

# Whole-Exome Sequencing Reveals a Recurrent D401N Mutation in the *COMP* gene that Causes Multiple Epiphyseal Dysplasia



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## Abstract

Multiple epiphyseal dysplasia (MED) is a rare osteochondrodysplasia characterized by moderate short limb dwarfism and early-onset osteoarthritis. By whole-exome sequencing (WES), we identified a dominantly inherited mutation (c.1201G>A; p.D401N) in cartilage oligomeric matrix protein (*COMP*) in a large four-generation Chinese family. Immunofluorescence analysis revealed mutant *COMP* secretion was severely impaired. Our result expands the mutational spectrum of *COMP* and provides strong evidence for the genotype-phenotype correlation of *COMP* pathogenicity in MED.

**Keywords:** MED; *COMP*; WES

**Abbreviations:** MED: Multiple Epiphyseal Dysplasia; WES: Whole-Exome Sequencing; GSDs: Genetic Skeletal Diseases

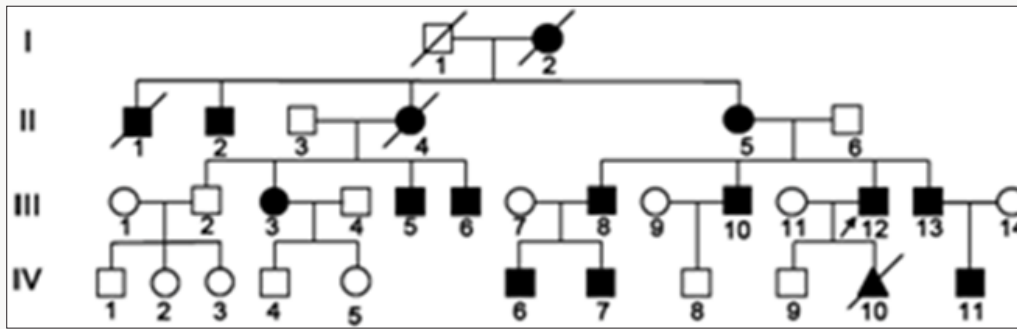
## Introduction

Human genetic skeletal diseases (GSDs) are an extremely diverse and *COMp*lex group of rare genetic condition that primarily affect the development and homeostasis of the osseous skeleton. GSDs not only cause patients in pain and disability, but also bring poor quality of life and high healthcare costs. According to the 2015 Nosology and Classification of the GSDs, there are 436 well-characterized skeletal diseases that are classified primarily on the basis of clinical, radiographic, and molecular criteria [1,2].

Multiple epiphyseal dysplasia (MED/EDM1, MIM no.132400) is a rare GSDs disease affecting the development of epiphyses. The main clinical features include mild short stature, lower limb deformities and early onset osteoarthritis [3,4]. MED is genetically heterogeneous and can result from mutations in *COMP*, *DTDST* (*SLC26A2*), *MATN3*, *COL9A1*, *COL9A2* or *COL9A3* or *CANT1* [5-7], with the majority of cases results from mutations in *COMP* gene. These mutations cause disorganized endochondral ossification

of the epiphysis, ultimately leading to destruction of the articular cartilage.

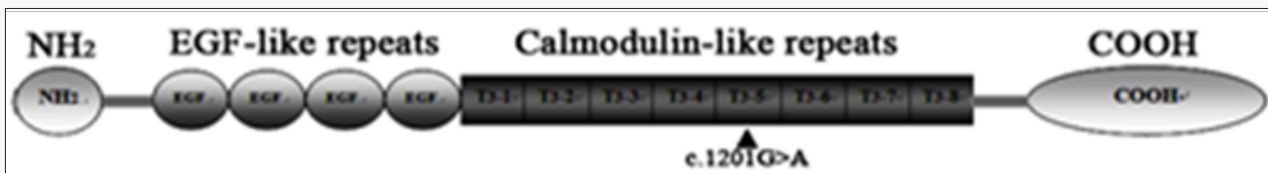
*COMP* is a 524kD secreted pentameric extracellular glycoprotein found in cartilage, tendon, and synovium, and plays an essential role in maintaining the extracellular matrix structure in cartilage [8]. Multiple functions have been suggested for *COMP*, including regulation of collagen fibril assembly, chondrocytes proliferation and interactions with other matrix proteins such as collagens type II, IX and matrilin-3 (*MATN3*) [9,10]. *COMP* mutation can affect the secretion of *COMP* protein and cause its retention in the ER [11]. In the current study, we identify a recurrent pathogenic SNV in *COMP* gene that cause MED by WES in a four-generation Chinese family this mutation is further validated using Sanger Sequencing. We examined the effects of mutation (D401N) in Hela cells and found this mutation affects *COMP* protein secretion and causes its retention in the ER.



