Identification of two novel COL10A1 heterozygous mutations in two Chinese pedigrees with Schmid-type metaphyseal chondrodysplasia

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Abstract

Background: Schmid-type metaphyseal chondrodysplasia (MCDS) is an autosomal dominant disorder caused by COL10A1 mutations, which is characterized by short stature, waddling gait, coxa vara and bowing of the long bones. However, descriptions of the expressivity of MCDS are rare. Methods: Two probands and available family members affected with MCDS were subjected to clinical and radiological examination. Genomic DNA of all affected individuals was subjected to whole-exome sequencing, and candidate mutations were verified by Sanger sequencing in all available family members and in 250 normal control donors. A spatial model of the type X collagen (α1) C-terminal noncollagenous (NC1) domain was further constructed. Results: We found that the phenotype of affected family members exhibited irregular dominance. Mutation analysis indicated that there were two novel heterozygous missense mutations, c.1765T>A (p.Phe589Ile) and c.1846A>G (p.Lys616Glu) in the COL10A1 gene in family 1 and 2, respectively. The two novel substitution sites were highly conserved and the mutations were predicted to be deleterious by in silico analysis. Furthermore, protein modeling revealed that the two substitutions were located in the NC1 domain of collagen X (α1), which potentially impacted the trimerization of collagen X (α1) and combination with molecules in the pericellular matrix. Conclusion: Two novel mutations were identified in the present study, which will facilitate diagnosis of MCDS and further expand the spectrum of the COL10A1 mutations associated with MCDS patients. In addition, our research revealed the phenomenon of irregular dominance in MCDS.

Background

Schmid-type metaphyseal chondrodysplasia (MCDS; MIM 156500), the most common type of metaphyseal chondrodysplasia, is an autosomal dominant congenital disorder that is
characterized by short stature, waddling gait, coxa vara and bowing of the long bones (primarily involving the femur) [1, 2]. The distinctive clinical features of MCDS also include leg pain, enlarged capital femoral epiphyses or partial metaphyseal abnormalities of the upper limbs [3]. The typical radiographic findings of MCDS are widening and irregularity of the growth plates, especially in the distal femur and proximal tibia [4, 5, 6], but only a small percentage of patients have involvement of the upper limbs [7]. In addition, it should be noted that all patients affected with MCDS exhibit normal extraskeletal manifestations [8].

COL10A1 (MIM 120110), located on chromosome 6q21-q22.3 [9], is closely associated with MCDS when pathogenic variants occur in this gene [10, 11, 12]. The product of the COL10A1 gene is the \( \alpha_1 \) chain of type X collagen, which composes the type X collagen by forming a homotrimer [5]. Type X collagen is a member of the collagen superfamily of structural macromolecules, which has a unique expression pattern localized to the hypertrophic chondrocytes of growth plate cartilage [13]. The function of type X collagen, as a short-chain minor collagen of cartilage, is to play an important role in fetal chondrogenesis and endochondral ossification [5, 14]. Therefore, specific mutations in COL10A1 are likely to result in occurrence of MCDS.

To date, over fifty kinds of mutations in COL10A1 have been reported in the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/), and 50 variants of those to our knowledge are directly associated with MCDS (Summarized in supplementary data) [15, 16]. Based on different mutation types, the mutations in COL10A1 can be divided into two categories: single amino acid residue lack or substitution resulting from missense mutations or small deletions (Class I) and truncated peptide attributed to nonsense mutations or frameshift mutations (Class II) [17, 18]. Of note, the underlying pathogenic mechanisms of the two types are completely different.
The present study describes the phenomenon of irregular dominance and summarizes several potential genetic patterns in two unrelated Chinese pedigrees with MCDS. Moreover, mutation screening used to support diagnosis was performed in the two unrelated Chinese MCDS families.

**Methods**

**Patient families**

Two independent non-consanguineous five-generation families (Fig. 1) containing nineteen MCDS patients, identified by two independent orthopedic surgeons, were recruited from the outpatient department of Shanghai Jiao Tong University Affiliated Sixth People’s Hospital (Shanghai, China) when they sought treatment for the probands. All the available patients underwent comprehensive clinical and radiological measurements for diagnosis (All individuals who participated in this study are labeled in Fig. 1). Written informed consent was obtained from all of the participants. The present study was approved by the Ethics Committees of Shanghai Jiao Tong University Affiliated Sixth People’s Hospital (Shanghai, China).

