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Characterization of a novel *COL10A1* variant associated with Schmid-type metaphyseal chondrodysplasia and a literature review

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Abstract

Background: Schmid-type metaphyseal chondrodysplasia (SMCD) is a rare autosomal dominant skeletal dysplasia caused by heterozygous mutations in *COL10A1*, the gene which encodes collagen type X alpha 1 chain. However, its genotype–phenotype relationship has not been fully determined.

Subjects and Methods

The proband is a 2-year-old boy, born of non-consanguineous Chinese parents. We conducted a systematic analysis of the clinical and radiological characteristics and a follow-up study of the proband. Whole-exome sequencing was applied for the genetic analysis, together with bioinformatic analysis of predicted consequences of the identified variant. A homotrimer model was built to visualize the affected region and predict possible outcomes of this variant. Furthermore, a literature review and genotype–phenotype analysis were performed by online searching all cases with SMCD.

Results: A novel heterozygous variant (NM_000493.4: c.1863_1866delAATG, NP_000484.2: p.(Met622 Thrfs*54)) was identified in *COL10A1* gene in the affected child. And it was predicted to be pathogenic by *in silico* analysis. Protein modeling revealed that the variant was located in the NC1 domain, which was predicted to produce truncated collagen and impair the trimerization of collagen type X alpha 1 chain and combination with molecules in the matrix. Moreover, genotype–phenotype

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correlation analysis demonstrated that patients with truncating variants or variants in NC1 domain often presented earlier onset and severer symptoms compared with those with non-truncating or variants in non-NC1 domains.
Conclusion: The NC1 domain of *COL10A1* was proved to be the hotspot region underlying SMCD, patients with variants in NC1 domain were more likely to present severer manifestations at an earlier age.

KEYWORDS
COL10A1, schmid-type metaphyseal chondrodysplasia, short stature, skeletal dysplasia, variant

1 | INTRODUCTION

Short stature (SS) in childhood is one of the most common chief complaints about a referral to pediatric endocrinologists, which is defined as actual height below 2 SDS of the average height for age, sex, and ethnic group established norm (Allen & Cuttler, 2013; Rogol & Hayden, 2014). There are a number of etiologies attributing to short stature which can be either congenital or acquired, and both can cause physical deformities and place a huge burden on patients and their families. Early diagnosis is quite necessary for these patients to accept early and effective intervention. Nevertheless, many disorders associated with short stature present similar phenotypes, making it more difficult to determine the cause.

Schmid-type metaphyseal chondrodysplasia (SMCD, OMIM 156500) is a rare autosomal dominant disease that is characterized by short stature, long bone deformities such as genu varum and genu valgum, and typical radiographic manifestations of metaphyseal dysplasia of the tubular bones especially the femur (e.g., splaying, flaring, widening, cupping; Richmond & Savarirayan, 2019). This skeletal dysplasia is attributed to the mutation of the *COL10A1* gene (OMIM 120110) which encodes collagen type X alpha 1 chain and is featured by the assembly defect of type X collagen in the growth plate (Warman et al., 1993; Wilson et al., 2002). Human type X collagen is a homotrimer of three collagen type X alpha 1 chains, each crude chain composed of a signal peptide (amino acids 1–18) at the N-terminal followed by a non-collagenous 2 (NC2) region (amino acids 19–56), a triple helix-forming Gly-X-Y repeats region (amino acids 57–519) and a non-collagenous 1 (NC1) region (amino acids 520–680) at the C-terminal responsible for initiating trimerization of type X collagen and forming specific supramolecular structures within the cartilage matrix, which plays an important role in fetal chondrogenesis and endochondral ossification (Bogin et al., 2002). Till now, only 59 heterozygous *COL10A1* variants have been reported to cause SMCD. Most SMCD-related variants identified recently cluster in the C-terminal NC1 trimerization domain (Bateman et al., 2005). Since the genotype–phenotype correlation in SMCD is still not clear, it will be helpful to better understand the genotype–phenotype relationship if more cases are found and studied.

Interestingly, here we found a SMCD patient with a new heterozygous frameshift variant of the *COL10A1* gene which was predicted to be pathogenic possibly through NMD. Moreover, we summarized all previously reported variants as well as their phenotypic information, and further analyzed the genotype–phenotype correlation. Our results enrich the gene mutation spectrum to help identify the *COL10A1* gene function and reveal the pathogenesis underlying SMCD.

2 | PATIENTS AND METHODS

2.1 | Ethical compliance

This study has been approved by the Ethics Committee of Shandong Provincial Hospital affiliated to Shandong University. The study protocol was in line with the Declaration of Helsinki (as revised in Brazil 2013). Informed consent was obtained from all individual participants included in the study and written informed consent was received from participants prior to inclusion in the study.

2.2 | Patient

A detailed history about the onset and progression of growth failure and lower limb deformities as well as family history were obtained. Physical examination, laboratory detection, and X-rays were performed to confirm the diagnosis. Peripheral blood samples were obtained from the patient and his family members for genetic testing.

