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ORIGINAL ARTICLE

Identification of a novel *COL10A1*: c.1952 G>T variant in a family with Schmid metaphyseal chondrodysplasia and development of a noninvasive prenatal testing method

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Abstract

Background: The collagen alpha-1(X) chain gene (*COL10A1*) is a known causative gene for Schmid metaphyseal chondrodysplasia (SMCD). This study clinically examined a Chinese family ($n = 42$) for SMCD and inheritance pattern. Fifteen individuals were diagnosed with SMCD based on characteristic skeletal phenotypes with autosomal dominant inheritance mode.

Methods: Four clinically diagnosed patients and three healthy relatives were selected for subsequent genetic tests. Trio-whole exome sequencing (Trio-WES) followed by Sanger sequencing and familial co-segregation analysis were performed to identify SMCD-associated variants.

Results: *COL10A1* (NM_000493.4):c.1952 G>T(p.Trp651Leu) variant was detected only in the four patients and not in the three healthy relatives. The variant was evaluated as "likely pathogenic" according to the American College of Medical Genetics and Genomics variation classification guidelines with evidence of PM2, PM5, PP1, and PP3. To test the presence of the target variant in proband's fetal offspring, we developed a noninvasive prenatal testing method by extracting cell-free fetal DNA in maternal plasma followed by high-depth sequencing. The variant was also detected in the fetus and later confirmed by amniocentesis.

Conclusion: We identified a new disease-causing variant in *COL10A1*. Cell-free fetal DNA in maternal peripheral blood can be used as the rapid and noninvasive prenatal diagnostic method to detect the pathogenic/or likely pathogenic variant.

KEYWORDS

COL10A1, noninvasive prenatal testing, pathogenic variant, Sanger sequencing, Schmid metaphyseal chondrodysplasia (SMCD), trio-whole exome sequencing

Yanchou Ye and Weihao Li contributed to this work equally.

1 | INTRODUCTION

Schmid metaphyseal chondrodysplasia (SMCD, OMIM#156500) is characterized by metaphyseal irregularities of the long bones, shortening of the tubular bones, widened growth plates(especially at the knees), coxa vara and anterior cupping, sclerosis, and splaying of the ribs (Chan et al., 1998). The prevalence of SMCD is estimated to be about 3–6/1 000 000 (Makitie et al., 2005). There are many types of metaphyseal chondrodysplasia (MCD) with various clinical characteristics (Bonafe et al., 2015; Hasegawa et al., 2015; Savarirayan et al., 2000; Zhang et al., 2018). Molecular testing for the causative genes and variants is the golden standard of diagnosis (Ikegawa et al., 1998; Monaghan et al., 2020). Because the phenotype of SMCD overlaps with that of other MCDs as well as several other inherited disorders with metaphyseal dysplasia, using whole exome sequencing is advisable for its genetic diagnosis (Pokharel et al., 1995). The collagen alpha-1(X) chain gene (*COL10A1*, OMIM: 120110) has been identified as the SMCD-causing gene with autosomal dominant inheritance pattern (Elliott et al., 2005).

There is a 50% chance of inheriting the pathogenic variant from a SMCD-affected parent (Flynn & Pauli, 2003; Richmond & Savarirayan, 1993). Currently, amniocentesis and chorionic villus sampling are the two commonly used invasive prenatal diagnostic procedures that have drawbacks such as injury to the fetus, miscarriage, and/or intrauterine infection (Levy & Stosic, 2019; Lo, 2015). Therefore, there is a need to develop a better molecular detection method for prenatal testing of the variant (Chiu et al., 2011; Lo et al., 1997; Zhu et al., 2017).

Recently, we examined a family of 42 members (15 patients with SMCD and 27 healthy relatives). We identified a novel disease-causing variant in *COL10A1*. Moreover, we developed a noninvasive prenatal testing method and verified the results with an invasive method.

