A Novel *COMP* Mutation in a Chinese Family with Multiple Epiphyseal Dysplasia

Jiashen Shao

Peking Union Medical College Hospital

Sen Zhao

Peking Union Medical College Hospital

Zihui Yan

Peking Union Medical College Hospital

Lianlei Wang

Peking Union Medical College Hospital

Yuanqiang Zhang

Peking Union Medical College Hospital

Mao Lin

Peking Union Medical College Hospital

Chenxi Yu

Peking Union Medical College Hospital

Shengru Wang

Peking Union Medical College Hospital

Yuchen Niu

Peking Union Medical College Hospital

Xiaoxin Li

Peking Union Medical College Hospital

Guixing Qiu

Peking Union Medical College Hospital

Jianguo Zhang

Peking Union Medical College Hospital

Zhihong Wu

Peking Union Medical College Hospital

Nan Wu (✉ dr.wunan@pumch.cn)

Peking Union Medical College Hospital <https://orcid.org/0000-0002-9429-2889>

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Abstract

Background: Multiple epiphyseal dysplasia (MED) is a skeletal disorder characterized by delayed and irregular ossification of the epiphyses and early-onset osteoarthritis. At least 66% of the reported autosomal dominant MED (AD-MED) cases are caused by *COMP* mutations.

Methods: We recruited a four-generation Chinese family with early-onset hip osteoarthritis, flatfoot, brachydactyly, and mild short stature. The assessment of family history, detailed physical examinations, and radiographic evaluations were performed on the proband and other family members, followed by the performance of whole-exome sequencing (WES). The pathogenicity of the candidate mutation was also analyzed.

Results: We recruited an AD-MED family with 10 affected members and 17 unaffected members. The main radiographic findings were symmetrical changes in the dysplastic acetabulum and femoral heads, irregular contours of the epiphyses, a shortened femoral neck, and flatfoot. Lower bone density was also observed in the ankle joints, wrist joints, and knees, as well as irregular vertebral end plates. In the proband, we identified the missense mutation c.1153G>T (p. Asp385Tyr), located in exon 11 of the *COMP* gene. This mutation was assessed as “pathogenic” because of its low allele frequency and its high likelihood of co-segregation with disease in the reported family. Sanger sequencing identified the novel heterozygous mutation c.1153G>T (p. Asp385Tyr) in exon 11 of *COMP* in all affected individuals in the family.

Conclusions: Our result underlined a key role of the Asp385 amino acid in the protein function of *COMP*, and confirmed the pathogenicity of the *COMP* (c.1153G>T; p. Asp385Tyr) mutation in AD-MED disease. We have therefore expanded the known mutational spectrum of *COMP* and revealed new phenotypic information for AD-MED.

Introduction

Multiple epiphyseal dysplasia (MED; MIM# 132400) is a skeletal disorder characterized by delayed and irregular ossification of the epiphyses as well as early-onset osteoarthritis [1]. To date, six genes have been associated with MED, including five genes that cause autosomal dominant MED (AD-MED; *COMP*, *COL9A1*, *COL9A2*, *COL9A3* and *MATN3*) and one gene that causes autosomal recessive MED (rMED; *SLC26A2*) [2-7]. The incidence of AD-MED is estimated to be 1 in 10,000 individuals, and at least 66% of reported AD-MED cases are caused by *COMP* mutations [8]. These cases are known as EDM1 (or *COMP*-MED), and are characterized by mild short stature, premature osteoarthritis of load-bearing joints, and abnormalities of the epiphyses of hands, long bones, and hips [9, 10].

The *COMP* gene encodes cartilage oligomeric matrix protein (COMP), made up of 757 amino acids [11]. COMP is a 552 kDa pentameric adhesive glycoprotein that is mainly found in synovium, tendons, ligaments, and the extracellular matrix of cartilage [12, 13]. The binding of COMP to extracellular matrix proteins is essential for cartilage and extracellular matrix integrity [11]. Since the 1990s, more than 80 novel mutations have been reported in *COMP* that are involved in MED pathogenesis [12, 14]. The locations of these mutations are predominantly concentrated in the highly conserved type III (T3) calcium-binding repeat domain, and it has been demonstrated that the T3₄ mutation is significantly associated with MED compared with other T3 repeats. These mutations affect the secretion of extracellular matrix proteins and extracellular matrix integrity, often leading to skeletal abnormalities including pseudoachondroplasia (PSACH) and MED [15].

