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>

Research article

Keywords: COMP, Multiple Epiphyseal Dysplasia, Femoral head necrosis, Whole exome Sequencing

Posted Date: February 24th, 2020

DOI: <https://doi.org/10.21203/rs.2.24293/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

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 : Here, we reported a four-generation Chinese family with early-onset hip osteoarthritis, flatfoot, brachydactyly, and mild short stature. Whole exome sequencing was performed on the proband followed by enquiring family history, detailed physical examination, and radiographic evaluation. After that, the pathogenicity of candidate mutation was also analyzed.

Results : We recruited a dominant MED family with 10 affected members and 17 unaffected members. The main radiographic findings were symmetric changes in dysplastic acetabulum and femoral head, irregular contours of epiphyses, shorted femoral neck, and flatfoot. Lower bone density of ankle joint, wrist joint, and knees were observed as well as irregular vertebral end plates. As a result, we found a missense mutation c.1153G>T (p. Asp385Tyr) located in the exon 11 of COMP gene. This mutation was assessed as “pathogenic” for the low allele frequency and a high likelihood of co-segregation with disease in the reported family. Sanger sequencing identified a novel heterozygous mutation c.1153G>T (p. Asp385Tyr), in exon 11 of COMP gene in all affected male individuals.

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

Introduction

Multiple Epiphyseal Dysplasia (MED; MIM# 132400) is a skeletal disorder characterized by delayed and irregular ossification of the epiphyses and 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, MATN3) and one gene that cause 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 the reported autosomal dominant MED cases are caused by COMP mutations [8], i.e. EDM1 (COMP-MED; MIM#132400), characterized by mild short stature, premature osteoarthritis of load bearing joints, and abnormalities of the epiphyses of hands, long bones and hips [9, 10].

COMP encodes cartilage oligomeric matrix protein (COMP) constituted with 757 amino acids [2]. COMP is a 552 KDa-pentameric-adhesive glycoprotein protein mainly existing in synovium, tendon, ligaments, and the extracellular matrix of the cartilage [11–13]. Binding of COMP to extracellular matrix proteins is essential for the integrity of cartilage and extracellular matrix. Since the 1990s, there are more than 80 novel mutations of COMP gene that involved the pathogenesis of MED reported [6, 14, 15]. The locations of these mutations are predominantly concentrated in the highly conserved type III (T3) calcium-binding repeat domain, and the mutation of T3₄ showed a significant association with MED compared with the other T3 repeats. These mutations affect the secretion of extracellular matrix proteins and integrity of extracellular matrix, which often leading to skeletal abnormalities including pseudoachondroplasia (PSACH) and MED [16].

Here, we reported a four-generation Chinese family with early-onset hip osteoarthritis, flatfoot, brachydactyly, and mild short stature. Whole Exome Sequencing (WES) was performed on the proband followed by enquiring family history, detailed physical examination, and radiographic evaluation. After that, the pathogenicity of candidate mutation was also analyzed.

Patients And Methods

Patients

The proband (Ⅱ-10) initially diagnosed as Legg-Calve-Perthes disease (LCPD) for the observed radiographic changes such as uneven density of bilateral femoral head and bilateral femoral head collapse, which indicates avascular necrosis of the femoral head. After the investigation of family history, a four-generation pedigree with 27 family members was recruited from Shan Xi province, China. 5 mL peripheral blood sample was collected from each of the 27 family members, including 17 unaffected individuals and 10 affected individuals (Ⅱ-9, Ⅱ-11, Ⅱ-12, Ⅱ-13, Ⅱ-1, Ⅱ-10, Ⅱ-16, Ⅱ-18, Ⅱ-22, Ⅱ-24). Detailed physical examination and radiographic assessment was conducted on the proband and family members (Table 1). Informed written consent was obtained from each individual. When the individuals younger than the age of 16, the written informed consent is 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. Whole-exome sequencing (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 [17].

The interpretation of variants was followed by American College of Medical genetics and Genomics (ACMG) [18]. 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'-GGTCATTTCTCTGGCAGTGT-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).