2.3 | DNA extraction and whole-exome sequencing

Genomic DNA was isolated from peripheral blood leukocytes using the QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's instructions. Whole-exome sequencing (WES) was performed on DNA from peripheral blood. After genomic DNA fragmentation, paired-end adaptor ligation, amplification, and purification, the human exons were captured by using the SeqCap EZ Med Exome Enrichment Kit (Roche NimbleGen). The DNA library was generated by postcapture amplification and purification and then sequenced on the Illumina HiSeq sequencing platform. Sequence data alignment to the human genome reference (hg19) and variant-calling were performed with NextGene V2.3.4 software to obtain the coverage and mean read depth of the target regions. The average coverage of the exome was >100×, which permitted the examination of the target region with enough depth to exactly match >99% of the target exome. To ensure the accuracy of data analysis, mutations with low coverage in the target area will be filtered out.

Additionally, annotation information, including the conservation of nucleotide bases and amino acids, predictions of biological functions, the frequency in normal populations (Genome Aggregation Database [GenomAD], Trans-Omics for Precision Medicine [TOPMED], the Exome Aggregation Consortium [ExAC]), and data from the Human Gene Mutation Database (HGMD), Clinvar and Online Mendelian Inheritance in Man (OMIM) databases, was performed by NextGene V2.3.4 and our in-house scripts. A variant was recognized as a mutant when it was not found in dbSNP (http://www.ncbi.nlm.nih.gov/snp/), in the exome variant server (http://evs.gs.washington.edu/EVS/), in the Ensembl database and in 500 Chinese controls, or alternatively, the allele frequency was found to be less than 0.001 in the database. Pathogenic variants were determined according to the Standards and Guidelines for the Interpretation of Sequence Variants published by the American College of Medical Genetics and Genomics (ACMG) in 2015 with the Human Genome Variation Society (HGVS) nomenclature (den Dunnen et al., 2016; Richards et al., 2015).

When the detected pathogenic or suspected pathogenic variants exists, the laboratory verified it by Sanger sequencing and ensured that the coverage of the gene coding sequence reached 100%. Using Primer3 version 1.1.4 (http://www.sourceforge.net) and GeneDistiller 2014 (http://www.genedistiller.org/), tagged sequencing primers of *COL10A1* were designed. Polymerase chain reaction (PCR) was performed in a 50 μL system including 4 μL genomic DNA, 1 μl forward and reverse primers, 5 μL 10 × PCR buffer, 4 μL dNTPs, and 0.3 μL Taq Hot Start (Takara Bio). The PCR conditions were as follows: an initial denaturation step (95°C for 5 min), followed by 40 cycles of denaturation (95°C for 30 s), annealing (65°C for 30 s), and elongation (72°C for 30 s). Amplicons were sequenced using an ABI 3730 system (Applied Biosystems), and sequence analysis was performed using the autoassembler software Chromas 2.6.6 (Technelysium Pty Ltd. Available at www.technelysium.com.au/chromas.html.) and visual inspection.

2.4 | Bioinformatic analysis

The bioinformatic analysis of *COL10A1* (Gene ID:1300, NCBI Reference sequence: NG_008032.1) variant was performed by two software tools, MutationTaster (http://www.mutationtaster.org/), PROVEAN (http://provean.jcvi.org) to predict disease-causing effects of the variant. Furthermore, SWISS-MODEL software was used to build a model of the homotrimer of type X collagen trimerization domain and DeepView software was used for visualizing the spatial structure and altered residues of the protein model.

2.5 | Follow-up study

After his first clinical evaluation, the patient was followed-up every 3 months in our hospital to closely keep track of his growth and development, therapeutic regimens, and potential complications as well as biochemical tests and radiographs performed if necessary. If there was an emergency (such as acute joint pain), the patient would be taken to the local hospital for examination and treatment.

2.6 | Statistical analysis

Statistical analysis was performed by SPSS 19.0 software package (SPSS Inc.). The Kolmogorov–Smirnov test was used to determine the distribution of continuous variables. Continuous variables with normal distribution were given as mean±SD and compared by independent samples Student's *t*-test, while those with non-normal distribution were given as median (25th, 75th percentiles) and compared by Mann–Whitney U-test. $p < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Clinical features

The patient, a 2-year-old Chinese boy, was first admitted to our hospital for genu varum and waddling gait. After a 40-week gestation without any abnormality, he was born with a body length of 51 cm (+0.3 SD), and a body weight of 3.8 kg (+1.2 SD). When he could first walk at the age of 1-year-old, he was found genu varum and waddling gait. He was only 81 cm (−2.1 SD) tall and weighed 13 kg (+0.3 SD) when he was 2 years old. The serum biochemical and hormone assays at the first clinical examination showed normal serum calcium, phosphate, vitamin D, parathyroid hormone, and a slightly increased alkaline phosphatase with normal bone mineral density and bone age (Table 1). The measurements of different body segments were listed in Table 2. Radiographs showed mild dorsal scoliosis, genu varum, and metaphyseal widening and irregularity (Figure 1). There were no extra-skeletal manifestations. The cytogenetic analysis confirmed a 46,XY karyotype. His parents were not consanguineous and there was no special medical or family history. His older brother was both physically and mentally healthy.