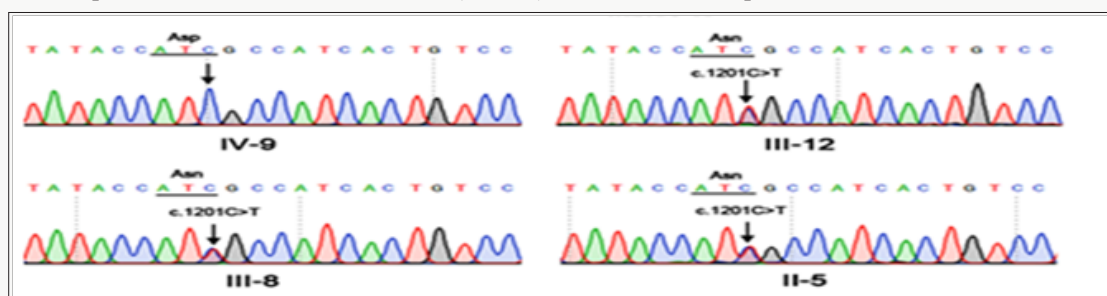
**Figure 1A:** Square indicates male, and circles indicate females. Blackened symbols denote affected individuals. The proband is indicated by arrows (↗).



**Figure 1B:** Right hand and left hand X ray of the proband showed bilateral short and small phalanges, markedly irregularities of the distal radial and ulnar epiphyses.



**Figure 1C:** The structure of a COMP monomer, showing the locations of the N-terminal domain (NH<sub>2</sub>), the EGF-like and calmodulin-like repeats, and the C-terminal domain (COOH), is shown at the top.



**Figure 1D:** Heterogenous c.1201C>T (minus strand) was detected in patient III-12 III-8, II-5, whilst this mutation is absent in healthy family members.

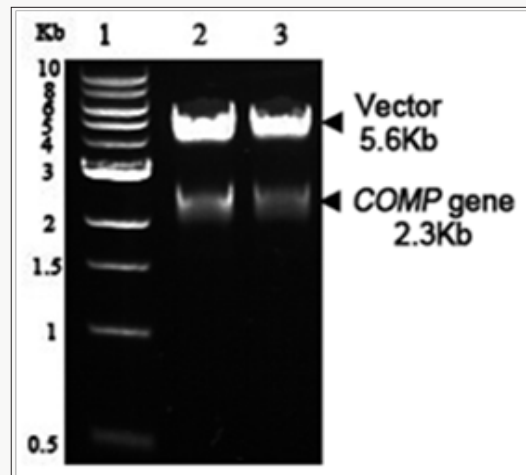
**Figure 1:** Family pedigree and sequencing results.

We investigated a four-generation Chinese family affected with MED (Figure 1A). Sixteen patients were included in the study; patients were identified and followed up for skeletal dysplasias at specialized clinics in participating institutions. Diagnosis of MED was made based on clinical and radiographic examinations, as described previously [12]. The proband had MED common signs:

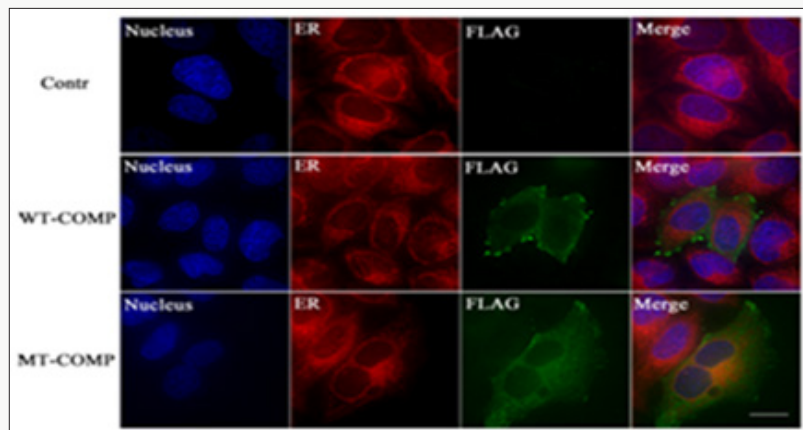
joint pain affecting the hip and knee joint, muscular hypotonic, joint laxity, mild genu vara. X-ray examination showed bilateral short and small phalanges, markedly irregularities of the distal radial and ulnar epiphyses (Figure 1B). In this family, all patients had the common features of MED.

