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Identification of a novel mutation in the *MAFB* gene in a pediatric patient with multicentric carpotarsal osteolysis syndrome using next-generation sequencing

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ABSTRACT

Multicentric carpotarsal osteolysis syndrome (MCTO) is a rare form of skeletal dysplasia characterized by progressive bone resorption, in the carpal and tarsal bones. Patients may develop chronic kidney disease, which eventually advances to end-stage renal disease (ESRD). Both sporadic and familial cases of autosomal-dominant inheritance are reported in literature. Here, we report a case of a 10.5-year-old boy who presented with CKD stage V, and who suffered from bone deformities and difficulty in walking at a younger age. He was diagnosed with MCTO and subjected to genetic analysis. We identified a novel mutation (NM_005461.5:c.173C > G) in the exon 1 of *MAFB* using next-generation sequencing. However, the mutation was not detected in his asymptomatic parents or siblings. This novel heterozygous mutation has not been reported previously. Our results show that the new mutation broadens the spectrum of disease phenotypes. This mutation may be helpful to confirm the potential cases of MCTO, which although can be identified through radiographic findings, stand a high chance of being misdiagnosed as rheumatological disease or as a metabolic bone disease secondary to CKD.

1. Introduction

Multicentric carpotarsal osteolysis syndrome (MCTO; OMIM 166300) was initially described as 'disappearing bone disease' (Jackson, 1838). Since its initial characterization, approximately one hundred cases with similar clinical features have been reported in the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php). The proper nomenclature of this disease has been disputed, with glossaries identifying it as idiopathic osteolysis, essential osteolysis, hereditary osteolysis, or familial osteolysis. The term 'idiopathic multicentric osteolysis' was proposed to distinguish this disorder from other osteolytic diseases (Tyler and Rosenbaum, 1976). The condition is divided into five types: I. autosomal dominant; II. autosomal recessive; III. sporadic; IV. Gorhan-Stout disease (an autosomal dominantly inherited disease characterized by massive bone resorption and diffuse cystic angiomatosis); and V. Winchester's syndrome (Hardegger et al., 1985). Idiopathic multi-centric osteolysis was eventually renamed as 'multicentric carpotarsal osteolysis syndrome' by the International Nomenclature and Classification of the Osteochondrodysplasias based on the bones that are primarily afflicted (Lachman, 1998).

MCTO is a rare etiology of chronic kidney disease (CKD) and displays radiographical features characteristic of osteolysis. Many previous reports have analyzed clusters of cases within the same family, following a pattern of autosomal dominant inheritance. Many MCTO cases, especially those which present at the later stages in life, have been mistaken for rheumatological illnesses. Affected individuals may also suffer subtle craniofacial abnormalities. The diagnosis can be confirmed by analyzing the *MAFB* gene, which plays a key role in activation and differentiation of osteoclasts, as well as in renal development (Moriguchi et al., 2006; Kim et al., 2006). In the present study, we discuss a pediatric case of MCTO and present a novel mutation identified by the genetic analysis of the patient.

2. Patient data

A 10.5-year-old boy presented with CKD stage V at our renal outpatient clinic. Medical attention was sought earlier for multiple bone deformities and an inability to walk when he was one-and-a-half years old. Bone biopsy was performed at age five at the local institution; the results stated, 'Broken cortical bone and yellow bone marrow can be

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seen in the specimens and no cartilage components are found'. Blood renal function tests revealed parameters within normal limits; however, urine analysis showed 1+ proteinuria without hematuria. No further follow-up was pursued until the age of ten, when he was admitted for care at another institution following a history of severe headache for preceding two months accompanied by vomiting. His body weight and height were 23 kg and 131 cm, respectively. According to his inpatient records, initial blood pressure (BP) was 246/163 mm Hg, and laboratory investigations showed deteriorated renal function. Serum creatinine was 210 μmol/L (normal range: 21–65 μmol/L) and blood urea nitrogen (BUN) was 21.6 mmol/L (normal range: 1.5–7 mmol/L). Alkaline phosphatase (ALP) was 269 IU/L (normal range: 40–500 IU/L). Parathyroid hormone (PTH) level was not tested at that time. Hemoglobin (Hb) was 106 g/L, and urine analysis showed 3+ proteinuria. Twenty-four hour urine collection showed a protein excretion of 2689 mg (approximately 100 mg/kg/24 h). During hospitalization, his BP was controlled at 90–130/60-90 mm Hg with sodium nitroprusside (infusion rate of 0.5–4.5 mcg/kg/min) and Enalapril (10 mg oral daily). During subsequent follow-up for one month, his serum creatinine, BUN, calcium, and hemoglobin levels further deteriorated to 570 μmol/L, 32.3 mmol/L, 2.07 mmol/L, and 70 g/L, respectively. Subsequently, he was referred to our center for further management. Upon admission to our unit, facial anomalies including triangular face, micrognathia, and exophthalmos were noted (covered as per the request of parents; Fig. 1A). Blood pressure was recorded at 132/94 mm Hg, and renal ultrasound showed bilateral renal atrophy and reduced renal blood flow. In contrast, both of his siblings, a younger brother and sister, exhibited normal renal functions and urine analysis. A bilateral X-ray of the hands and feet revealed osteolysis-related changes, mainly in the peripheral joints (Fig. 1B-E).

