A Novel COMP Mutated Allele Identified in a Chinese Family with Pseudoachondroplasia

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1. Introduction

Pseudoachondroplasia (PSACH, OMIM 177170), whose incidence is estimated to be ~1/60000 (http://www.orpha.net/), is a relatively common osteochondrodysplasia. Its clinical features include disproportionate short stature, brachydactyly, joint laxity, and early-onset osteoarthritis. COMP encodes the cartilage oligomeric matrix protein, which is expressed predominantly in the extracellular matrix (ECM) surrounding the cells that make up cartilage, ligaments, and tendons. Mutations in COMP are known to give rise to PSACH. In this study, we identified a novel nucleotide mutation (NM_000095.2: c.1317C>G, p.D439E) in COMP responsible for PSACH in a Chinese family by employing whole-exome sequencing (WES) and built the structure model of the mutant protein to clarify its pathogenicity. The novel mutation cosegregated with the affected individuals. Our study expands the spectrum of COMP mutations and further provides additional genetic testing information for other PSACH patients.

PSACH is an autosomal dominant disease that is considered to result from mutations in the gene encoding the cartilage oligomeric matrix protein (COMP) [1, 6, 7]. The human COMP gene localizes on chromosome 19p13.1 and is a member of the thrombospondin gene family. It contains 19 exons, encoding an amino-terminal coiled-coil oligomerization domain, four type II epidermal growth factor-like repeats (EGF-like), eight type 3 calmodulin-like repeats (CLRs/T3 repeats), and a globular carboxyl terminal domain (CTD). It is abundantly expressed in the extracellular matrix (ECM) surrounding the cells that make up cartilage, ligaments, synovium, and tendons. Recent work suggests that COMP may stimulate chondrocyte proliferation by directly binding to the granulin/epithelin precursor (GEP) [8, 9]. Numerous COMP mutations have been identified in PSACH patients, and most of them were in the highly conserved T3 repeats[10, 11]. Point mutations resulting in single amino acid substitutions for conserved residues and in-frame deletions that delete codon(s) for one or more residues are the dominant incident [12].
Based solely on the family history, a detailed physical examination, and the radiographic method, it is difficult to accurately diagnose individuals with PSACH. For example, multiple epiphyseal dysplasia (MED, OMIM 132400) has semblable phenotypes and inherited patterns. With the application of next-generation sequencing techniques, such as whole-exome sequencing (WES), the discovery of the genetic architecture of individuals encountering heterogeneous conditions, such as PSACH, has extended, and it has also enabled the diagnosis of PSACH with more confidence[13].

In this study, we detected a novel mutated allele in COMP (NM_000095.2: c.1317C>G, p.D439E) in a Chinese family diagnosed with highly suspected PSACH based on clinical and radiologic results. To the best of our knowledge, there have been no reports of this variant in previous studies that have also not been presented in various single nucleotide polymorphism (SNP) databases.

2. Materials and Methods

2.1. Patients and Subjects. This research was approved by the Review Board of Xiangya Hospital of Central South University. Written informed consent was obtained from the proband and her guardians, in which all subjects consented to this study and the publication of images.

2.2. DNA Extraction. Peripheral blood samples of the proband and her family were collected to extract genomic DNA using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA).

2.3. Whole-Exome Sequencing. We followed the methods of Jin et al. [14] and Tång et al. [15]. The Berry Genomics Co., Ltd. (Chengdu, China) provided exome capture using the cBot Cluster Generation System and HiSeq PE Cluster Kit (Illumina, San Diego, CA, USA) and high-throughput sequencing by the Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA). The common variants (frequency ≥ 0.05) were filtered according to the Genome Aggregation Database (GnomAD; http://gnomad.broadinstitute.org) and the 1000 Genomes Project database (1000G; https://www.genome.gov/27528684/1000-genomes-project/). Candidate disease-causing variants were screened by the skeletal dysplasia-related gene list (Table S1), and then, pathogenicity prediction was predicted by MutationTaster software (http://www.mutationtaster.org), the SIFT server (http://provean.jcvi.org/index.php), and Polymorphism Phenotyping v2 server (Polyphen-2; http://genetics.bwh.harvard.edu/pph2/). The annotation of inheritance patterns, clinical phenotypes, and gene functions was conducted by Online Mendelian Inheritance in Man (OMIM; https://www.omim.org).