**Whole-exome sequencing and mutation confirmation**

Peripheral blood samples from all of the available family members and 250 unrelated healthy donors aged 18 to 45 years (125 males and 125 females) were collected and stored at -80°C. Genomic DNA was extracted from samples using the QuickGene DNA whole blood kit (Kurabo Industries Ltd., Osaka, Japan). A BioAnalyzer 2100 was subsequently employed to assess nucleic acid quantity and quality. We sequenced the whole-exome of all available affected individuals and individual II:1 in family 1 in order to determine the mutated gene resulting in this disorder. The SureSelect Human All Exon 57Mb Kit (Agilent Technologies, Inc., Santa Clara, CA, USA) and the HiSeq 2000 platform (Illumina, Inc., San Diego, CA, USA) were used to capture the whole-exome sequence.
following the manufacturer’s protocol. Illumina base-calling software v1.7 was then used to convert the raw image files into 90-base-paired-end reads and all of the variants were validated.

In the first step, all detected variants were filtered against six single-nucleotide polymorphism databases: the dbSNP144 (hgdownload.cse.ucsc.edu/goldenPath/hg19/database/snp144), the HapMap Project (ftp.ncbi.nlm.nih.gov/hapmap), the 1000 Genomes Project (www.internationalgenome.org), the YanHuang database (yh.genomics.org.cn), the Exome Variant Server (evs.gs.washington.edu/EVS/) and the Exome Aggregation Consortium database (exac.broadinstitute.org), which rejected common single nucleotide polymorphism (SNP) sites (MAF>1%). In the second step, common single nucleotide variants (SNV) among all family members in each family were of interest but synonymous or intronic variants not located within splice sites were excluded. In the third step, we further checked the conservation of remaining mutations using the UCSC database (https://genome.ucsc.edu/) and the causality of the altered amino acid residues by utilizing Polymorphism Phenotyping version 2 (Polyphen-2; genetics.bwh.harvard.edu/pph2/) and Protein Variation Effect Analyzer (PROVEAN; provean.jcvi.org/index.php/). Finally, the most likely pathogenic candidate mutation was presented in accordance with gene functions and previous studies (Details in supplementary data).

The identified mutation regions and flanking sequence of the COL10A1 gene were amplified using a standard polymerase chain reaction protocol to facilitate Sanger sequencing among all available family members and donors. The primers were designed using Primer-3 software (bioinfo.ut.ee/primer.3-0.4.0/). Direct sequencing was performed using the BigDye Terminator Cycle Sequencing Ready Reaction Kit, version 3.1 (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA), and the sequence was
analyzed with an ABI Prism 3130 automated sequencer. Mutation was checked using the Polyphred program (droog.mbt.washington.edu/poly_get.html).

**Protein spatial model construction**

The wild-type ribbon structure of the type X collagen (α1) C-terminal noncollagenous (NC1) domain was initially constructed using DeepView and SWISS-MODEL (swissmodel.expasy.org). In addition, to illustrate the positions of the pathogenic mutation and flanking regions, the amino acid residue substitutions were further incorporated into the model.

**Results**

**Clinical features**

Clinical findings in two MCDS pedigrees are primarily summarized in Table 1. The typical manifestations, including short stature, coxa vara, bowing of the femur and widening and irregularity of the growth plates of the distal femur and proximal tibia observed in X-ray images, were partly found in all patients sooner or later after birth. However, the severity of symptoms varied between individuals. The morbidity rates of males and females in the two families were 54% and 31%, respectively ($P = 0.21$, Fisher’s exact test).

Family 1:

Apart from those typical clinical features, proband 1 (V:2), a 2-year-old boy, and his grandfather (III:1) also presented with waddling gait and their affected growth plates appeared “cup”-shaped in X-ray images (Fig. 2A-C). Interestingly, the mother (IV:4) of proband 1 only exhibited relatively short stature without any of the other aforementioned clinical and radiological manifestations. Furthermore, the development and appearance of II:1 were absolutely normal without any mental or physical deformity, as were all other unaffected family members.

Family 2:
Proband 2, an 18 month-old boy, had exhibited typical clinical manifestations and slow growth since 6 months after birth (Fig. 2D E). However, the clinical features of his mother (IV:4) remained mild, similar to IV:4 in family 1 (Fig. 2D F). Notably, most of the affected members in family 2 were males and male patients were significantly more severely affected than females. In contrast to family 1, there was no phenomenon of atavism in family 2.