3. Methods

Informed consent was obtained from the patient's parents following institutional guidelines. Genomic DNA was extracted from the peripheral blood leukocytes of the proband using the QIAamp DNA Blood Midi Kit (Qiagen Hilden, Germany). The exon regions of 4239 genes, including the *MAFB* gene, were specifically enriched using a biotinylated capture probe (MyGenostics, Beijing, China). The enriched libraries were sequenced using an Illumina HiSeq 2000 sequencer. Data analysis and bioinformatics processes were performed following the standard Illumina procedure. Clean reads were aligned to the human reference genome (hg19) using the BWA Multi-Vision software package (Li and Durbin, 2009). Sequence variations, including single nucleotide polymorphisms (SNPs) and insertions or deletions (InDels) were identified using the GATK program (https://www.broadinstitute.org/gatk/). Potentially pathogenic variants were defined for allele frequencies less than 0.01 (determined by databases including the 1000 genome variants database and ESP6500 database) regardless of nonsense, missense, splice-site, or InDel variants.

Polymerase chain reaction (PCR) and Sanger sequencing were used to confirm the mutations. The primer pair F (5′-CTAAGACTAGGGCGTCTGCACR-3′) and R (5′-TGCTTGGACATCCACACG-3′) was used in PCR. The PCR products were sequenced using an ABI3730Xl DNA Analyzer (Applied Biosystems). Data obtained were compared with the reference sequences (GenBank accession number: NM_005461). One missense mutation (NM_005461.5:c.173C > G) in exon 1 of *MAFB* was identified by PCR and subjected to Sanger DNA sequencing; samples from all family members of the patient were used to check if the disease was hereditary (Fig. 2). Further, pathogenicity was predicted with several algorithms (SIFT [http://sift.jcvi.org], Mutation Taster [http://www.mutationtaster.org], PolyPhen-2 [http://genetics.bwh.harvard.edu/pph2/]).

4. Results

4.1. Clinical treatment and outcome

The patient received hemodialysis (HD) as per parental wish following the diagnosis of end stage renal failure. An HD protocol of three hours per session, three times per week was prescribed. A Kt/V of at least 1.2 was maintained, and a urea reduction ratio (URR) of at least 60% was targeted, following the Kidney Disease: Improving Global

