enzyme for protein folding that can assist with the correct formation of disulfide bridges in nascent polypeptide chains. Moreover, PDI is a ubiquitously expressed, prototypical member of the disulfide isomerase family of proteins.^[8,9] Therefore, if PDI is influenced by a deletion mutation in the *P4HB* gene, a series of downstream reactions will be influenced as well. Rauch et al^[2] functionally verified the missense mutation in the *P4HB* gene from the initial 2 patients. The mutation site in these patients was located in the C-terminal disulfide isomerase domain of PDI, which is sterically close to the enzymatic center and was found to affect its disulfide isomerase activity *in vitro*. Our patient exhibited a large deletion mutation that was located in the PDI domain. As a result, it is likely that the disulfide isomerase activity of PDI was impaired in this patient.

We speculate that CLCRP1 is an autosomal dominant disease given the distinct genotype and phenotype of both the patient and her parents. Although her grandfather's brother exhibited ocular proptosis, his death prevented us from researching the relationship between that case and ours. To the best of our knowledge, only 3 mutation sites in the *P4HB* and *CRTAP* genes

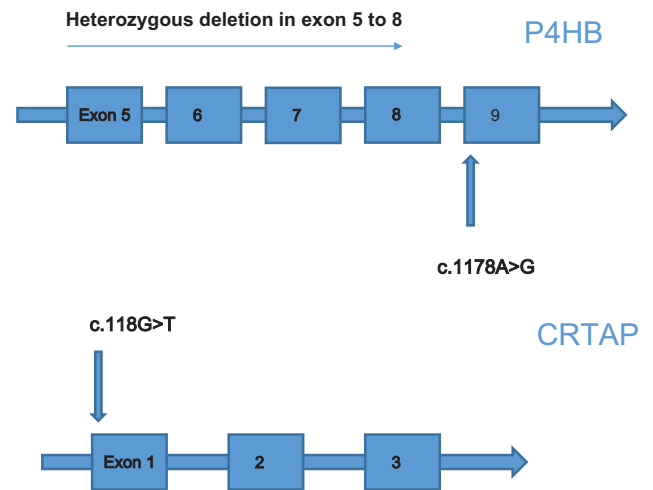


Figure 8. Rauch et al^[2] reported a heterozygous missense mutation in exon 9 of the *P4HB* gene (NM_000918.3: c.1178A>G [p.Tyr393Cys]) in the initial 2 cases. Balasubramanian et al^[6] reported a homozygous c.118G>T mutation in exon 1 of the *CRTAP* gene. Our case presented with a heterozygous deletion of exons 5 to 8 in the *P4HB* gene.

of Cole–Carpenter syndrome have been discovered, including 2 missense mutation, as reported previously, and 1 deletion mutation, as reported in this patient (Fig. 8).

In conclusion, the present case reports a Chinese patient with a novel, *de novo* heterozygous deletion mutation of exons 5 to 8 in the *P4HB* gene. This patient exhibited notable ocular proptosis and skeletal system abnormalities. This study expands the mutant spectrum of the *P4HB* gene and enhances the understanding of CLCRP1. To the best of our knowledge, this is the 1st case report of a heterozygous deletion mutation of CLCRP1 in Asia.

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