2.4. Cosegregation Analysis. Primer pairs (COMP 5′→3′: GACACGGATACAGACCGATAG; COMP 5′→3′: CACACGTCGATCTCTTGCTCC) were designed by Integrated DNA Technologies (https://sg.idtdna.com/pages). The target fragment was amplified by polymerase chain reaction (PCR) and analyzed using the ABI 3100 Genetic Analyzer (ABI, Foster City, CA, USA).

2.5. Modeling of the COMP Mutant. The structure of the COMP complex (PDB ID: 3FBY) was obtained from the Protein Data Bank in Europe database (ePDB; https://www.ebi.ac.uk/pdbe/?tsource=tp-tpcytq_omg). PyMol was used to build the D439E mutant model according to the wild-type COMP structure.

3. Results

3.1. Patients’ Characteristics. We enrolled a Chinese family with highly suspected PSACH (Figure 1(a)). The proband (II-3), a 14-year-old girl from Hunan Province of central south China with short stature (140 cm, <3%, no hormonal abnormalities), was primarily diagnosed with congenital dysplasia of the hip and required total hip replacement surgery after reaching an adult age. A medical history investigation revealed that the proband had suffered from waddling gait for ~12 years and bilateral hip joint pain for a year with aggravation for 5 months. Hip movement was restricted, particularly in flexion and abduction. The knees were positioned in the genu varum. Other studies with a positive Allis sign and Patrick sign suggested that the patient may have sacroiliac joint disease, but muscle tension was normal. Radiographs showed acetabular dysplasia with distorted acetabular shape, small epiphyses and femoral head deformity (Figures 1(b) and 1(c)). The right lower limb was shortened by 2 cm. The patient also had mild scoliosis with a Cobb angle of 17°, without brachydactyly and shoulder malformation (Figure 1(e)). Family history examination showed that her father (I-1, 155 cm) had necrosis of bilateral femoral heads and had undergone right hip replacement surgery in 2020. In addition, all of her sisters (II-1, II-2, and II-4) were short and presented unusual gait and hip dysplasia (Table 1). Her mother (I-1) and brother (II-5) were in good health.

3.2. Genetic Analysis. Common variants were filtered according to various SNP databases, and 776 unique SNPs were detected. After screening against a series of skeletal dysplasia causative genes, eight variants were identified in the proband (Table 2, Table S1). We classified these variants based on the American College of Medical Genetics (ACMG) guidelines [16] and strongly suspected that the heterozygous nucleotide variant of COMP (NM_000095.2: c.1317C>G, p.D439E) was the causative mutation in the family.

Sanger sequencing revealed that a novel mutated allele in COMP (c.1317C>G, p.D439E) was identified in the proband (II-3), which was inherited from her father (I-1), and her sisters (II-1, II-2, and II-4) also harbored this mutation (Figure 2(a)). The COMP mutation cosegregated with the affected family members. Cross-species alignment analysis of COMP showed that this mutated site had high conservation for 5 months. Hip movement was restricted, particularly in flexion and abduction. The knees were positioned in the genu varum. Other studies with a positive Allis sign and Patrick sign suggested that the patient may have sacroiliac joint disease, but muscle tension was normal. Radiographs showed acetabular dysplasia with distorted acetabular shape, small epiphyses and femoral head deformity (Figures 1(b) and 1(c)). The right lower limb was shortened by 2 cm. The patient also had mild scoliosis with a Cobb angle of 17°, without brachydactyly and shoulder malformation (Figure 1(e)). Family history examination showed that her father (I-1, 155 cm) had necrosis of bilateral femoral heads and had undergone right hip replacement surgery in 2020. In addition, all of her sisters (II-1, II-2, and II-4) were short and presented unusual gait and hip dysplasia (Table 1). Her mother (I-1) and brother (II-5) were in good health.