Table 1
Clinical and genetic 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)
Ⅱ-9	M	58	160	+	+	+ (9 y)	Left side (28 y)	Left knee (28 y)	Hip (-)	+ (-)
Ⅱ-11	F	No information	160	+	No information	+ (-)	Bilateral (38 y)	Lumbar vertebra (38 y)	Ankle, hip (40 y)	+ (-)
Ⅱ-12	M	No information	No information	+	No information	+ (-)	-	Bilateral knees (56 y)	-	-
Ⅱ-13	M	54	155	+	+	+ (9 y)	Bilateral (-)	Bilateral knees (8 y)	Knees (26 y)	+ (8 y)
Ⅱ-1	M	49	162	-	-	-	Bilateral (47 y)	-	-	-
Ⅱ-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)
Ⅱ-16	F	31	164	+	-	+ (12 y)	Bilateral (29 y)	Left knee (29 y)	Hip (29 y)	+ (12 y)
Ⅱ-18	M	25	165	+	-	+ (8 y)	Left side (13 y) bilateral (20 y)	Lumbar vertebra (18 y)	Hip (18 y)	+ (8 y)
Ⅱ-22	F	34	162	+	-	+ (10 y)	Bilateral (28 y)	-	Hip (30 y)	+ (10 y)
Ⅱ-24	F	11	140	+	-	+ (10 y)	-	-	-	-
The members in bold had the COMP mutation.										

Results

Patients' characteristics

We recruited a dominant MED family with 10 affected members and 17 unaffected members (Fig. 1a). The proband (**Ⅱ-10**) was a 38-year-old woman who had waddling gait when she was six-year-old. The sense of pain in hip, lumbar vertebra, bilateral knees was obvious around 22 years old. The height of proband is normal (156 cm), and no clues demonstrate growth retardation in the childhood. The main radiographic findings were symmetric changes in dysplastic acetabulum and femoral head (Fig. 2a), irregular contours of epiphyses, shorted femoral neck (Fig. 2b), and flatfoot (Fig. 2d). Lower bone density of ankle joint, wrist joint, and knees were observed as well as irregular vertebral end plates. Her fingers and elbows are normal. Referring to the other affected family members, we found a patient (**Ⅱ-1**) has mild phenotype (only presented with the symptoms of hip pain), one patient (**Ⅱ-18**) has mild sacroiliitis, and two patients has brachydactyly (**Ⅱ-9**, **Ⅱ-13**) (Fig. 2c). It is worth mentioning that the proband and all affect individuals in this family have flatfoot except one patient (**Ⅱ-1**) (Table 1). Furthermore, compare to female patients, male patients in this family have generally shorter stature (≤ 165 cm).

Mutation analysis

After the raw sequencing data were dealt through analytical pipeline [17, 19], we found the proband carried 3185 different type of single nucleotide variants (SNV), including missense, frameshift, splicing, nonsense and unknown influence of synonymous or non-coding variant. All pathogenic and likely pathogenic variants were manually reviewed according ACMG guide and OMIM database. As a result, we found a missense mutation c.1153G > T (p. Asp385Tyr) located in the exon 11 of COMP gene. This mutation was assessed as “pathogenic” for the low allele frequency and a high likelihood of co-segregation with disease in the reported family. Sanger sequencing was performed in 26 family members. The result shows that all the affected patients (Ⅱ-9, Ⅱ-13, Ⅱ-10, Ⅱ-16, Ⅱ-18, Ⅱ-22, Ⅱ-24) carry the heterozygous mutation c.1153G > T (p. Asp385Tyr) in the exon 11 of COMP gene while the unaffected family members were not (Fig. 1b). That further indicates this mutation is co-segregated in our family, as a potent supportive evidence for the pathogenicity of this mutation. The three-dimensional structure of the COMP protein provided further evidence of pathogenicity, as the replacement of the long side chain of Aspartate 385 by a phenolic hydroxy of tyrosine (Fig. 3).