3.2 | Follow-up

During the follow-up, the patient has taken long-acting recombinant growth hormone at a dose of 0.17 IU/kg/day once a week together with daily calcium and vitamin D supplements since July 2019. The height of the patient was increased obviously after treatment without side effects. At the last follow-up, his height was up to 86 cm at the age of 2 years and 6 months old (−1.9 SD) with a weight of 16.1 kg (+1.5 SD). Besides, the results of biochemical and hormonal test were normal and the radiograph showed similar results compared to that of the first examination mentioned before. There was no evidence for treatment with recombinant growth hormone systematically evaluated in children with SMCD yet. Therefore, growth hormone seems to be effective, at least in our present study, to treat short stature in SMCD children.

3.3 | Genetic analysis of *COL10A1* gene

The patient's clinical features, as well as biochemical and hormone profiles, were typical of SMCD. The patient was subject to whole-exome sequencing and the detected variant was further confirmed via Sanger sequencing in all available family members. We found the patient carried a novel heterozygous variant c.1863_1866delAATG, p.(Met622 Thrfs*54) while it was absent in his family members (Figure 2a,b), indicating that this variant was probably a *de novo* variant (PS2). And this variant has not been reported before in the HGMD, TOPMED, ExAC, and 1000Genome databases (PM2). The novel c.1863_1866delAATG, p.(Met622 Thrfs*54) frameshift variant was located in the NC1 domain (Figure 2c). It caused a shift in the normal open reading frame of mRNA codons and created a premature stop codon at amino acid 675, producing a slightly truncated protein, which was 6 amino acids shorter than the wild type (PVS1). Moreover, this frameshift variant was strongly predicted to be pathogenic and deleterious using two online bioinformatic software—MutationTaster and PROVEAN with a PROVEAN score of −93.9 far below the cutoff (cutoff = −2.5). This might cause NMD according to MutationTaster. All of the above suggested that the novel *COL10A1* variant of the patient was pathogenic (PVS1+PS2+PM2) according to the criteria for classifying pathogenic variants established by American College of Medical Genetics and Genomics (ACMG; Richards et al., 2015).

3.4 | Protein structural model

The model of type X collagen NC1 homotrimer with three identical chains was built by SWISSMODEL automatically based on the template and visualized by DeepView. The NC1 trimers were formed by very tight interaction of each subunit, creating a central solvent-filled channel lined predominantly by strand F, which becomes more hydrophilic at the apex of the trimer where a cluster of four Ca^{2+} is presently contributing to the stability of type X collagen NC1 trimer, and an interaction surface composed of three strips of exposed aromatic residues, which are likely to be involved in the higher-order assembly of type X collagen in the extracellular matrix. The NC1 region consists of a 10-stranded β-sandwich with a jellyroll topology similar to the globular domain (gC1q) of the complement protein C1q. Ten β-strands are respectively labeled A, A', B, B',C, D, E, F, G, and H, among of which strands A, H, C, and F are mostly buried inside, whereas strands A', B, B', G, D, and E form the solvent-accessible surface of the NC1

TABLE 1 Laboratory parameters of the patient at the first clinical evaluation

Parameters	Results	Reference range
Serum calcium (mmol/L)	2.44	2.2–2.7
Serum phosphate (mmol/L)	1.49	0.85–1.51
Vitamin D (ng/mL)	51.04	15–100
Parathyroid hormone (pg/mL)	16.7	15–65
Alkaline phosphatase (U/L)	333	45–125
Creatinine (μmol/L)	25	40–135

TABLE 2 Body segments measurements in standard deviation (±SD)

Body segments	Results	SD
Height (cm)	81.3	−2.1
Weight (kg)	13	+0.3
Head circumference (cm)	47	−1.0
Upper segment (cm)	47	/
Lower segment (cm)	36	/

Note: Age of the patient is 2 years old.