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4. Discussion

In this research, we used WES to identify a novel nucleotide variant (c.1317C>G, p.D439E) in exon 13 of COMP in a girl (II-3) with PSACH. Her father (I-1) and sisters (II-1, II-2, and II-4) carried the same mutation and had similar symptoms with an onset in early childhood. COMP is secreted by the endoplasmic reticulum of chondrocytes, and the misfolding of COMP inhibits its secretion, thereby causing toxicity to cells and leading to cell death [11, 17, 18]. Moreover, COMP has a significant effect on maintaining cartilage and ECM integrity, especially affecting the assembly of collagen fibers [11, 19]. There is a recent hypothesis that scoliosis with or without vertebral malformations may occur in association with arthrogryposis and can be triggered by defects in connective tissue matrix proteins. It was confirmed again in the proband and her young sister (II-3 and II-4), who harbored a COMP mutation and suffered from mild scoliosis and hip contractions.

COMP consists of a coiled-coil domain, four EGF-like repeats, eight T3 repeats, and a CTD domain. The mutations located at the T3 repeats region of COMP seem to cause a more severe phenotype. The T3 repeats region is a Ca\(^{2+}\)-binding pocket [8]. A high proportion of specific residues, such as aspartic acid, glycine, cysteine, and proline, in this region also promotes protein folding and Ca\(^{2+}\) binding [20]. In the D439E mutant, aspartic acid was substituted with glutamic acid, possibly reducing the stability of the mutant and inhibiting the binding ability of Ca\(^{2+}\). Mupro (https://www.ics.uci.edu/~baldig/mutation.html) predicted that D439E can

### Table 1: The clinical symptoms of patients in the present PSACH family.

| Patients | Sex | Age (years) | Height (cm) | Onset ages (years) | Gait abnormalities | Hip dysplasia | Knee contractions | Scoliosis | Brachydactyly | Myasthenia |
|----------|-----|-------------|-------------|--------------------|--------------------|---------------|------------------|-----------|--------------|-----------|
| I-1      | M   | 51          | 155         | Unknown            | +                  | +             | +                | —         | —            | —         |
| II-1     | F   | 17          | 145         | 3                  | +                  | +             | Unknown          | Unknown   | —            | —         |
| II-2     | F   | 15          | 140         | 2                  | +                  | +             | +                | —         | —            | —         |
| Proband  | F   | 14          | 140         | 2                  | +                  | +             | +                | —         | —            | —         |
| II-4     | F   | 12          | 135         | 3                  | +                  | +             | +                | —         | +            | —         |

M: male; F: female; +: positive phenotype; -: negative phenotype.
decrease the stability (ΔΔG = −0.7849), as expected. It has also been mentioned that chondrocyte attachment may be altered, for the three-dimensional Ca2+-dependent structure of mutant COMP get changed [21]. All of these functions are closely associated with the generation and development of PSACH. The present mutation (c.1317C>G, p.D439E) occurred on the T3 repeat domain, which may affect the structure and function of COMP, and our proband (I-3) was diagnosed with PSACH.

MED is also a skeletal dysplasia similar to PSACH, but with milder severity. MED patients present short statures (final adult height: 145-170 cm), hip osteoarthritis, mild genu varum, and mild irregularity of vertebral endplates [18]. It is difficult to distinguish PSACH and MED. Currently, most studies think that the biggest difference is that MED hardly leads to spine dysplasia [21, 22]. Most mutations of MED that have been reported involve COMP mutations. In addition to COMP, some forms of MED can also be caused by mutations in MATN3, COL9A1, COL9A2, COL9A3, COL2A1, FGFR1, SLC26A2 and DTDS [23–25]. In our study, the proband was highly suspected of having PSACH based on the following points: (1) Only the COMP mutation was found as a possible disease-causing gene by WES and cosegregated with the affected family members. (2) The major phenotypes of the proband include short stature, gait wadding, and early-onset osteoarthrosis, which are consistent with PSACH. Although vertebral anomalies usually resolved with age, mild scoliosis was also found in the patient.