Here, we reported a four-generation Chinese family with early-onset hip osteoarthritis, flatfoot, brachydactyly, and mild short stature. The assessment of family history, detailed physical examinations, and radiographic evaluations were performed on the proband and other family members, followed by the performance of whole-exome sequencing (WES). The pathogenicity of the candidate mutation was then analyzed.

Methods

Patients

The proband (II-10) was initially diagnosed with Legg–Calvé–Perthes disease based on the observed radiographic changes, including uneven density of the bilateral femoral head and bilateral femoral head collapse, which indicates avascular necrosis of the femoral head. After an investigation of the proband’s family history, a four-generation pedigree with 27 family members was recruited from Shanxi Province, China. Clinical evaluation including medical history, physical examination, and radiographic assessment, as well as peripheral venous blood (EDTA-K2 anticoagulant) was collected from all individual. For patients cannot visit our hospital for clinical evaluation, we collected their clinical information by telephone interviews. Their consent forms and anteroposterior radiographs of pelvis were delivered by the express. Written informed consent was obtained from each participant; if the participant was younger than 16 years old, written informed consent was obtained from their parents or legal guardians. The Ethics Committee of Peking Union Medical College and Chinese Academy of Medical Sciences (PUMCH) approved this study.

DNA preparation and WES

According to the manufacturer's protocols, genomic DNA samples were extracted from peripheral blood leukocytes of each family member by peripheral blood DNA extraction kit (QIAamp DNA Blood Mini Kit; Qiagen, Germany). Purified DNA was qualified by Nanodrop2000 (Thermo Fisher Scientific, Waltham, MA, USA) and quantified by Qubit 3.0 using the dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA, USA).

The genomic DNA was broken into 180 to 280 bp fragments by ultrasonoscope and Illumina paired-end libraries were prepared from DNA samples. WES was performed on genome DNA of the proband (III-10) and individual II-6, II-9, III-6, III-18, and IV-12. The exome captured by a SureSelect Human All Exon V6+UTR r2 core design (91 Mb, Agilent), then sequenced on an Illumina HiSeq 4000 platform (Illumina, San Diego, CA, USA). The raw sequencing data were analyzed through Genome Analysis Toolkit (GATK, Version 3.4.0), and error assessment, variants calling were performed through in-house developed analytical pipeline [16].

The interpretation of variants was followed by American College of Medical genetics and Genomics (ACMG) [17]. The variants were filtered through the following procedures: (1) the allele frequency of variants is far less than 1% or absent from the database like 1000 Genomes Project (The 1000 Genomes Project Consortium 2015), Exome Aggregation Consortium (ExAC) (<http://exac.broadinstitute.org/>). (2) Variants were filtered out when they were synonymous mutation or located in introns without influence on splicing and biological function. (3) Variants were reviewed by phenotype evaluation, inheritance model, reported documents as well databases like Human Gene Variant Database (HGMD) and Online Mendelian Inheritance in Man (OMIM) (available at: <https://omim.org/>).

Sanger validation

A pair of PCR (Polymerase chain reaction) primer (F: 5'-CCATGAAGTTGGGACTCTGT-3', R: 5'-GGTCATTCTCTGGCAGTGT-3') were designed via Primer 3.0 (<http://primer3.ut.ee/>) to amplify the Exon 11 of *COMP* gene. The PCR program was 95°C for 3 min, followed by 38 cycles at 94°C for 30 sec, 58-60°C for 30 sec, 72°C for 50 sec, and a final extension at 72°C for 8 min. All the collected samples in this family were used as DNA template, the products of PCR analyzed in 2% agarose gels and purified by QIA quick PCR purification kit (Qiagen, Germantown, USA). Sequencing was performed on ABI3700 sequence Detection System (Applied Biosystems, Inc., Foster City, CA, USA). The reference sequence of candidate gene was obtained from UCSC Genome Browser (<http://genome.ucsc.edu>), and compared with sequencing data through CodonCode Aligner (version 6.0.2.6; CondonCode, Centerville, MA, USA).