2 | MATERIALS AND METHODS

2.1 | Subjects

A total of 42 members from a Chinese family affected by SMCD from Shantou (Guangdong, China), were examined. Fifteen family members were diagnosed with SMCD based on the characteristic skeletal phenotypes. Twenty-seven family members did not have features of the condition. The inheritance mode of SMCD in the family was identified as autosomal dominant. The Proband and his wife needed genetic counseling whether their fetus(V: 1) would develop SMCD, we selected him and another three affected patients, IV: 1 was proband, male, 32 years old, III: 2 was his mother, III:7 was his uncle, II:3 was his great-uncle, and three unaffected members with an age range of 26–63 years, including the proband's healthy father, sister and wife (Figure 1). They were recruited in December 2019 at the Department of Obstetrics, The Third Affiliated Hospital of Sun Yat-sen University. All participants provided written informed consent before. The study protocol was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University.

2.2 | Sample collection and DNA extraction

Approximately 2 mL peripheral blood samples from the recruited patients and unaffected relatives were collected in EDTA-containing tubes. Amniotic fluid sample was collected from IV: 2 at fetal age of 16 weeks. For each sample, genomic DNA from 200 μl peripheral blood was extracted using the DNeasy Blood and Tissue Kit (Cat. No. 51306, QIAGEN DNA Mini Kit; QIAGEN) according to the manufacturer's instructions. DNA was quantified using a Qubit®fluorometer (Life Technologies) and stored at –20°C until further use.

2.3 | Targeted next-generation sequencing

Trio-WES was performed on DNA extracted from individuals to identify the causal variants in the family trio of III: 1, III: 2, and IV: 1. In brief, the extracted genomic DNA was hybridized and enriched. First, 1 μg genomic DNA was fragmented to approximately 200 bp using a Biorupter UCD-200 (Cat. No. UCD-200, Diagenode). The DNA fragments were then repaired at the end, and the 3'end was added one A base. Second, the DNA fragments were connected with sequencing adaptors, and fragments about 320bp were collected by XP beads. After PCR amplification, the DNA fragments were hybridized and captured by IDT's xGen Exome research panel (Integrated DNA Technologies) according to the manufacturer's protocol. The hybrid products were eluted and collected. Then DNA was amplified using PCR and purified. Next, the libraries were tested for enrichment by qPCR, and size distribution and concentration were determined using an Agilent Bioanalyzer 2100 (Agilent Technologies). Finally, 150-bp pair-end reads were used for sequencing the genomic DNA of the study participants with Novaseq6000 platform (Illumina). Raw image files were processed using CASAVA v1.82 for base calling and generating raw data.