(3) Mutations in the T3 repeats domain of COMP were more likely to cause a severe phenotype in PSACH patients. In our case, the mutation (c.1317C>G, p.D439E) located at the 6th repeat of T3 repeats (T3_6), and according to the hypothesis of Briggs et al., missense mutations in T3_6 had a greater frequency of PSACH than MED[20].

To date, there are at least 191 mutations in COMP that have been reported (http://www.hgmd.cf.ac.uk/ac/search.php). Among these mutations, the vast majority was connected with PSACH and was mostly located in the T3 repeats domain (Figure 3). Thus, the domain is a mutation hot spot, where the COMP mutation we identified was located. In addition to a large number of missense mutations, it also includes in-frame small deletions, insertions, or indels [12, 26, 27]. Three COMP mutations (c.1315G>A, p.D439N; c.1315G>A, p.D439N; c.1317C>G, p.D439E) were identified in MED cases, which altered the 439th amino acid (AA) of COMP[20, 28, 29]. These mutations indicated the pathogenicity of AA alterations, and the present mutation

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**Table 2: Variants identified by WES in combination with skeletal dysplasia-related gene-filtering in the proband.**

| Gene     | Mutation          | Mutation taster | PolyPhen-2 | SIFT | 1000G gnomAD | OMIM clinical phenotype                                                                 | ACMG classification |
|----------|-------------------|-----------------|------------|------|--------------|----------------------------------------------------------------------------------------|---------------------|
| GNPTAB   | c.673C>T, p.Q225K | D               | B          | T    | —            | AR, mucolipidosis 2 alpha/beta; AR, mucolipidosis 3 alpha/beta. AD, brachyolmia type 3; AD, digital arthropathy-brachydactyly, familial; AD, hereditary motor and sensory neuropathy, type 2c; AD, scapuloperoneal spinal muscular atrophy; AD, SED, Maroteaux type. | BS (PM2, BS4, BP4, BP5) |
| TRPV4    | c.2569C>T, p.Q857K| P               | B          | T    | —            | —                                                                                      | BP (BP4, BP5)       |
| TGFB1    | c.77C>T, p.P26L   | D               | B          | T    | —            | —                                                                                      | BP (PM2, BP4, BP5)  |
| FLNB     | c.2671G>T, p.D991N| P               | B          | T    | —            | —                                                                                      | BP (BP4, BP5)       |
| COL9A1   | c.674A>T, p.D225V | D               | B          | D    | 0.00040      | 0.00025 AD, epiphyseal dysplasia, multiple. 6. AD, epiphyseal dysplasia, multiple. 6. | BP (BP4, BP5)       |
| RECQL4   | c.1396C>T, p.P466T| D               | B          | T    | —            | AR, Baller-Gerold syndrome; AR, Rothmund-Thomson syndrome, type 2. AR, Baller-Gerold syndrome; AR, Rothmund-Thomson syndrome, type 2. | BS (PM2, BS4, BP4, BP5) |
| COMP     | c.1317C>G, p.D439E| D               | D          | D    | —            | AD, epiphyseal dysplasia, multiple. 6. AD, epiphyseal dysplasia, multiple. 6. AD, epiphyseal dysplasia, multiple. 1; AD, pseudoachondroplasias. | PS (PS1, PM1, PM2, PP1, PP3, PP4) |
| EVC      | c.769delinsTTAC, p.Y258H | P             | B          | T    | —            | AD, Weyers acrofacial dysostosis; AR, Ellis-van Creveld syndrome. AD, Weyers acrofacial dysostosis; AR, Ellis-van Creveld syndrome. | BP (PM2, BP4, BP5)  |