In a previous study by Mabuchi et al., a different mutation at the same position (c.1153G > A, p. Asp385Asn) have been described as the cause of the MED phenotype [20]. Meanwhile, Jackson et al. also identified the recurrent (c.1153G > A, p. Asp385Asn) mutation in two British families and one Dutch family with MED. Recently, Liu et al. found an AD-MED family, in which multiple members had been diagnosed as ANFH. After WES, they found the heterozygous variant in COMP (c.1153G > A), contributed to the occurrence of phenotype.

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

Discussion

In the current study, we report a four-generation family of early-onset hip osteoarthritis caused by a heterozygous mutation of c.1153G > T. Their prominent symptoms include severe osteoarthritis, knees pain, and femoral head necrosis, same as COMP-associated MED. According to the ACMG and our study, this variation a) is absent from the records of any frequency database like 1000 Genomes Project and ExAC, indicates this mutation is a rare mutation (PM2); b) located in a well-established functional domain (PM1); c) co-segregated with disease in multiple affected family members (PP1); d) have computational evidence supporting a deleterious effect on the gene (PP3). Basing on the evidence above, we assessed this mutation as pathogenic, and played an important role in the genetic etiology of this family.

A different mutation at the same position (c.1153G > A, p. D385N) has been shown to cause MED [20]. In that reported sporadic case, the patients have mild short stature and early onset osteoarthritis, diagnosed as the severe form of MED- “Fairbank type”. In another study, the authors reported a pedigree with severe hip osteoarthritis. After WES, the c.1153G > A mutation of the COMP gene was identified as related to the MED phenotype of this family. The phenotype of pedigrees reported by Liu et al. is partially similar to our patient [21]. They are mainly manifested as necrosis of the femoral head, but there is no phenotype such as flatfoot. In the current study, the c.1153G > T mutation was predicted to lead to amino acid substitution from Asp to Tyr, which implies that other substitution of this position can also lead to the occurrence of classical phenotype of MED.

The precise function and pathogenic mechanism of mutant COMP in the MED have not been fully defined. However compelling evidence indicates that COMP play an important role in maintaining the integrity of cartilage and extracellular matrix. The misfolding of the mutant COMP affects its normal secretion from the endoplasmic reticulum of the affected chondrocytes, and this intracellular retention is toxic to chondrocytes, resulting in premature chondrocyte death [22]. These events which reduce the number of chondrocytes in the growth plate ultimately reduce linear growth with the phenotypic outcome being dwarfism. Moreover, the reduction of COMP secretion would affect the assembly of collagen fibers [23], leading to a decrease in articular cartilage mechanical strength and the occurrence of early-onset osteoarthritis [24–26]. At the meantime, the calcium binding T3 repeat of COMP has been shown to provide support for chondrocyte attachment [27]. The changes in the 3-D calcium-dependent structure of the mutant COMP may impress chondrocyte attachment and contribute to the development of MED phenotype. Briggs et al. [28] confirmed that both PSACH and MED mutations are predominantly located within the type III repeat domain of COMP (90% of mutation), meanwhile, they found that missense mutations and in-frame insertion/deletion of single residue in T3₅₋₇ usually cause PSACH, while missense mutations in T3₃₋₄ more often cause MED. Our mutation c.1153G > A is located in the T3₃₋₄ repeat of COMP. Therefore, the mechanisms mentioned above may explain some phenotypes of our family, such as mild short stature and early-onset osteoarthritis.