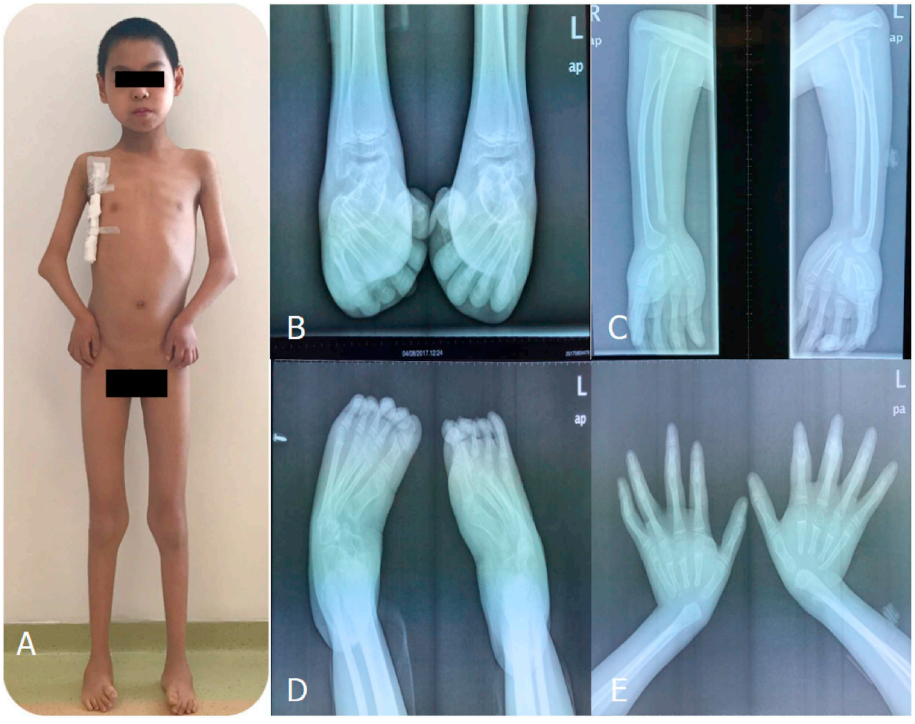

Fig. 1. (A): A photo of the patient. (B) and (D): Various deformities of ankle joints are observed in bilateral X-rays. The first metatarsals of both feet are short and thick. (C): Bilateral elbow dislocation. Bilateral proximal ulna and radius show morphological malformations. Bilateral radius and distal ulna are thin. Both distal radii are slightly curved toward the adjacent ulna side. (E): Bilateral wrist joint subluxation. Proximal interphalangeal joints are replaced by synostosis.

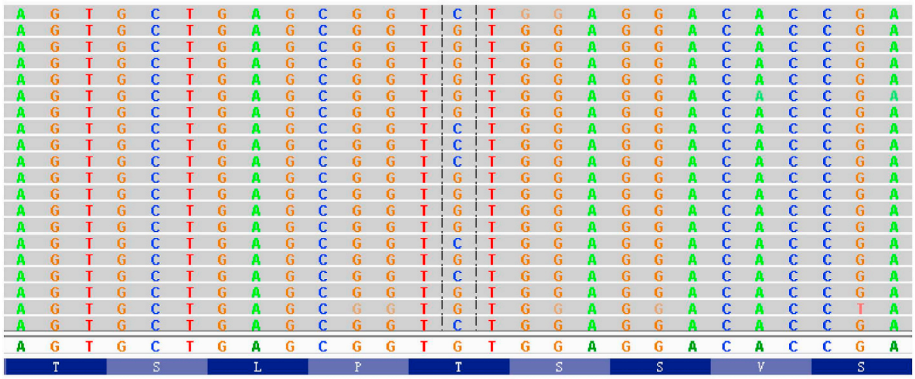

Fig. 2. A pathogenic variant of the *MAFB* gene was identified by whole-exome sequencing.

Outcomes (KDIGO) guidelines. After one year of HD therapy, the patient received a new kidney from an unrelated deceased donor. The transplanted kidney was compatible with his body and no post-transplantation rejection or infection occurred. Proteinuria disappeared and blood pressure and renal function returned to normal ranges. Nevertheless, due to the unavailability of medication capable of halting or reversing osteolysis, the patient's bones and joints will continue to deteriorate gradually over time.