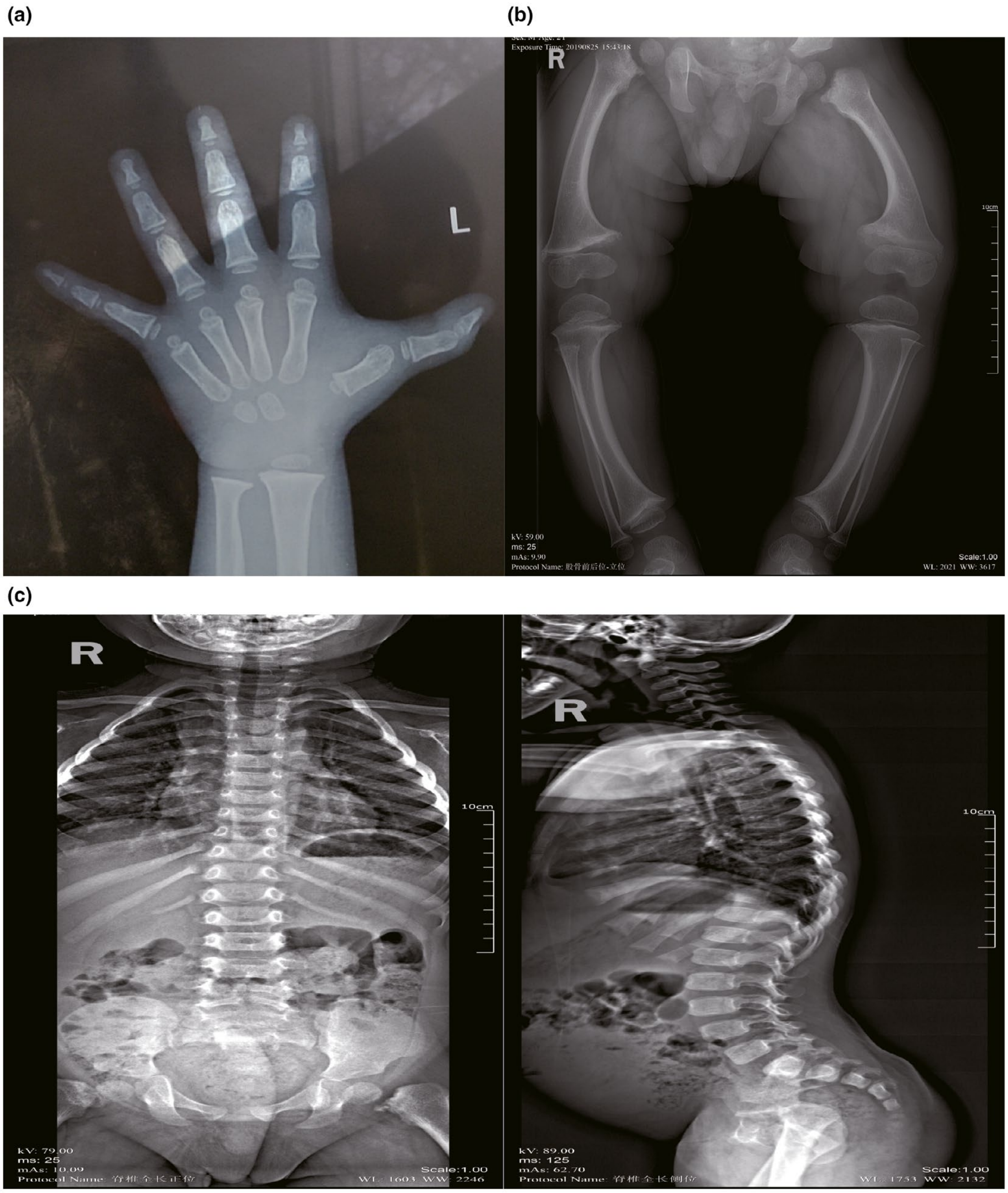

FIGURE 1 Radiographs of the patient at the first clinical examination. The results showed metaphyseal cupping of the proximal phalanges (a), coxa vara, metaphyseal widening, and irregularity of both femur and tibia (b) as well as mild dorsal scoliosis with a lumbosacral cleft in L4/5, S1 (c)

trimer. It is of note that the mutated region started from amino acid 622 to the end of *COL10A1* (Figure 3a), including both solvent-filled channels and the surface of the homotrimer (Figure 3b,c). The slightly truncated mutant protein lacking normal E-H strands (Figure 3a) will cause misfolding and trimer assembly failure of *COL10A1* subunits, so we naturally predict this variant to cause SMCD by haploinsufficiency mechanism.

Therefore, all of the information indicates that the p.(Met622 Thrfs*54) variant is predicted to impair the hydrophobicity of the subunit core residues to prevent protein folding and the collagen X supra-structure in a haploinsufficiency manner via changing the structure of both central solvent-filled channel and patches of aromatic residues on the surface of NC1 trimer.