3-D protein structures of both wild and variant type COMP protein were predicted using an online modeling server, SWISS-MODEL program (<https://swissmodel.expasy.org/>), which were then viewed and edited by the molecular visualization system PyMOL (PyMOL Molecular Graphics System, Version 2.3.3, Schrödinger, LLC).

Results

Patients' characteristics

We recruited an AD-MED family with 10 affected members and 17 unaffected members (Figure 1a). The proband (II-10) was a 38-year-old woman who had developed a waddling gait at the age of 6 years (Figure 2). At around the age of 22 years, the patient reported pain in the bilateral hip, lumbar vertebrae, and bilateral knees. The height of the proband was normal (152 cm), and there was no evidence to suggest growth retardation in childhood. The main radiographic findings were symmetrical changes in the dysplastic acetabulum and femoral heads, irregular contours of the epiphyses, a shortened femoral neck, and flatfoot (Figure 2). Lower bone density of the [ankle joints](#), wrist joints, and knees was observed, as well as irregular vertebral end plates. Her fingers and elbows were normal. When examining the other affected family members, we found one patient (II-2) with a mild phenotype (only presenting with the symptoms of hip pain), one patient (II-18) with mild sacroiliitis, and two patients with brachydactyly (II-9, II-13) (Figure 3). The proband and all affected individuals in this family had flatfoot, except for one patient (II-2) (Table 1). Furthermore, compare with the general Chinese male population (average height = 170 cm), male patients in this family have generally shorter stature (≤ 165 cm).

Mutation analysis

After the raw sequencing data were dealt through the analytical pipeline [16, 18], we identified that the proband carried 3185 different types of single nucleotide variants (SNVs), including missense, frameshift, splicing, and nonsense SNVs, as well as those with unknown influence of the synonymous or non-coding variants. All pathogenic and likely pathogenic variants were manually reviewed according to ACMG guidelines and the OMIM database. As a result, we identified the missense mutation, c.1153G>T (p. Asp385Tyr), located in exon 11 of *COMP*. This mutation was assessed as "pathogenic" based on its low allele frequency and its high likelihood of co-segregation with disease in the reported family. Sanger sequencing was performed in 24 family members, including 7 affected members and 17 unaffected members (Figure 1a). All affected family members carried the heterozygous mutation c.1153G>T (p. Asp385Tyr) in exon 11 of *COMP*, while the unaffected family members did not (Figure 1b). These results further indicated that this mutation was co-segregated in our family, and provides strong evidence for the pathogenicity of this mutation. The three-dimensional structure of the COMP protein provided further evidence of pathogenicity, with the mutation resulting in the replacement of the long side chain of Asp385 by a phenolic hydroxyl of tyrosine (Figure 4).

In a previous study by Mabuchi et al., a different mutation at the same position (c.1153G>A, p. Asp385Asn) has been reported to cause the MED phenotype [19]. Furthermore, Jackson et al. [20] also identified this recurrent mutation (c.1153G>A, p. Asp385Asn) in two British families and one Dutch family with MED. In addition, Liu et al. recently described an AD-MED family in which multiple members had been diagnosed with ANFH. Using WES, they identified a heterozygous variant in *COMP* (c.1153G>A) that contributed to the disease phenotype [10].

Consistent with the previously reported phenotypes, our pedigree cases also showed symptoms of classical AD-MED, such as femoral head necrosis, mild short stature, early-onset osteoarthritis of the knee and hip, and brachydactyly. These symptoms highlight the key role for Asp385 amino acid in the protein function of *COMP*, and confirm the pathogenicity of *COMP* (c.1153G>T; p. Asp385Tyr) in AD-MED disease.

Discussion

In the current study, we investigated a four-generation family with early-onset hip osteoarthritis caused by a heterozygous c.1153G>T mutation. Their prominent symptoms included severe osteoarthritis, knee pain, and femoral head necrosis, which is consistent with *COMP*-associated MED. According to the ACMG and our study, this variation: a) is absent from the records of any frequency database, such as the 1000 Genomes Project and ExAC, indicating that this mutation is rare (ACMG pathogenicity criteria: PM2); b) is located in a well-established functional domain (PM1); c) is co-segregated with disease in multiple affected family members (PP1); and d) has computational evidence supporting a deleterious effect on the gene (PP3). Based on this evidence, we classified this mutation as pathogenic, with an important role in the genetic etiology of our family.