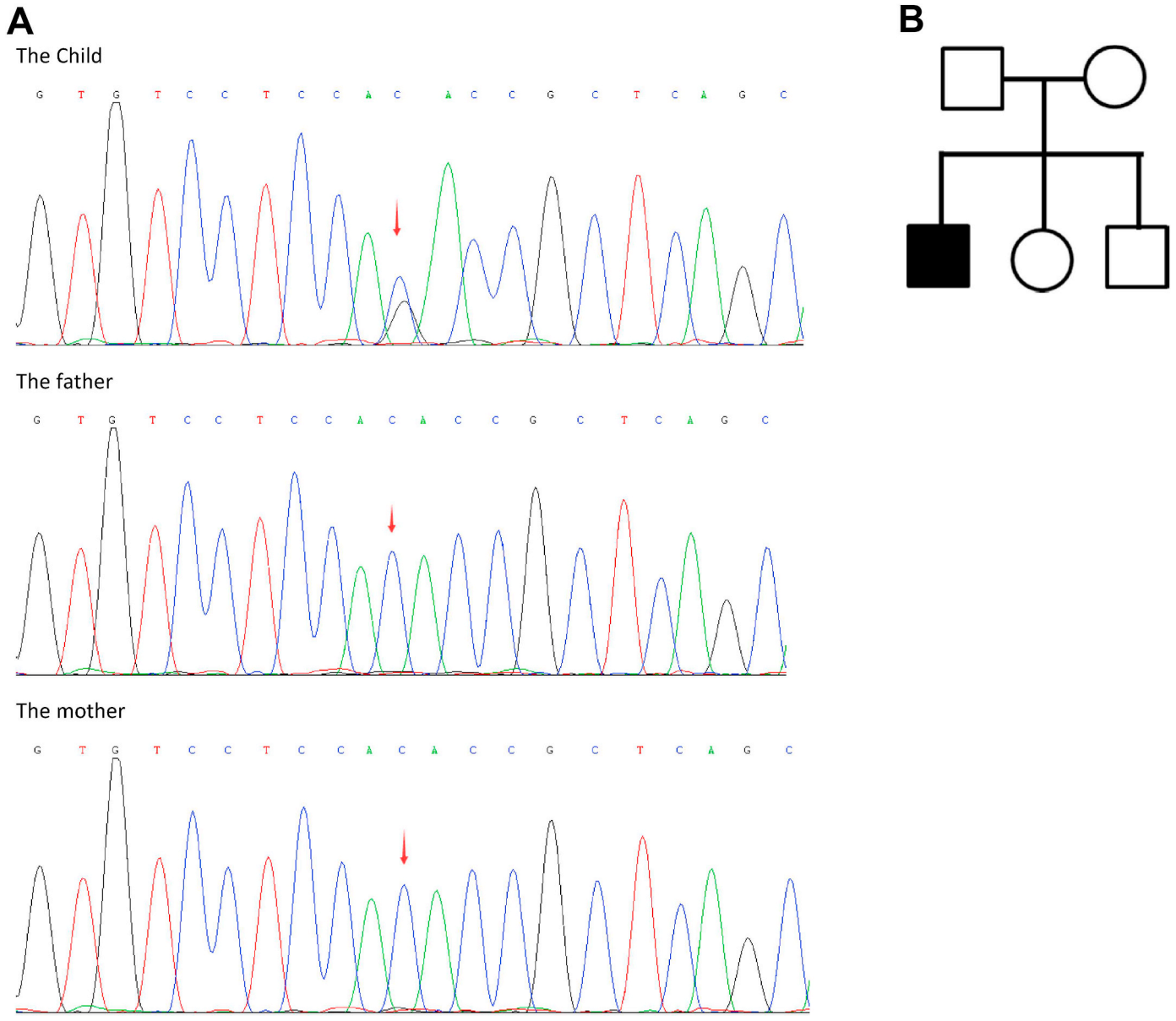

Fig. 3. a) The mutation of c.173C > G (p.T58R) was not detected in the patient's asymptomatic parents. b). Pedigree of the family.

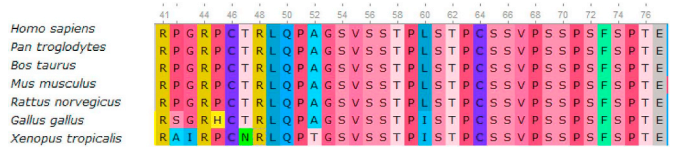

Fig. 4. Comparative genomics revealed the conservation of threonine 58 residue in the transactivation domain of the MafB protein.

Table 1
The p.T58R mutation is predicted to be causative by PolyPhen-2, SIFT, and Mutation Taster.

Variant observed in MafB (NM_005461.5) and mutation prediction				
Variant	Amino Acid substitution	functional prediction		
		Polyphen-2 (Humvar)	SIFT	Mutation Taster
c.173C > G	p.Thr58Arg	possibly damaging	deleterious	disease causing

4.2. Molecular diagnosis

Targeted sequence capture and next-generation sequencing were used for genetic analysis. Sanger sequencing was used to confirm the mutation. One mutation was a G-to-C substitution at nucleotide 173 of the *MAFB* gene, which led to an amino acid replacement from threonine to arginine at codon 58 (Fig. 2). This exchanged an uncharged residue with a positively charged residue. Significantly, Thr58 has been conserved at its position among multiple vertebrate species (Fig. 4). Pathogenicity prediction with several algorithms (SIFT, Mutation Taster, PolyPhen-2) assessed this variant to be disease-causing or at least possibly damaging (Table 1).

A paternity test by microsatellite analysis confirmed that the patient was the biological offspring of his parents. The mutation mentioned above was not detected in his asymptomatic parents or siblings (Fig. 3A and B) and was speculated to be de novo. This variant has not been reported previously and was submitted to the ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar) with the submission name SUB6199365 and accession number SCV000965692.