**Genetic analysis**

Whole-exome sequencing was followed by systematic screening and bioinformatic analysis. After rejecting common SNP sites (MAF>1%), SNV were captured. Considering the function of the mutant genes and the results of previous studies, the present study focused on the novel heterozygous missense mutation c.1765T>A (p.Phe589Ile) (NM_000493.3) in exon 3 of the COL10A1 gene in family 1 and another novel mutation c.1846A>G (p.Lys616Glu) (NM_000493.3) in the identical region of the COL10A1 gene in family 2. The two novel mutations were not found in the ExAC and gnomAD databases, so the results further supported the conclusion that the two mutations were novel and rare. Of note, the changed amino acid residues were highly conserved among several species (Fig. 3E). The Polyphen-2 and PROVEAN scores of the variant c.1765T>A (p.Phe589Ile) were 1.000 and -2.69, respectively, which indicated a deleterious function; and the corresponding scores of the other variant c.1846A>G (p.Lys616Glu) were 1.000 and -1.633.

To confirm the identification of COL10A1 mutations revealed by whole-exome sequencing, we performed Sanger sequencing of all of the available family members and the 250 healthy donors. In accordance with the results of whole-exome sequencing, the mutations c.1765T>A (p.Phe589Ile) and c.1846A>G (p.Lys616Glu) were identified in the eight affected individuals and individual II:1 in family 1 and in nine affected individuals in family
2, but not in other available unaffected family members or the 250 healthy donors (Fig. 3A-D).

**Protein structural model**

According to the spatial ribbon structure of the protein, both mutations p.Phe589Ile and p.Lys616Glu were located in the NC1 domain of collagen X (α1) (Fig. 4A B), where three identical regions interact to form a collagen X homotrimer. Moreover, the replaced wild type residues are conserved among various collagens. It is of note that the two substitutions are located in the hydrophobic area and the surface of the assembled homotrimer, respectively. The mutation p.Phe589Ile weakens the hydrophobicity of the wild type residue, and the other substitution p.Lys616Glu changes the residue site from strongly alkaline to acidic, both of which potentially destroy the interaction between collagen X (α1) peptide chains or between collagen X (α1) peptide chains and other molecules.

**Discussion**

There is no doubt that MCDS is an autosomal dominant inherited disorder, resulting from heterozygous mutation of the COL10A1 gene [4, 10, 17]. In the present study, probands with MCDS and affected family members were identified in two large independent Chinese pedigrees by means of typical clinical findings and genetic analysis. Interestingly, although these individuals suffered from identical disease, there were still differences in the severity of clinical manifestations among all the patients in each family. Moreover, atavism, the reappearance of an ancestral character in a descendant individual whose immediate ancestors lacked this character, was present in family 1. The presentation of II:1 in family 1 was absolutely normal without any deformity except moderate stature, but no radiological examination was performed for him due to individual’s reluctance, so we failed to acquire further evidence of clinical diagnosis. Of note, the mutation c.1765T>A in
the COL10A1 gene was confirmed in the genome of II:1 in family 1, which was similar to a person who carried a COL10A1 variant, but exhibited normal phenotype in a previous report [19]. We attributed the phenomenon of atavism and differential performance to irregular dominance based on the presence of an identical pathogenic mutation in each family, possibly caused by the existence of a modifier gene and environmental differences [20]. Recently, Forouhan et al. proposed that ATF6α and ATF6β play important roles in modulating disease severity in MCDS mice by positively or negatively regulating the endoplasmic reticulum stress response [21], which we considered to be the associated mechanism of the irregular dominance phenomenon. However, further molecular experiments are needed.

Depending on the differential expressivity of all affected members in two Chinese families (Table 1), we have summarized the following possible rules on the pedigrees affected with MCDS [6, 16]. First, based on the onset age, these patients were characterized by delayed dominance, which only occurred months or even a year after birth, at 6 to 18 months old in this study, rather than at birth. Furthermore, the severity of disease was closely associated with onset age presenting a negative correlation, that is, the earlier the onset age, the more severe the condition. For example, decreased quality of life, including unstable standing or waddling gait was observed in patients whose onset ages were only around 6 months old. Conversely, only short stature without other deformities was exhibited in those whose onset ages were 10 months old or later. In addition, we found that there were possible, but not significant, potential differences in gender susceptibility in MCDS. Moreover, despite the trend that male patients were more severely affected than females, as observed in family 2, we still could not draw a firm conclusion due to the rather small sample size.

The molecular structure of type X collagen is a homotrimer of three X (α1) chains, each
comprised of a 463 amino acid Gly-X-Y collagenous domain (COL1) flanked by a 38-residue N-terminal noncollagenous domain (NC2) and a 161-residue C-terminal noncollagenous domain (NC1) (Fig. 4C) [9]. In addition, there is an 18-residue signal peptide ahead of the NC2 domain. To date, a total of 50 mutations of the COL10A1 gene resulting in MCDS have been reported (Supplementary data). All of the identified mutation sites of COL10A1 associated with MCDS, including mutations in the present study, are located in the NC1 domain [4, 8, 12, 22, 23, 24, 25, 26, 27, 28], except for two missense mutations in the signal peptide and one in the triple helical domain. As for genotype-phenotype correlations, the three variants that are not located in the NC1 domain are associated with late-onset ages and mild manifestations of MCDS, but most of those located in the NC1 domain lead to the severe forms of this disease.