3.5 | Literature review on *COL10A1* variants related to SMCD

Here, we listed and summarized all the published literature about *COL10A1* variants related to SMCD including our newly discovered variant (Table S1). As only the proband's information was given detailedly in most literature, mutation frequencies and the sample size in the genotype–phenotype correlation were both calculated by pedigrees, rather than

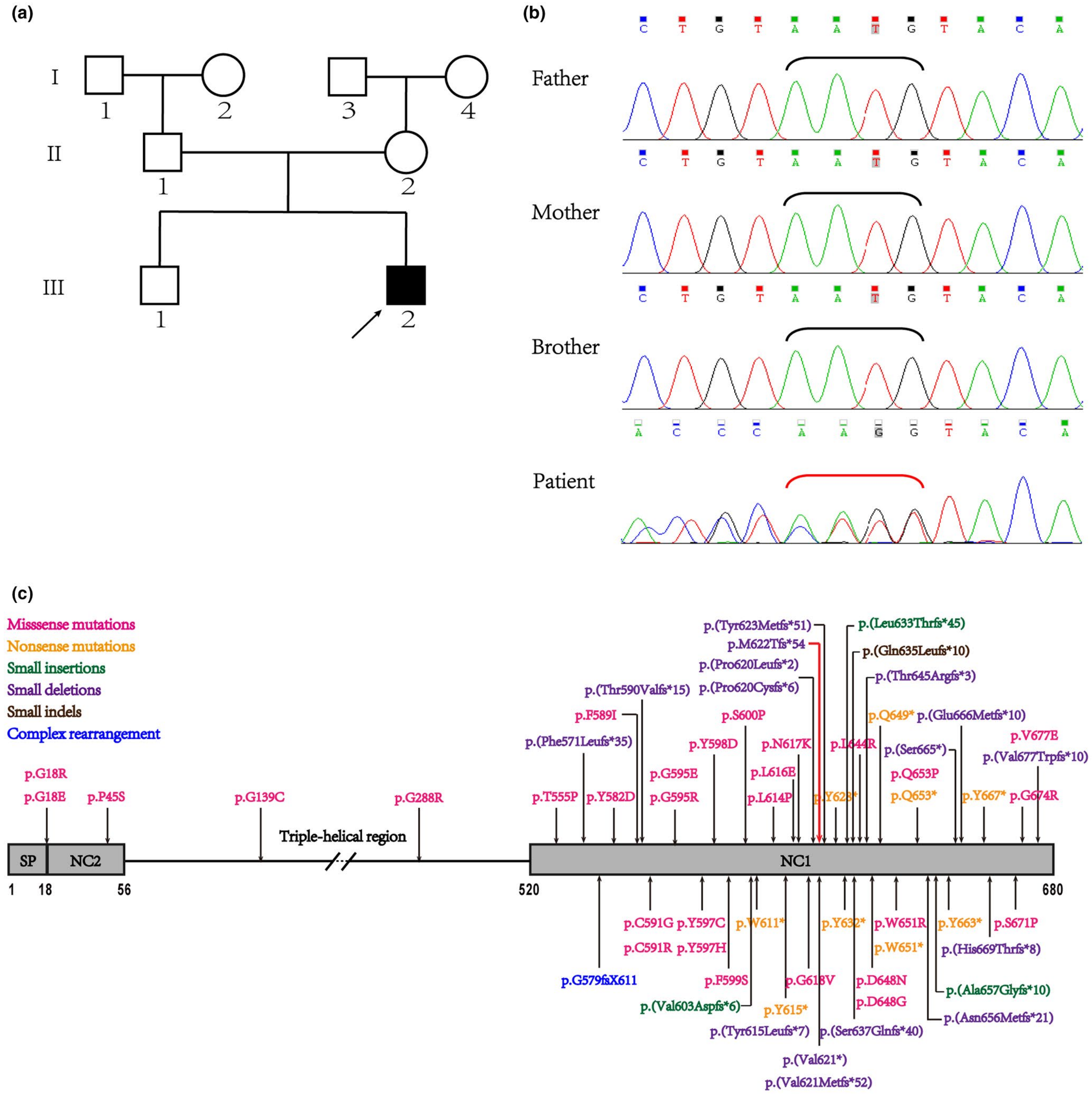

FIGURE 2 The pedigree of the family with the c.1863_1866delAATG, p.(Met622 Thrfs*54) variant, and schematic representation of *COL10A1* (Gene ID:1300, NCBI Reference sequence: NG_008032.1) variants. (a) Pedigree of a Chinese SMCD family. Males and females are indicated by squares and circles. The affected individual is represented by filled symbols. The proband is represented by arrows. (b) Partial DNA sequence of the deletion site in the *COL10A1* gene. Arcs indicate the deletion site. (c) Schematic representation of *COL10A1* and distribution of all *COL10A1* variants recorded in HGMD. The amino acid numbers defining each domain are shown below. Arrows show all fifty variants of *COL10A1* in the signal peptide, NC2 region, triple helical region as well as NC1 region. The newly identified variant is indicated by red arrow. NC1, non-collagenous domain 1; NC2, non-collagenous domain 2; S, signal peptide. Red represents missense variants; yellow represents nonsense variants; green represents small insertions; purple represents small deletions; brown represents small indels; blue represents complex rearrangement

individuals. All variants could be grouped based on different criteria (Table 3). There were 41 point variants and 19 frameshift variants, these respectively including 29 missense, 10 nonsense, 2 single-base deletions as well as 3 large insertions, 14 large deletions, and 2 complex rearrangements. 91.7% of the variants were located in NC1 domain of collagen type X alpha 1 chain with only 5 variants in non-NC1 domains. Since blood samples of the proband's parents were only available in 49 of 60 pedigrees, among which inherited and *de novo* variants took a nearly identical share. The vast