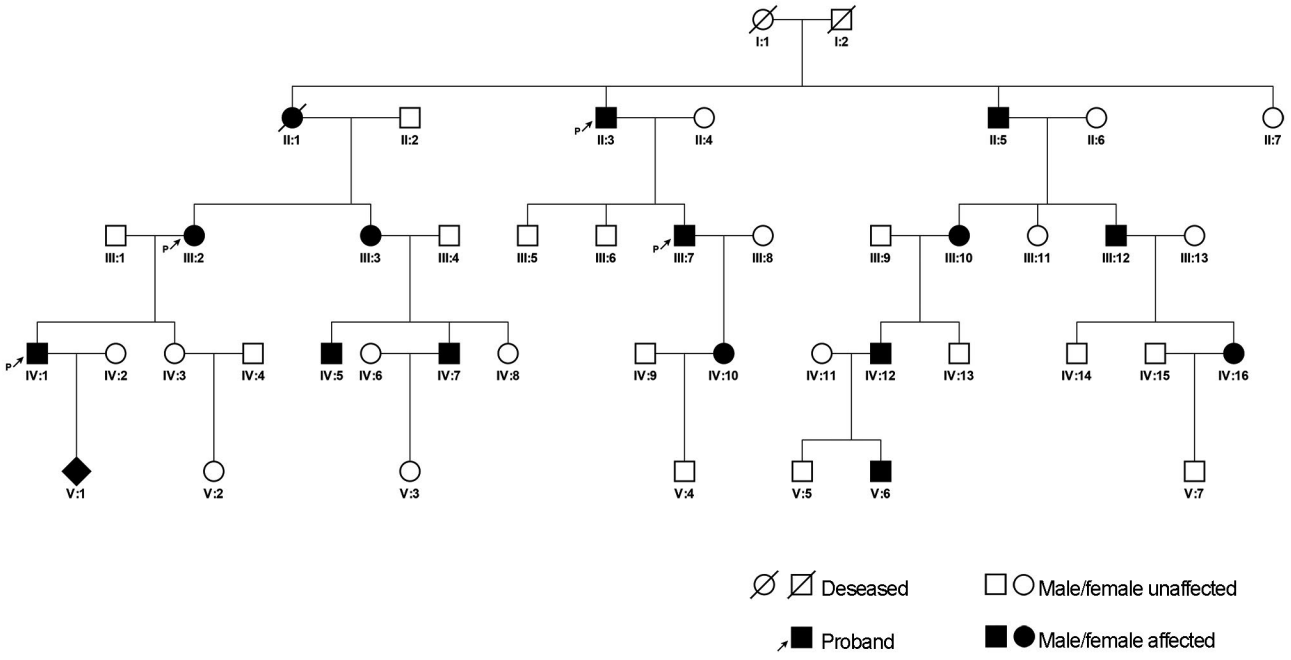

FIGURE 1 Pedigree chart of a large Chinese family affected by Schmid metaphyseal chondrodysplasia

2.4 | DNA analysis

The sequencing reads were aligned to the human reference genome (hg19/GRCh37) using Burrows–Wheeler Aligner tool(Kimura & Koike, 2015) and PCR duplicates were removed using Picard v1.57 (http://picard.sourceforge.net/). Variants Detection System by Berry Genomics and the third-party software GATK (https://software.broadinstitute.org/gatk/) were employed for variant calling. Variant annotation and interpretation were conducted by ANNOVAR and the Enliven®Variants Annotation Interpretation System authorized by Berry Genomics. Annotation databases mainly included (Wang et al., 2010):

(i)human population databases, such as gnomAD (http://gnomad.broadinstitute.org/), the 1000 Genome Project (http://browser.1000genomes.org), Berrybig data population database, dbSNP (http://www.ncbi.nlm.nih.gov/snp) etc;
(ii) in silico prediction algorithms, such as SIFT (http://sift.jcvi.org), FATHMM (http://fathmm.biocompute.org.uk), Mutation Assessor(http://mutationassessor.org), CADD (http://cadd.gs.washington.edu), SPIDEX (Xiong et al., 2015), etc;
(iii)disease and phenotype databases, such as OMIM (http://www.omim.org), ClinVar (http://www.ncbi.nlm.nih.gov/ClinVar), HGMD (http://www.hgmd.org), HPO (https://hpo.jax.org/app/) etc.

For trio-analysis, potential monogenetic inheritance patterns including de novo, autosomal recessive, autosomal dominant, X-linked recessive inheritance, mitochondrial, and, where possible, imprinted gene variation were analyzed. Full penetrance was assumed for the potentially causal variants and variants that were found in the parents or were recorded in any of the abovementioned databases or in our in-house control exomes were excluded as etiology. Once a variant was considered as the etiology of a recessive disorder, manual inspection for coverage, and additional variants of the entire coding domain was undertaken using Integrated Genomics Viewer.

The variants were classified to five categories—"pathogenic,""likely pathogenic,""uncertain significance,""likely benign," and"benign"—according to the American College of Medical Genetics and Genomics (ACMG) guidelines for interpretation of genetic variants (Richards et al., 2015). Variants with minor allele frequencies (MAF) <1% in exonic region or with splicing impact were taken for deep interpretation considering ACMG category, evidence of pathogenicity, and clinical synopsis and inheritance model of the associated disease.

2.5 | Sanger sequencing

To determine that the same variant was present in the patients and healthy controls simultaneously and to perform familial co-segregation analysis, the variant was specifically amplified using Sanger sequencing. Fetal DNA from amniotic fluid sample obtained from IV: 2 was also amplified and sequenced. The primer sequences were as follows: forward, 5′-ACGTGCATGTGAAAGGGAC-3′

and reverse, 5′-AGGGTGGGGTAGAGTTAGAGA-3′. The cycling conditions were 96°C for 5 min, 98°C for 30 s, and 58°C for 1.5 min, 72°C for 3 min for 30 cycles, and finally 13 min at 72°C. The total reaction volume was 25 μl. The specificity of PCR products was determined by 1.5% agarose gel electrophoresis. The results of Sanger sequencing were analyzed by Mutation Surveyor V3.97 (Soft Genetics) and Chromas (Technelysium Pty Ltd.).