In the present study, two novel variants resulted in severe clinical features in parts of affected individuals, which was consistent with previous study [4, 12]. However, the current study is the first to report the phenomenon of irregular dominance in MCDS patients. The function of the NC1 domain is mainly to assist the folding of the peptide chain so that it can combine to form a homotrimer [5]. Therefore the NC1 domain becomes impaired, the collagen X (α1) chains are prevented from aggregating and instead form non-functional polymers, which tend to promote harmful accumulation of invalid products and even initiate the endoplasmic reticulum stress response [5, 29]. Meanwhile, the quantity of correctly-folded collagen X is reduced, and therefore functional haploinsufficiency was the most likely cause of the MCDS [14, 30, 31].

According to previous experiments, COL10A1 nonsense mutations in cartilage tissue lead to removal of the mutant mRNA by nonsense-mediated mRNA decay (NMD), which is the pathogenic molecular mechanism of nonsense mutations in MCDS [18, 32]. Nevertheless, the process via which COL10A1 missense mutations result in MCDS is completely different
In the case of COL10A1 missense mutations, a common consequence appears to be disruption of collagen X trimerization, homeostasis, secretion and combination, with consequent intracellular or pericellular degradation [6, 17]. In the present study, one of the substitutions (p.Phe589Ile) affects a hydrophobic area and the other (p.Lys616Glu) is predicted to affect the surface of the assembled trimer (Fig. 4A B). The substitution p.Phe589Ile weakens the hydrophobicity of the wild type residue, which is likely to seriously impact the assembly and stability of the hydrophobic channel and thus hinder collagen X trimerization. The other substitution (p.Lys616Glu) changes the residue site from alkaline to acidic, potentially destroying the combination of trimeric collagen X into supramolecular structures within the cartilage pericellular matrix. Together, these biochemical and pathophysiological processes may explain the underlying mechanisms of MCDS in the two present pedigrees.

To our knowledge, the present study is the first report of COL10A1 missense mutations in Chinese pedigrees with MCDS. In addition, the dominant negative effect may be playing an important role in the development of MCDS [17, 33], but this undetermined mechanism will need further experimental verification.

Conclusions

In summary, we identified two novel COL10A1 heterozygous missense mutations [c.1765T>A (p.Phe589Ile)] and [c.1846A>G (p.Lys616Glu)] in two relatively large unrelated Chinese pedigrees with MCDS. The genetic analysis facilitated diagnosis of the disease and further expanded the spectrum of COL10A1 mutations associated with MCDS patients. In addition, our research revealed the phenomenon of irregular dominance and summarized several potential genetic patterns in the two Chinese pedigrees with MCDS.

Abbreviations
MCDS: schmid-type metaphyseal chondrodysplasia; COL10A1: type X collagen (α1); COL1: collagenous domain; NC1: C-terminal noncollagenous domain; NC2: N-terminal noncollagenous domain; MAF: minor allele frequencies; NMD: nonsense-mediated mRNA decay

Declarations

Acknowledgement

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Authors’ contributions

L.C.K primarily analyzed the outcomes of whole-exome sequencing and Sanger sequencing, and was a major contributor in writing the manuscript. L.S collected the clinical information and peripheral blood samples of all of the participants, and contributed to the analysis of sequencing outcomes. W.B.W, R.T.Z and M.W.W contributed to the conducting of genetic analysis for the participants. Q.L.K conceived and designed the study and experimental methods. All authors read and approved the final manuscript.

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Availability of data and materials

Most of data generated or analyzed during this study are included in the article and supplementary files. The extra data used and analyzed in the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
Written informed consent was obtained from all of the adult participants and the guardians of participants under 16 years old. The present study was approved by the Ethics Committees of Shanghai Jiao Tong University Affiliated Sixth People’s Hospital (Shanghai, China).

Consent for publication
Written informed consent was obtained from all of the participants approving this non-commercial research and the publication of any associated data/images.