(a)

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1   MLPQIPFLLLVSLNLVHGVFYAERYQMPTGIKGPLPNTKTQFFIPYTIKSKGIAVRGEQG
    ............................................................
61  TPGPPGPAGPRGHPGPSGPPGKPGYGSPGLQGEPGLPGPPGPSAVGKPGVPGLPGKPGER
    ............................................................
121 GPYGPKGDVGPAGLPGPRGPPGPPGIPGPAGISVPGKPGQQGPTGAPGPRGFPGEKGAPG
    ............................................................
181 VPGMNGQKGEMGYGAPGRPGERGLPGPQGPTGPSGPPGVGKRGENGVPGQPGIKGDRGFP
    ............................................................
241 GEMGPIGPPGPQGPPGERGPEGIGKPGAAGAPGQPGIPGTKGLPGAPGIAGPPGPPGFGK
    ............................................................
301 PGLPGLKGERGPAGLPGGPGAKGEQGPAGLPGKPGLTGPPGNMGPQGPKGIPGSHGLPGP
    ............................................................
361 KGETGPAGPAGYPGAKGERGSPGSDGKPGYPGKPGLDGPKGNPGLPGPKGDPGVGGPPGL
    ............................................................
421 PGPVGPAGAKGMPGHNGEAGPRGAPGIPGTRGPIGPPGIPGFPGSKGDPGSPGPPGPAGI
    ............................................................
481 ATKGLNGPTGPPGPPGPRGHSGEPGLPGPPGPPGPPGQAVMPEGFIKAGQRPSLSGTPLV
                A                     A'      B'     B      C
    ............................................................
541 SANQGVTG[MPVSAFTVILSKAYPAIGTPIPFDKILYNRQQHYDPRTGIFTCQIPGIYYFS
              D         E              F             G
    ............................................................
601 YHVHVKGTHVWVGLYKNGTPVMYTYDEYTKGYLDQASGSAIIDLTENDQVWLQLPNAESN
              H
    ...................TPMMNTPKATWIRLQGVPSSISQKMTRCGSSFPMPSQMA
661 GLYSSEYVHSSFSGFLVAPM]
    YTPLSMSTPLSQDS*
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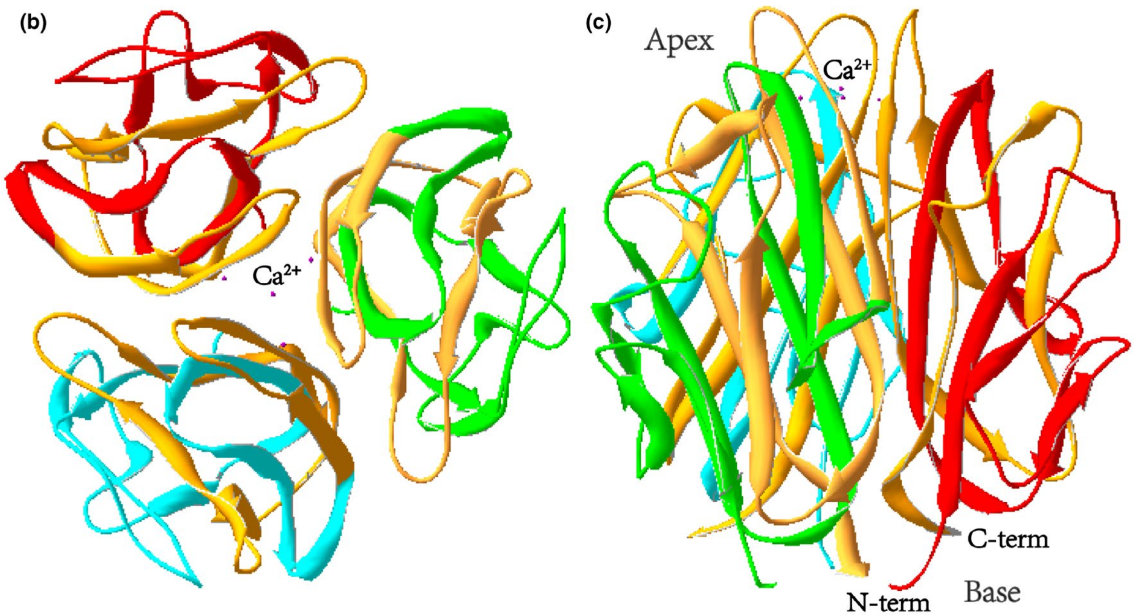