A different mutation at the same position (c.1153G>A, p. D385N) has been reported to cause MED [19]. In this previous study, the reported sporadic case had mild short stature and early onset osteoarthritis, and was diagnosed with a severe form of MED—the “Fairbank type”. In another study, the authors reported a pedigree with severe hip osteoarthritis. Using WES, a c.1153G>A mutation of the *COMP* gene was identified to be related to the MED phenotype of this family [20]. The phenotypes of the pedigrees reported by Liu et al. [10] were partially similar to that of our patient. They mainly manifested as necrosis of the femoral head, but their phenotype did not include flatfoot. In the current study, the c.1153G>T mutation was predicted to lead to an amino acid substitution from Asp to Tyr, implying that other substitutions at this position can also lead to the classical MED phenotype.

The precise function and pathogenic mechanisms of mutant *COMP* in MED have not been fully defined. However, compelling evidence indicates that *COMP* proteins play an important role in maintaining cartilage and extracellular matrix integrity. The misfolding of mutant *COMP* affects its normal secretion from the endoplasmic reticulum of chondrocytes, and this intracellular retention is toxic to these cells, resulting in premature chondrocyte death [21, 22]. These events reduce the number of chondrocytes in the growth plate, ultimately reducing linear growth, and the phenotypic outcome is dwarfism. Moreover, the reduction of *COMP* secretion also affects the assembly of collagen fibers [23], leading to a decrease in articular cartilage mechanical strength and the occurrence of early-onset osteoarthritis [24-26]. In addition, the calcium-binding T3 repeat of *COMP* has been found to provide support for chondrocyte attachment [27]. Changes in the three-dimensional calcium-dependent structure of the mutant *COMP* may therefore alter chondrocyte attachment, thereby contributing to MED phenotype development. Briggs et al. [12] previously confirmed that both PSACH and MED mutations are predominantly located within the T3 repeat domain of *COMP* (90% of mutations). They also reported that missense mutations and in-frame insertions/deletions of single residues in T3₅₋₇ usually cause PSACH, while missense mutations in T3₃₋₄ are more likely to cause MED. Our novel c.1153G>A mutation is located in the T3₃₋₄ repeat of *COMP*. The aforementioned mechanisms may explain some of the phenotypes in our family, such as mild short stature and early-onset osteoarthritis.

Clubfoot is a rare radiological finding that has been observed mostly in association with rMED. In a previous study [28], rMED patients with clubfoot were reported to carry homozygous/compound heterozygous mutations in *SLC26A2* at birth. In addition, Superti-Furga et al. [29] described rMED patients with normal stature, clubfoot, and double-layered patellae caused by a *DTDST* mutation. In our study, all affected individuals in the family had flatfoot except for a single patient (II-2). However, other intra-familial differences in phenotype were observed in our family. For example, one patient (II-18) had mild sacroiliitis, with lower back pain at the age of 18 years, and also had gait abnormalities, mild short stature, flatfoot, and hip osteoarthritis. In addition, two male patients (II-9, II-13) had brachydactyly, but the other patients in this family did not have this phenotype. Furthermore, the male patients in this family were generally shorter in height, but it is unclear if this is related to the c.1153G>T mutation. In a pedigree study conducted by Sakamoto et al. [30], intra-familial differences in severity were also observed in their four-generation family: radiological manifestations in the knees were more severe in the proband's father than in the proband, and the proband's young sister was of shorter stature than the proband. Liu et al. [10] also reported intra-familial differences; for example, a twin brother in their reported family had more severe walking limitations than other family members. These intra-familial phenotypic differences may be difficult to explain by genetic factors, but may be explained by the effects of environmental factors [1, 31].

Conclusions

We identified a novel heterozygous pathogenic mutation in *COMP* from an AD-MED family that exhibited *COMP*-associated MED, and other phenotypes including flatfoot. Our results expanded both the mutational and phenotypic spectra of *COMP* and suggested that this mutation of a

key amino acid residue is disease-causing.