Club foot is a rare and peculiar radiological finding which has been observed mostly in association with recessive multiple epiphyseal dysplasia (rMED). In Makitie's study, they found that rMED patients with club foot carry homozygous/compound heterozygous mutations in SLC26A2 at birth [29]. Superti-Furga et al. [30] found the rMED patients with normal stature, club foot, and double layered patella caused by a DTDST mutation. Interestingly, in our study, we found that all affected individuals in our family have flatfoot except single patient (Ⅱ-1). Meanwhile, other intra-familial differences of phenotype could be observed in our family. For example, one patient (Ⅱ-18) have mild sacroiliitis, low back pain at 18 years old, who also have gait abnormalities, mild short stature, flatfoot, and hip osteoarthritis. In addition, two male patients (Ⅱ-9, Ⅱ-13) have brachydactyly, but other patients do not have this phenotype. Furthermore, male patients in this family are generally shorter in height, but we are not sure if this is related to the mutation of c.1153G > T. In the pedigree study conducted by Sakamoto et al., they also observed the intra-familial differences in the severity of their four-generation family: the radiological manifestation of the knees were more severe in the proband's father than in the proband, and the stature of young sister is shorter than of proband [31]. Otherwise, Liu et al. [21] also found similar characteristics of intra-

familial differences, a twin brother of their family has more severe symptoms of walking limitation than other family member. These intra-familial differences of phenotype are difficult to explain by genetic factors but can be explained by the effects of environmental factors [1, 3].

In conclusion, we identified a novel heterozygous pathogenic mutation in COMP from an AD-MED family, exhibiting COMP-associated MED, and other phenotypes like flatfoot. Our results expanded the mutational and phenotypic spectrum of COMP and suggested that the mutation of the key amino acid residues would be disease-causing.

Declarations

Ethics approval and consent to participate: The study was approved by the Ethical Review Board of the Peking Union Medical College Hospital. 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: All of the patient's medical record and images are kept in Peking Union Medical College Hospital. For the review, please refer to the method section.

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

Funding: This work was supported by the National Natural Science Foundation of China (81822030 to N.W., 81772299 to Z.W.); Beijing Natural Science Foundation (7172175 to N.W.); the National Key Research and Development Program of China (No. 2018YFC0910500 to Z.W. and N.W.), the Central Level Public Interest Program for Scientific Research Institute (2018RC31003 to N.W.), and the CAMS Initiative Fund for Medical Sciences (2016-I2M-3-003 to N.W.).

Conflict of Interest: The authors declare no conflict of interest, financial or otherwise.

Authors' contributions: All authors searched the literature, designed the study, interpreted the findings. Jiashen Shao, Sen Zhao, and Zihui Yan participated in the experiment and data collection/interpretation for the study. Jiashen Shao, Sen Zhao, Mao Lin, and Chenxi Yu analyzed data and drafted the manuscript. Nan Wu and Zhihong Wu conceived the project. Nan Wu and Zhihong Wu helped to revise the manuscript. Yuanqiang Zhang and Lianlei Wang helped with data management and statistical analysis. Xiaoxin Li and Yuchen Niu provided technique support. Shengru Wang, Jianguo Zhang, and Guixing Qiu offered professional discussions and instructions.

Acknowledgements: We are grateful to the patients, their families, clinical surgeons, and genetic counselors for providing samples and clinical histories.

References

1. Dahlqvist J, Orlen H, Matsson H, Dahl N, Lonnerholm T, Gustavson KH. Multiple epiphyseal dysplasia. *Acta Orthop*. 2009; 80(6):711-715.
2. Briggs MD, Chapman KL. Pseudoachondroplasia and multiple epiphyseal dysplasia: mutation review, molecular interactions, and genotype to phenotype correlations. *Human mutation*. 2002; 19(5):465-478.
3. Chapman KL, Briggs MD, Mortier GR. Review: Clinical Variability and Genetic Heterogeneity in Multiple Epiphyseal Dysplasia. *Pediatric Pathology*. 22(1):53-75.
4. Czarny-Ratajczak M, Lohiniva J, Rogala P, Kozlowski K, Perala M, Carter L, et al. A mutation in *COL9A1* causes multiple epiphyseal dysplasia: further evidence for locus heterogeneity. *American journal of human genetics*. 2001; 69(5):969-980.
5. Paasilta P, Lohiniva J, Annunen S, Bonaventure J, Merrer ML, Pai L, et al. *COL9A3*: A Third Locus for Multiple Epiphyseal Dysplasia. 64(4):0-1044.
6. Unger SL, Briggs MD, Holden P, Zabel B, Ala-Kokko L, Paasilta P, et al. Multiple epiphyseal dysplasia: radiographic abnormalities correlated with genotype. *Pediatric radiology*. 2001; 31(1):10-18.
7. Muragaki Y, Mariman ECM, van Beersum SEC, Per? M, van Mourik JBA, Warman ML, et al. A mutation in the gene encoding the $\alpha 2$ chain of the fibril-associated collagen IX, *COL9A2*, causes multiple epiphyseal dysplasia (EDM2). 12(1):103-105.
8. Anthony S, Munk R, Skakun W, Masini M. Multiple epiphyseal dysplasia. *The Journal of the American Academy of Orthopaedic Surgeons*. 2015; 23(3):164-172.
9. Cohn DH, Briggs MD, King LM, Rimoin DL, Wilcox WR, Lachman RS, et al. Mutations in the cartilage oligomeric matrix protein (COMP) gene in pseudoachondroplasia and multiple epiphyseal dysplasia. *Annals of the New York Academy of Sciences*. 2010; 785(1):188-194.