FIGURE 3 The amino acid sequence and crystal structure of type X collagen trimerization domain. (a) Comparison of the sequences between the wild type *COL10A1* (upper line) and the mutant protein (lower line). Dots indicate the same amino acid. Red brackets indicate the amino acid sequence of the trimerization-forming domain of *COL10A1*. And black boxes mark the sequences of ten β-strands (A, A', B, B', C, D, E, F, G, and H). The sequence variant altered the reading frame at the amino acid 622 and induced multiple incorrect codons (denoted in red letters) and a premature stop codon at amino acid 675. (b) Cartoon representation of a modeled homotrimer of *COL10A1* viewed from the apex which is formed by the tight association of three loops of each monomer, among which are four calcium ions. (c) Cartoon representation of a modeled homotrimer of *COL10A1* with highlighted Apex and Base region. (c) Cartoon representation is formed through rotating (b) by 90° on the horizontal axis. Calcium ions are represented as purple spheres. Red, green, and blue ribbons represent three identical regions of NC1 homotrimer. Yellow ribbons mark the region of altered residues

majority of patients had short stature, bowed legs, waddling gait and metaphyseal irregularities and genu varum was the most prevalent lower limb deformity.

Then we performed a simple genotype–phenotype correlation analysis. Based on different variant types, we further stratified patients into subgroups. It was shown in Table 4 that there existed significant phenotypic differences between different groups of patients. On the one hand, patients with truncating variants presented lower limb deformity at a significantly earlier age ($p = .020$) compared to those with non-truncating variants. In addition, patients carrying truncating variants demonstrated slightly earlier onset ($p = .149$) and shorter stature (p = .082) in comparison with those carrying non-truncating variants. On the other hand, patients with variants in NC1 domain showed significantly earlier onset (p = .008) and shorter stature (p = .022) compared with those with variants in non-NC1 domains. However, due to the unavailability of patient information in some pedigrees, the sample size included in the analysis was a bit small.

4 | DISCUSSION

As it is widely known that SMCD is a rare skeletal disorder caused by *COL10A1* gene mutation and inherited in an autosomal dominant manner (Bateman et al., 2005), our study reported a SMCD patient who carried a novel frameshift variant c.1863_1866delAATG,p.(Met622 Thrfs*54) with the heterozygous substitution of a large segment in the NC1 domain of collagen type X alpha 1 chain, leading to early onset at birth in this male child patient. The SMCD diagnosis was established in the proband with characteristic clinical, laboratory, and radiographic features together with a genetic test. In addition, the novel *COL10A1* variant of the patient was pathogenic according to the criteria for classifying pathogenic variants established by ACMG and it was predicted to induce NMD which is the pathogenic mechanism of nonsense variants in SMCD development.

To our knowledge, our study is the first to review all published papers on *COL10A1* variants associated with SMCD and analyze the genotype–phenotype correlation, thus providing a comprehensive characterization of patients with SMCD. Our results indicated that significant phenotypic differences existed between different subgroups of patients. Patients with truncating or NC1 domain variants tended to show earlier onset and severer symptoms compared with those with non-truncating or non-NC1 domains variants. Nevertheless, as a result of the small sample size in some subgroups, more cases need to be found and studied in further studies to have a deeper understanding of SMCD.

Up to date, all reported dominantly inherited variants in SMCD patients are located in the NC1 region of *COL10A1* except five variants, two in the signal peptide (p.Gly18Arg, p.Gly18Glu; Ikegawa et al., 1997), one in the NC2 domain (p.P45S; ul Ain et al., 2018) and two in the triple helical domain (p.G139C, p.Gly288Arg) of *COL10A1* (Park et al., 2015; Zhang et al., 2019; Figure 2c), which are associated with later onset and milder symptoms of MCDS (Marks et al., 1999; Park et al., 2015). Almost half variants detected in the probands were inherited from parents while the other half were *de novo* variants. Frameshift variants were much less frequent than point variants, among which missense variants took a dominant share. Most patients with *COL10A1* variants previously reported presented similar phenotypes seen in our case, such as short stature, genu varum, waddling gait, and metaphyseal irregularities. But the severity and some uncommon manifestations like lumbar lordosis and arthralgia demonstrated great variability between individuals and families.

Since clinical phenotypes vary among SMCD patients with different *COL10A1* variants, there maybe great complexity in the pathogenic mechanism (Bateman et al.,

TABLE 3 Number and frequency distribution of different variant types of *COL10A1*

Variant type	Numbers	Percentage (%)
Point variants		
Missense variants	29	48.4
Nonsensevariants	10	16.7
Single-base deletions	2	3.3
Frameshift variants		
Large insertions	3	5
Large deletions	14	23.3
Complex variants	2	3.3
NC1 domain variants	55	91.7
Non-NC1 domain variants	5	8.3
Inherited variants	26	43.3
***De novo* variants**	23	38.3
Variants of unclear origin	11	18.4