Abbreviations

MED: Multiple epiphyseal dysplasia; AD-MED: autosomal dominant MED; WES: Whole-exome sequencing; COMP: Cartilage oligomeric matrix protein; PSACH: Pseudoachondroplasia; PUMCH: Peking Union Medical College and Chinese Academy of Medical Sciences; ACMG: American College of Medical genetics and Genomics; OMIM: Online Mendelian Inheritance in Man; HGMD: Human Gene Variant Database; PCR: Polymerase chain reaction; SNVs: Single nucleotide variants; rMED: Autosomal recessive MED.

Declarations

Ethics approval and consent to participate: The study was approved by the Ethical Review Board of the Peking Union Medical College Hospital (2012/03/14, JS-908). Written informed consent was provided by each participant. When the individuals younger than the age of 16, the written informed consent is obtained from their parents or legal guardians.

Consent for publication: Written informed consent for publication of clinical details and/or clinical images was obtained from the all of the participants. When the individuals younger than the age of 18, the written informed consent is obtained from their parents or legal guardians.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: NW and ZW conceived the project. JS, SZ, and ZY participated in the experiment and data collection/interpretation for the study. JS, SZ, ML, and CY analyzed data and drafted the manuscript. NW and ZW helped to revise the manuscript. YZ and LW helped with data management and statistical analysis. XL and YN provided technique support. SW, JZ, and GQ offered professional discussions and instructions. All authors read and approved the final manuscript.

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Table

Table 1 Clinical information of affected family members

Patient	Gender	Age at examination	Height	Flatfoot	Brachydactyly	Gait abnormalities (onset year)	Hip pain (onset year)	Other joint pain, bilateral or unilateral (onset year)	Morning stiffness (onset year)	Fatigue with long-distance walking (onset year)
▯-9*	M	58	160	+	+	+ (9 y)	Left side (28 y)	Left knee (28 y)	Hip (-)	+ (-)
▯-11	F	60	160	+	Data unavailable	+ (-)	Bilateral (38 y)	Lumbar vertebra (38 y)	Ankle, hip (40 y)	+ (-)
▯-7	M	62	152	+	Data unavailable	+ (-)	-	Bilateral knees (56 y)	-	-
▯-13*	M	54	155	+	+	+ (9 y)	Bilateral (-)	Bilateral knees (8 y)	Knees (26 y)▯	+ (8 y)
▯-2	M	49	162	-	-	-	Bilateral (47 y)	-	-	-
▯-10*	F	39	162	+	-	+ (6 y)	Left side (22 y) bilateral (25 y)	Lumbar vertebra, bilateral knees (25 y)	Ankle, knees (20 y)	+ (12 y)
▯-16*	F	31	164	+	-	+ (12 y)	Bilateral (29 y)	Left knee (29 y)	Hip (29 y)	+ (12 y)
▯-18*	M	25	165	+	-	+ (8 y)	Left side (13 y) bilateral (20 y)	Lumbar vertebra (18 y)	Hip (18 y)	+ (8 y)
▯-22*	F	34	162	+	-	+ (10 y)	Bilateral (28 y)	-	Hip (30 y)▯	+ (10 y)
▯-24*	F	11	140	+	-	+ (10 y)	-	-	-	-

*DNA sequence analysis was performed on those affected family members.

Figures

Figure 1

The pedigree of the Chinese family with MED and the result of Sanger sequencing. (a)*DNA sequence analysis was performed on those family members; shaded symbols represent the affected individuals; black arrow represents proband, and slanting lines represent deceased individuals; (b) Electropherograms of Sanger sequencing showing the heterozygous c.1153G>T.

Figure 2

The clinical and radiological characteristics of proband. Clinical and radiological appearance of proband (III-10) showing (a) the limitation of movement in the lumbar spine due to the pain of lumbar vertebra and hip, (b) flatfoot on both sides, and (c) the avascular necrosis of the bilateral femoral heads.

Figure 3

The radiological characteristics of an affected patient. Radiological assessment of a patient (X-13) showing (a) the brachydactyly of the both hands, (b) shallow femoral trochlear grooves and slightly squared femoral condyle in the bilateral knee, (c) the avascular necrosis of the bilateral femoral heads, and (d) flatfoot on both sides.

Figure 4

Protein structure predicted by SWISS-MODEL. Protein structure predicted by SWISS-MODEL shows replacement of the long side chain of Aspartate 385 by a phenolic hydroxy of tyrosine.