Italicized words: mutations identified in this study; D: disease causing; B: benign; T: tolerated; P: polymorphism; AR: autosomal recessive; AD: autosomal dominant. Pathogenic: PVS1> PS1>...> PS4> PM1-6> PP1-5; benign: BA1> BS1-4> BP1-7. PVS: pathogenic very strong; PS: pathogenic strong; PM: pathogenic moderate; PP: pathogenic supporting; BA: benign stand-alone; BS, benign strong; BP, benign supporting.
Figure 2: (a) The sequencing result of the COMP mutation. Sequence chromatograms indicate the heterozygous variant (c.1317C>G, p.D439E) in all affected members of this family. (b) The mutated site (D439E) is highly evolutionary conserved across species. The red graph represents mutated amino acids, and the black box emphasizes these sites across species for comparison. (c) The protein complex of COMP with or without mutants. Green balls represent Ca\textsuperscript{2+}. 

| Species         | p.D439         | p.D439         |
|-----------------|----------------|----------------|
| Human           | Q D G          | D G H Q D S R D N C P T |
| Mutated         | Q D G          | E G H O D S R D N C P T |
| Mmulatta        | Q D G          | D G H Q D S R D N C P T |
| Mmusculus       | Q D G          | D G H Q D S R D N C P T |
| Ggallus         | S D G          | D G H Q D T R D N C P S |
| Trubripes       | S D G          | D G H Q D S R D N C P A |
| Drerio          | S D G          | D G H Q D S R D N C P A |
| Dmelanogaster   | G D D          | D G V P N S L D N C P M |
| X tropicalis    | R D G          | D G H Q D T S D N C P S |
(c.1317C>G, p.D439E) also occurred at this AA site. By analyzing these three mutant protein models, we considered that D439N and D439G alter the electric charge of a Ca\(^{2+}\)-binding site and that D439E breaks the spatial configuration of this binding region (Figure 2(c)). Unlike MED cases with these three known mutations, our proband had PSACH. This may be caused by ethnic or individual differences. In fact, many researchers thought that these two malformations should be classified as the same disease with different severities. Interestingly, it seems that these mutations are more likely to disrupt the folding and tertiary structure of COMP[20]. Studies in COMP knocked out mice had suggested that it is not a reduction in COMP but a dysfunctional mutated COMP that results in PSACH[22, 30].

Actually, the diagnosis of PSACH mostly relies on patients’ characteristics, family history, clinical symptoms, and radiological features [31]. There is currently no appropriate therapy for this genetic disease, and only symptomatic treatments are available [32]. Moreover, the boundary between PSACH and MED is indefinite. Recently, with the application of next-generation sequencing techniques, such as WES, the discovery of the genetic pathogenetic factor of individuals in heterogeneous conditions has facilitated the diagnosis of skeletal disorder disease, and it is also a powerful and cost-efficient method to verify the causative gene [13, 26]. In the future, further efforts will be made to provide assistance in genetic counseling and disease prognosis.
5. Conclusions
In conclusion, we detected a novel nucleotide variant (NM_000095.2: c.1317C>G, p.D439E) in COMP in a proband diagnosed with mild PSACH. Our study expands the spectrum of COMP mutations. Although we still know little about the exact mechanism and progression of the disease, this data may contribute to genetic diagnosis and counseling of families with PSACH.

Data Availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest
The authors declare no conflict of interest.

Authors’ Contributions
Lei Zeng collected and provided clinical information. Bing-Bing Guo and Zhuang-Zhuang Yuan carried out whole-exome sequencing and data analysis. Jie-Yuan Jin and Rong Xiang designed experiments and wrote the manuscript. Bing-Bing Guo and Jie-Yuan Jin contributed equally to this work.

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Supplementary Materials
For supplementary material file “Table S1”: we collected 224 skeletal dysplasia-related genes as candidate genes of the proband’s disease, and we made a list as a supplementary table. (Supplementary Materials)

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