10. Thur J, Rosenberg K, Nitsche DP, Pihlajamaa T, Ala-Kokko L, Heinegard D, et al. Mutations in cartilage oligomeric matrix protein causing pseudoachondroplasia and multiple epiphyseal dysplasia affect binding of calcium and collagen I, II, and IX. *J Biol Chem*. 2001; 276(9):6083-6092.
11. Morgelin M. Electron microscopy of native cartilage oligomeric matrix protein purified from the Swarm rat chondrosarcoma reveals a five-armed structure. *Journal of Biological Chemistry*. 1992; 267(9):6137-6141.
12. Oldberg A, Antonsson P, Lindblom KK, Heinegård D. COMP (Cartilage Oligomeric Matrix Protein) is structurally related to the thrombospondins. *Journal of Biological Chemistry*. 1992; 267(31):22346-22350.
13. Hedbom E, Antonsson P, Hjerpe A, Aeschlimann D, Paulsson M, Rosa-Pimentel E, et al. Cartilage matrix proteins. An acidic oligomeric protein (COMP) detected only in cartilage. *Journal of Biological Chemistry*. 1992; 267(9):6132.
14. Briggs MD, Hoffman SMG, King LM, Olsen AS, Mohrenweiser H, Leroy JG, et al. Pseudoachondroplasia and multiple epiphyseal dysplasia due to mutations in the cartilage oligomeric matrix protein gene. 10(3):330-336.
15. Kennedy J, Jackson G, Ramsden S, Taylor J, Newman W, Wright MJ, et al. *COMP* mutation screening as an aid for the clinical diagnosis and counselling of patients with a suspected diagnosis of pseudoachondroplasia or multiple epiphyseal dysplasia. *European journal of human genetics : EJHG*. 2005; 13(5):547-555.
16. Acharya C, Yik JH, Kishore A, Van Dinh V, Di Cesare PE, Haudenschild DR. Cartilage oligomeric matrix protein and its binding partners in the cartilage extracellular matrix: interaction, regulation and role in chondrogenesis. *Matrix Biol*. 2014; 37:102-111.
17. Wang K, Zhao S, Zhang Q, Yuan J, Liu J, Ding X, et al. Whole-exome sequencing reveals known and novel variants in a cohort of intracranial vertebral-basilar artery dissection (IVAD). *Journal of human genetics*. 2018; 63(11):1119-1128.
18. Liu J, Zhou Y, Qi X, Chen J, Chen W, Qiu G, et al. CRISPR/Cas9 in zebrafish: an efficient combination for human genetic diseases modeling. *Human genetics*. 2017; 136(1):1-12.
19. Wang K, Zhao S, Liu B, Zhang Q, Li Y, Liu J, et al. Perturbations of BMP/TGF-beta and VEGF/VEGFR signalling pathways in non-syndromic sporadic brain arteriovenous malformations (BAVM). *Journal of medical genetics*. 2018; 55(10):675-684.
20. Mabuchi A, Manabe N, Haga N, Kitoh H, Ikeda T, Kawaji H, et al. Novel types of *COMP* mutations and genotype-phenotype association in pseudoachondroplasia and multiple epiphyseal dysplasia. *Human genetics*. 2003; 112(1):84-90.
21. Liu HY, Xiao JF, Huang J, Wang Y, Wu D, Li T, et al. Diagnosis with Multiple Epiphyseal Dysplasia Using Whole-exome Sequencing in a Chinese Family. *Chin Med J (Engl)*. 2017; 130(1):104-107.
22. Y H, T T, Y Y, H M. Mutation (D472Y) in the type 3 repeat domain of cartilage oligomeric matrix protein affects its early vesicle trafficking in endoplasmic reticulum and induces apoptosis. *The American journal of pathology*. 2003; 163(1):101-110.