TABLE 4 Genotype–phenotype correlation in patients with SMCD reported

	Truncating variants	Non-truncating variants	p value	NC1 domain variant	Non-NC1 domain variant	p value
Age when signs and symptoms were first noticed	19.5 (12, 24) (N = 16)	38.56 ± 25.76 (N = 16)	.149	21(12, 36) (N = 28)	60 (60, 78) (N = 4)	.008*
Age when lower limb deformity first appeared	12 (12,24) (N = 14)	35.92 ± 25.99 (N = 13)	.020*	17(12,32.5) (N = 24)	60 ± 0 (N = 3)	.014*
Height SDS	−3.38 ± 1.13 (N = 15)	−2.72 ± 0.92 (N = 16)	.082	−3.18 ± 1.01 (N = 28)	−1.73 ± 0.60 (N = 3)	.022*

Note: Ages are displayed in months.

*Significant difference exists between subgroups (p < .05).

2003). And the pathogenic mechanism through which the frameshift variant p.(Met622 Thrfs*54) leads to lower limb deformity in our patient remains to be elucidated. NC1 homotrimer formation creates a central solvent-filled channel and an external surface of each NC1 trimer containing three hydrophobic patches which participate in initiating the supramolecular assembly between collagen type X alpha 1 chains or between collagen type X alpha 1 chains and other molecules for the higher-order structure of the collagen X network in bone matrix (Bateman et al., 2004). Critical hydrophobic interface residues near the base include Ala-553, Val-556, Ile-557, Leu-575, Ile-641, Phe-675, Val-677, and Ala-678 which accounted for the close packing between the NC1 subunits to generate a tight and stable collagen X protein structure (McLaughlin et al., 1999). And the most prominent feature of the trimer external surface is the presence of three strips of aromatic residues extending across each subunit interface (Figure 3b,c), with each strip containing eight partially exposed residues which are Trp-611 (9%-63% of the side chain accessible to a 1.4 A° probe), Tyr-615, Tyr-623, Tyr-625, and Trp-651 from one subunit as well as Tyr-562, Tyr-663, and Tyr-667 from the adjacent subunit (Shapiro & Scherer, 1998). So the novel frameshift variant c.1863_1866delAATG, p.(Met622 Thrfs*54) which altered most of the amino acid sequence after residue 621 (residue 622–680 altered) covering the entire central channel and partially exposed surface (Figure 3b,c), and introduced a premature stop codon in the NC1 domain to produce a shortened collagen type X alpha 1 chain (Figure 3a), was likely to tremendously damage collagen X structure and function by inhibiting collagen X homotrimer assembly preceding adverse clinical symptoms like lower limb deformity in patients with SMCD.

Haploinsufficiency has been recently recognized as one of the most probable cause of SMCD (Bateman et al., 2003; Chen et al., 2020). NC1 missense variants lead to misfolding, improper trimerization, intracellular retention of mutated collagen, and eventual collagen X haploinsufficiency (Mullan et al., 2017; Wilson et al., 2005). In addition, the process of nonsense-mediated decay (NMD) in which mutant mRNAs are determined to be completely degraded in chondrocytes gets involved in the molecular mechanism of SMCD caused by nonsense variants (Ho et al., 2007; Tan et al., 2008). Besides haploinsufficiency, the dominant-negative impairment of protein folding has been proposed to be involved in the pathogenesis of SMCD but this still needs more evidence to verify (Bateman et al., 2003; Gregory et al., 2000; Makitie et al., 2005). Compared to missense variants, patients with non-sense or frameshift variants generally show earlier onset and more severe manifestations, as present in the patient of our study (Higuchi et al., 2016; Makitie et al., 2005; Woelfle et al., 2011).

The lack of in vitro functional study was an obvious limitation in our study and we will investigate the mutant type X collagen biosynthesis and assembly in our further study. Not all *COL10A1* variants have been studied in detail and there was no protein analysis on mutant collagen X in cartilage tissue or chondrocytes from the patients, therefore studying the function of the variants on collagen X secretion and assembly had to depend on in vitro experiments at present.

In summary, our study is the first to identify and report a novel *COL10A1* heterozygous frameshift variant c.1863_1866delAATG (p.Met622 Thrfs*54) in a Chinese pedigree with SMCD which expands the mutation spectrum of *COL10A1* related to SMCD and promotes the understanding of genotype–phenotype correlations. Moreover, predicted pathophysiological consequences secondary to the variant help explain the underlying mechanisms of SMCD in this patient. Further functional studies remain to be completed to elucidate the pathogenic mechanism of SMCD.

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CONFLICTS OF INTEREST

All authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

C.X. and Y.W. designed and supervised the study as well as revised the manuscript. H.W. performed data analysis and literature review and wrote the manuscript. S.W. assisted data analysis. G.L. and J.Z. made the diagnosis. Y.Y. and X.J. collected clinical data. N.W. collected peripheral blood sample and contributed to genetic analysis. J.Z., X.S., and L.F. assisted the acquisition of data. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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