2.6 | Noninvasive prenatal testing for SMCD

The fetal DNA in the maternal blood was extracted for noninvasive prenatal testing assays. Approximately 5 ml of peripheral blood from IV: 2, at fetal age of 12 weeks, was collected in EDTA-containing tubes. Approximately 2 ml of plasma was separated in two steps: First, 10 ml of peripheral blood from IV: 2 was centrifuged at 1 600× *g* for 15 min. Then, approximately 2 ml of the supernatant was centrifuged at 16 000× *g* for 10 min at 4°C. The plasma cell-free fetal DNA (cfDNA) was extracted using a QIAamp Circulating Nucleic Acid Kit (Cat. No. 51306, QIAGEN DNA Mini Kit; QIAGEN) according to manufacturer's instructions. Finally, the DNA sample was quantified using a Qubit®fluorometer (Life Technologies) and stored at −20°C until further use. Overlapping PCR amplicons covering *COL10A1*: c.1952 G>T were generated, pooled, and sequenced using Novaseq6000 platform (Illumina). Fastq files were used to detect the target variant in high-depth sequencing and verified with amniotic fluid test.

3 | RESULTS

3.1 | Clinical phenotype

All the family members were clinically diagnosed with SMCD. Two clinically diagnosed patients (III: 2 and IV: 1) were chosen for x-ray tests. The radiographs showed metaphyseal irregularities of the long bones, shortening of the tubular bones, widened growth plates, coxa vara and anterior cupping, sclerosis, and splaying of the ribs (Figure 2). The clinical characteristics of the affected patients is provided in Table 1.