23. Halasz K, Kassner A, Morgelin M, Heinegard D. COMP Acts as a Catalyst in Collagen Fibrillogenesis. *Journal of Biological Chemistry*. 282(43):31166-31173.
24. Posey KL, Hankenson K, Veerisetty AC, Bornstein P, Lawler J, Hecht JT. Skeletal Abnormalities in Mice Lacking Extracellular Matrix Proteins, Thrombospondin-1, Thrombospondin-3, Thrombospondin-5, and Type IX Collagen. *American Journal of Pathology*. 172(6).
25. Mann HH, Özbek S, Engel J, Paulsson M, Wagener R. Interactions between the cartilage oligomeric matrix protein and matrilins. Implications for matrix assembly and the pathogenesis of chondrodysplasias. 2004; 279(24):25294-25298.
26. Holden P, Meadows RS, Chapman KL, Grant ME, Kadler KE, Briggs MD. Cartilage Oligomeric Matrix Protein Interacts with Type IX Collagen, and Disruptions to These Interactions Identify a Pathogenetic Mechanism in a Bone Dysplasia Family. *Journal of Biological Chemistry*. 276(8):6046-6055.
27. Chen FH, Thomas AO, Hecht JT, Goldring MB, Lawler J. Cartilage Oligomeric Matrix Protein/Thrombospondin 5 Supports Chondrocyte Attachment through Interaction with Integrins. *Journal of Biological Chemistry*. 280(38):32655-32661.
28. Briggs MD, Brock J, Ramsden SC, Bell PA. Genotype to phenotype correlations in cartilage oligomeric matrix protein associated chondrodysplasias. *European Journal of Human Genetics Ejhg*. 2014; 22(11):1278-1282.
29. Makitie O, Geiberger S, Horemuzova E, Hagenas L, Mostrom E, Nordenskjöld M, et al. *SLC26A2* disease spectrum in Sweden - high frequency of recessive multiple epiphyseal dysplasia (rMED). *Clin Genet*. 2015; 87(3):273-278.
30. Superti-Furga A, Neumann L, Riebel T, Eich G, Steinmann B, Spranger J, et al. Recessively inherited multiple epiphyseal dysplasia with normal stature, club foot, and double layered patella caused by a *DTDST* mutation. *Journal of medical genetics*. 1999; 36(8):621-.
31. Sakamoto Y, Yamamoto T, Kajino Y, Kabata T, Tsuchiya H, Miyake N, et al. Multiple epiphyseal dysplasia mimicking osteoarthritis due to acetabular dysplasia: A report of a familial case with a *COMP* mutation. *J Orthop Sci*. 2017; 22(5):967-971.

Figures

Figure 1

Pedigree of the studied Chinese family with MED and the result of Sanger sequencing. The pedigree of 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

Skeletal characteristics of patients carrying novel variants in COMP genes. (a) Plain radiographs of pelvis confirm the avascular necrosis of the bilateral femoral head of the proband. X-ray show flattening of the femoral heads and narrowing of joint spaces bilaterally (b) Plain radiographs of knee show shallow femoral trochlear grooves and slightly squared femoral condyle in the bilateral knee. (c) Brachydactyly (B-13). (d) Flatfoot on both sides.

Figure 3

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.