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

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Table 1

Clinical and genetic features of affected individuals.
| Pedigree | Patient | Gender | Age (years old) | Onset age (months old) | Height (cm) | Severity of disease* | Pathogenic mutation |
|----------|---------|--------|----------------|------------------------|------------|----------------------|---------------------|
| Family 1 | I₂      | Female | Death          | 12-18                  | 152        | Moderate             | Not available       |
|          | II₁     | Male   | 82             | /                      | 170        | Unaffected           | c.1765T>A           |
|          | III₁    | Male   | 57             | 6-8                    | 153        | Severe               | c.1765T>A           |
|          | III₉    | Male   | 52             | ~12                    | 160        | Moderate             | c.1765T>A           |
|          | III₁₁   | Male   | 50             | 10-12                  | 157        | Moderate             | c.1765T>A           |
|          | IV₁     | Male   | 33             | 10-12                  | 165        | Mild                 | c.1765T>A           |
|          | IV₄     | Female | 28             | ~12                    | 149        | Mild                 | c.1765T>A           |
|          | IV₁₃    | Male   | 29             | 6-8                    | 168        | Moderate             | c.1765T>A           |
|          | V₂ #    | Male   | 2              | ~6                     | 76         | Severe               | c.1765T>A           |
| Family 2 | V₄      | Male   | 4              | 6-8                    | 95         | Moderate             | c.1765T>A           |
|          | I₁      | Male   | Death          | Not available          | 165        | Moderate             | Not available       |
|          | II₂     | Female | 77             | ~12                    | 152        | Mild                 | c.1846A>G           |
|          | II₃     | Male   | 72             | 10-12                  | 158        | Moderate             | c.1846A>G           |
|          | III₁    | Male   | 51             | ~12                    | 160        | Moderate             | c.1846A>G           |
|          | III₃    | Male   | 48             | 10-12                  | 156        | Moderate             | c.1846A>G           |
|          | III₉    | Male   | 49             | 8-10                   | 155        | Moderate             | c.1846A>G           |
|          | IV₁     | Male   | 24             | 6-8                    | 158        | Severe               | c.1846A>G           |
|          | IV₄     | Female | 27             | ~12                    | 153        | Mild                 | c.1846A>G           |
|          | IV₉     | Female | 25             | 10-12                  | 150        | Mild                 | c.1846A>G           |
|          | V₁ #    | Male   | 1.5            | 6-8                    | 72         | Severe               | c.1846A>G           |

* “Mild” indicates that the patients only exhibit short stature without any other evident
abnormal manifestations. “Moderate” represents that patients have additional phenotypes
such as coxa vara and bowing of the femur. “Severe” patients show decreased quality of
life including unstable standing or waddling gait. # Proband of each pedigree.

Figures
The pedigrees of the MCDS patients. The phenomenon of atavism was presented in family 1 (A), but not in family 2 (B). Genetic analysis was performed among all patients and available unaffected family members. All individuals who participated in this study were labeled with "*".
Figure 2

The clinical and radiological features of patients in two affected families. The legs of proband in family 1 exhibited evident “O” shape (A) and bowing of the femurs and widening and irregularity of the growth plates of distal femur (white arrows) are shown in X-ray image (B). Moreover, the X-ray image of III:1 in family 1 also presented bowing of the femurs, deformity of growth plates and hips (white arrows) (C). In family 2, the proband showed deformity of the lower extremities (D) and similar radiological presentation (white arrows) (E) to proband in family
1. However, the proband’s mother in family 2 exhibited normal appearance in X-ray image (F).
Figure 3

Genetic analysis of patients and unaffected family members. (A) The novel heterozygous mutation site c.1765T>A (black arrows) of patients and II:1 in family 1 and (B) corresponding sequence in the other unaffected individuals. (C) The other mutation site c.1846A>G (black arrows) was identified in family 2 patients and (D) wild type site in unaffected family members. (E) Both of the substitution loci p.Phe589 and p.Lys616 were conserved in different species according to the UCSC database.
Protein modeling of type X collagen (α1) NC1 domain and stylized structure of collagen X. (A) (B) As illustrated in the ribbon protein model, both of the novel mutations are located in the NC1 domain of type X collagen (α1). One of the substitutions (p.Phe589Ile) affects a hydrophobic area and the other (p.Lys616Glu) is predicted to affect the surface of the assembled trimer. (C) The stylized structure of type X collagen (α1) is composed of a 18 amino acid signal peptide (S) and a 463 amino acid collagenous domain (HELIX) flanked by a 38-residue NC2 domain and a 161-residue NC1 domain. Furthermore, changes in the present study and most previous variants (supplementary data) are located in NC1 domain.

Supplementary Files
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Supplementary Materials. 2 WES QC statistics.xls
Supplementary Materials. 3 Workflow figure.jpg
Revised Supplementary Materials. 1 Mutations in COL10A1.pdf