We obtained written informed consent to perform molecular studies, which were approved by Institutional Review Board of Shanxi Medical University. We performed WES on genomic DNA sample of the proband (III-12) of the Chinese Han family with MED in order to identify the causal genes. A total of 134,056 genetic variants, including 14,990 non-synonymous changes, were occurred at the coding sequence or the canonical dinucleotide of the splice site junctions. Variants were functionally annotated and filtered using in-house cloud-based rare disease NGS analysis platform with builds in public databases (dbSNP, OMIM, ESP, Clinvar,

1,000 Genomes) as previously described [13]. Exonic sequence alterations and intronic variants at exon-intron boundaries, with unknown frequency or minor allele frequency (MAF) <1% and not present in the homozygous state in those databases were retained. Subsequently, a missense heterozygous mutation in the *COMP* gene, with a G to A transition at position 1201, resulting in a substitution of aspartic acid for asparagines at amino acid position 401 (c.1201G>A; p.D401N), in the T3 motifs of *COMP*, was identified as the potential disease-causing gene of MED (Figure 1C).



**Figure 2A:** *COMP* construct verification by double-digestion with AgeI/EcoRI. 1. Marker; 2. pcDNA-2Flag-WT-*COMP*; 3. pcDNA-2Flag-MT-*COMP*, harboring c.1201G>A mutation.



**Figure 2B:** HeLa cells were transfected with WT-*COMP* and MT-*COMP* and cultured for 24 hours at 37°C. The cells were fixed and incubated with the primary antibody (anti-FLAG) followed by FITC-labeled secondary antibodies. No *COMP* staining was detected in untransfected cells. Scale bar=15µm.

**Figure 2:** MT-*COMP* secretion was impaired.

To confirm the WES results, we performed Sanger sequencing for 11 family members to validate the potential disease causing variant. Sequencing results showed co-segregation of the pathogenic variant (c.1201G>A) with the disease phenotype (Figure 1D). Primers used for *COMP* causative variant validation were as follows: 11F: 5'-GAAGTCATTCTGGCCTGGTC-3' and 12R: 5'-GGTAGCCTTTGACAAACGCT-3'. To study the functional consequences of D401N mutation in *COMP*, we constructed the

FLAG-tagged full-length wild-type *COMP* (WT-*COMP*) and the mutant construct carrying D401N mutation, which was confirmed by Sanger sequencing that the correct mutation had been introduced and other PCR-generated errors were not incorporated (Figure 2A). Immunofluorescence was performed using anti-FLAG antibody that specifically recognizes recombinant *COMP* (Figure 2B) and cytopainter ER staining kit to stain the ER. As shown in the merged images, at 24 h post-transfection, a significant portion of

WT-COMP was localized to the cell surface; In contrast, MT-COMP protein was retained in the cytoplasm and co-localized with ER marker (red), suggesting that MT-COMP was retained in the ER and recapitulates the trafficking pathology in the mouse model of pseudoachondroplasia [14].

MED is a genetically heterogeneous disorder with marked clinical variability. Mutations in COMP gene is the most common form, accounting for at least half of the cases, with the majority of cases results from mutations in COMP gene and 85% of the COMP mutations in the C-type motif of the linker and T3 repeats [15,16]. In our study, we identified an autosomal dominant MED in a large Chinese family carry COMP D401N mutation. A single case of D401N mutation was previously reported in a COMP mutation screening study of 100 families in UK [17]. We hypothesize that the mutation identified disrupt calcium binding, as the mutation identified falls in the calmodulin-like domain of COMP, which constitute calcium-binding pockets and its binding to calcium is reported to be essential for the COMP conformation [18]. The previous study show that the disruption of matrix formation and cell-matrix interaction by mutated COMP on may be a major element in the pathogenesis of COMP-associated chondrodysplasias [9,11].

In conclusion, we identified a missense dominantly inherited recurrent variant in the COMP gene in a large family. This is the first report of COMP D401N mutation in Asian population. We extended the mutation repertoire of COMP gene in patients with MED, paving the way for gene therapy and prenatal diagnosis of MED.

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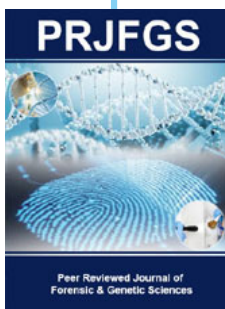


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