5. Discussion and conclusion

Usually presenting in early childhood, MCTO is a rare skeletal disorder with the clinical features mimicking juvenile rheumatoid arthritis. Progressive destruction of the carpal and tarsal bones occurs, and other bones may also be affected. Chronic renal failure is frequent associated with the syndrome. Mental retardation and minor facial anomalies have also been noted in some patients, and autosomal dominant inheritance has been documented in many families.

Although many MCTO cases have been reported, an underlying causative gene was not confirmed until recently. Several missense mutations clustering in the amino-terminal transcription activation domain of the *MAFB* gene located on chromosome 20q11.2-q13.1, were detected in five unrelated patients suffering from MCTO using whole-exome gene sequencing (Zankl et al., 2012).

A high degree of conservation of T58 residue is observed in the transcription factor V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MafB) encoded by *MAFB*. Moreover, the threonine residue at position 58 can be phosphorylated by the Ser/Thr kinase GSK3 (Rocques et al., 2007). Mutation of the above site could affect the activity of Maf proteins via dephosphorylation (Herath et al., 2014), and might therefore contribute to deregulation of osteoclastogenesis (Kim et al., 2006). This could further interfere with the podocyte development, which is a key component of the glomerular filtration barrier (Moriguchi et al., 2006).

Diseases with genotype-phenotype correlation has been reported and provides evidence for clinical heterogeneity (Mehawej et al., 2013). Among the eight patients reported, only six presented with renal manifestation and three of these underwent a renal biopsy. Focal segmental glomerulosclerosis (FSGS) was revealed by renal biopsy in two of the three unrelated patients (Park et al., 2018). A c.167C > T transition (pSer56Phe) discovered by us. in the *MAFB* gene near the point of the mutation, has been previously reported (Dworschak et al., 2013). The clinicians noticed Arnold-Chiari malformation type I and androgenetic alopecia in the patient and suspected these to be new phenotypic features; however, these were not observed in our patient or other reported cases. Further studies are warranted to establish if there is a relationship between these two phenotypes and MCTO.

We believe that the novel *MAFB* variant is responsible for the MCTO phenotype in our patient. The distinct differences between the patient in our study and those described in the literature may be due to the effect of a modifier, incomplete penetrance, epigenetic mechanisms, or environmental factors.

So far, twenty-one missense/nonsense mutations, three small deletions, and one gross deletion of the *MAFB* gene have been registered in Human Gene Mutation Database. We hereby report a de novo missense mutation in the amino-terminal transcriptional activation domain of *MAFB* (NM_005461.5:c.173C > G); this mutation is predicted to be damaging. The patient's presentation of CKD with other clinical features prompted us to investigate for MCTO and perform genetic analysis to accurately diagnose his case. As MCTO is a rare form of skeletal dysplasia, and not all patients demonstrate renal involvement, this disorder may be confused with rheumatological diseases, especially polyarticular juvenile arthritis. This increases the risk of delayed diagnosis and improper treatment (Eisenstein and Berkun, 2014). However, the frequent presence of abnormal facial features and the absence of biochemical markers should alert caregivers that further investigations, including genetic studies, may be necessary to confirm the underlying etiology of the bone deformities. Further, extended patient follow-up will also reveal the characteristic preferential involvement of the carpal and tarsal bones associated with MCTO. Hence, careful and vigilant tracking of patients will enable health care providers to differentiate MCTO from other disorders with a similar presentation.

Declaration of competing interest

The authors declare that they have no competing interests.

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Dr. Lina Shi contributes equally to the article. Authors would like to thank the patient and all physicians and medical staff involved in the assessment of the patient, and for their collaboration to this report. Authors also thank Dr. Man Chun Chiu (Princess Margaret Hospital, HK) and Dr. Brian Chung (Queen Mary Hospital, HK) for their advice and emendation. No funding was received for this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2020.103902.

Accession numbers

SCV000965692.

Availability of data and materials

All data gathered and analyzed during the preparation of this case report have been included in this published manuscript.

Ethics approval and consent to participate

The study was approved by the Research Ethics Board of Shenzhen Children's Hospital, Shenzhen, in the province of Guangdong, China. Written informed consent for the use of medical information and genetic analysis was obtained from the parents of the patient.

Consent for publication

Written consent was obtained from the parents of the patient for the publication of medical information.

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