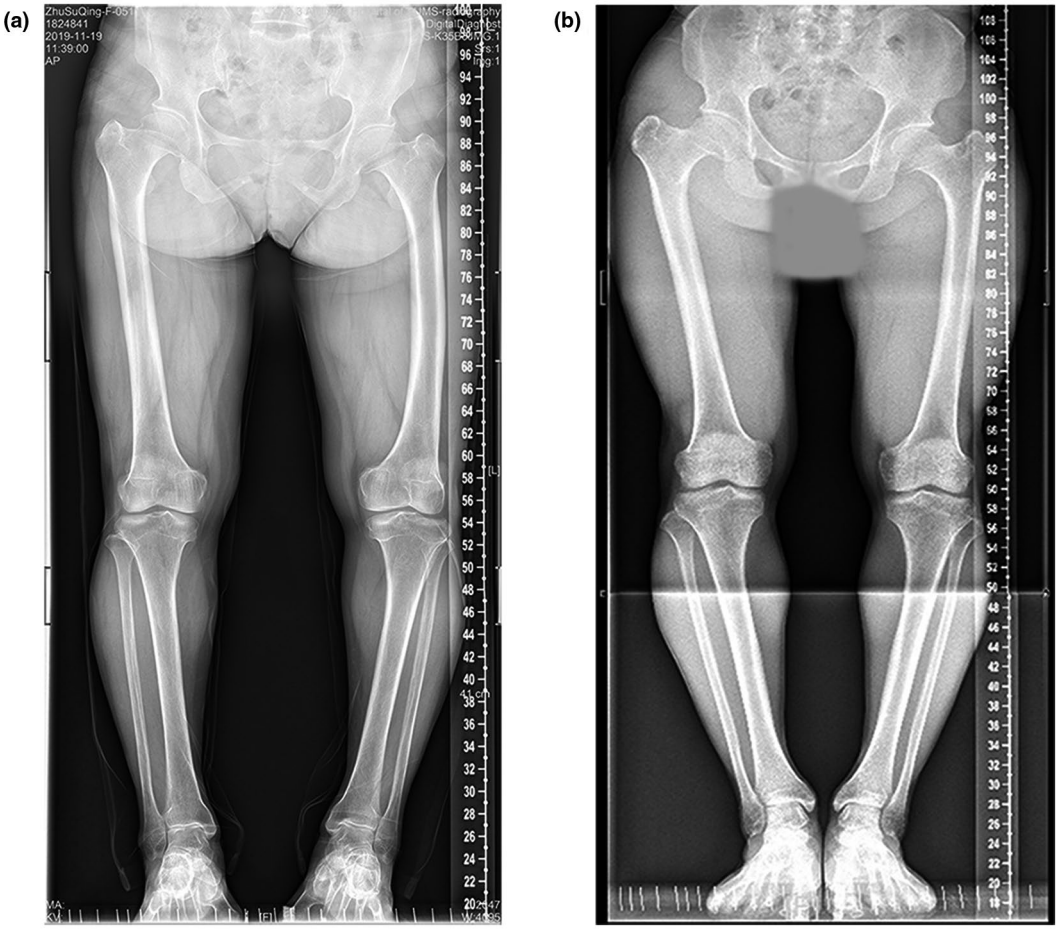

FIGURE 2 Standing lower-limb radiograph of patients with SMCD. Left panel, III: 2; Right panel, IV:1. Severe bilateral coxa vara with marked metaphyseal widening at the hips, metaphyseal widening and irregularity of the distal femurs with femoral bowing is seen

TABLE 1 Clinical features of patients with SMCD in a Chinese family

ID	Sex	Age (years)	Short stature	Waddling gait	Genu varum	Metaphyseal irregularities of the long bones	Coxa vara	Cupped &/or sclerotic anterior rib ends
II:3	Male	77	150cm	Yes	Yes	Yes	Yes	Yes
III:2	Female	58	145cm	Yes	Yes	Yes	Yes	Yes
III:7	Male	35	150cm	Yes	Yes	Yes	Yes	Yes
IV:1	Male	32	153cm	Yes	Yes	Yes	Yes	Yes

3.2 | Variant analysis

After mapping the sequences to the human reference genome (hg19/GRCh37 version) and removal of low-quality and polyclonal reads, an average coverage depth of 120× (range: 90–200×) was obtained for all the 93-kilo single nucleotide polymorphisms in blood samples of the three patients. Following Illumina sequence analysis, no large-fragmental deletions or duplications were detected in the gene-coding regions. The missense variant *COL10A1*:c.1952 G>T (p.Trp651Leu) was detected in the gene coding region of *COL10A1* in the male proband and his mother who exhibited SMCD phenotype, as illustrated in Table 2.

3.3 | Sanger sequencing analysis

Sanger sequencing revealed that out of the seven family members, four SMCD-affected individuals carried *COL10A1*:c.1952 G>T (p.Trp651Leu) variant, while the three unaffected controls carried the wild genotype. All results are shown in Table 2 and Figure 3.

3.4 | cfDNA variant analysis

The average length of the read was 120 bp, and the mean percentage of sequencing reads mapped to the human reference genome (hg19/GRCh37version) was 98%. After filtering out low-quality, polyclonal, and primer dimer reads, the percentage of fetal DNA was 17.8%, cfDNA *COL10A1*: c.1952 G>T site depth was 5000×, T base depth was 800×, and G base depth was 4200×. The T base depth frequency was 16% of the total depth, which corresponded to the fetal DNA concentration. Therefore, we inferred that the T base was derived from the fetus.

3.5 | Accuracy of cfDNA variant analysis

To verify the accuracy of nonnivasive prenatal testing (NIPT), the results obtained from cfDNA sequencing were validated by Sanger sequencing of fetal DNA collected from amniotic fluid cells. The noninvasive prenatal testing result was consistent with that of amniotic fluid testing.

3.6 | Discussion

This study is the first to report a novel disease-causing *COL10A1*:c.1952 G>T (p.Trp651Leu) variant. *COL10A1*,

ID	Sex	Age (years)	Height	SMCD	*COL10A1* (NM_000493.4) Variant	Classification of pathogenic
II:3	Male	77	150 cm	Yes	c.1952G>T p.W651L	Likely pathogenic
III:2	Female	58	145 cm	Yes	c.1952G>T p.W651L	Likely pathogenic
III:7	Male	35	150 cm	Yes	c.1952G>T p.W651L	Likely pathogenic
IV:1	Male	32	153 cm	Yes	c.1952G>T p.W651L	Likely pathogenic
III:1	Male	60	160 cm	NO	Wild type	—
IV:2	Female	30	152 cm	NO	Wild type	—
IV:3	Female	25	158 cm	NO	Wild type	—

TABLE 2 Seven persons Sanger Sequencing results with *COL10A1*:c.1952G>T: p.W651L

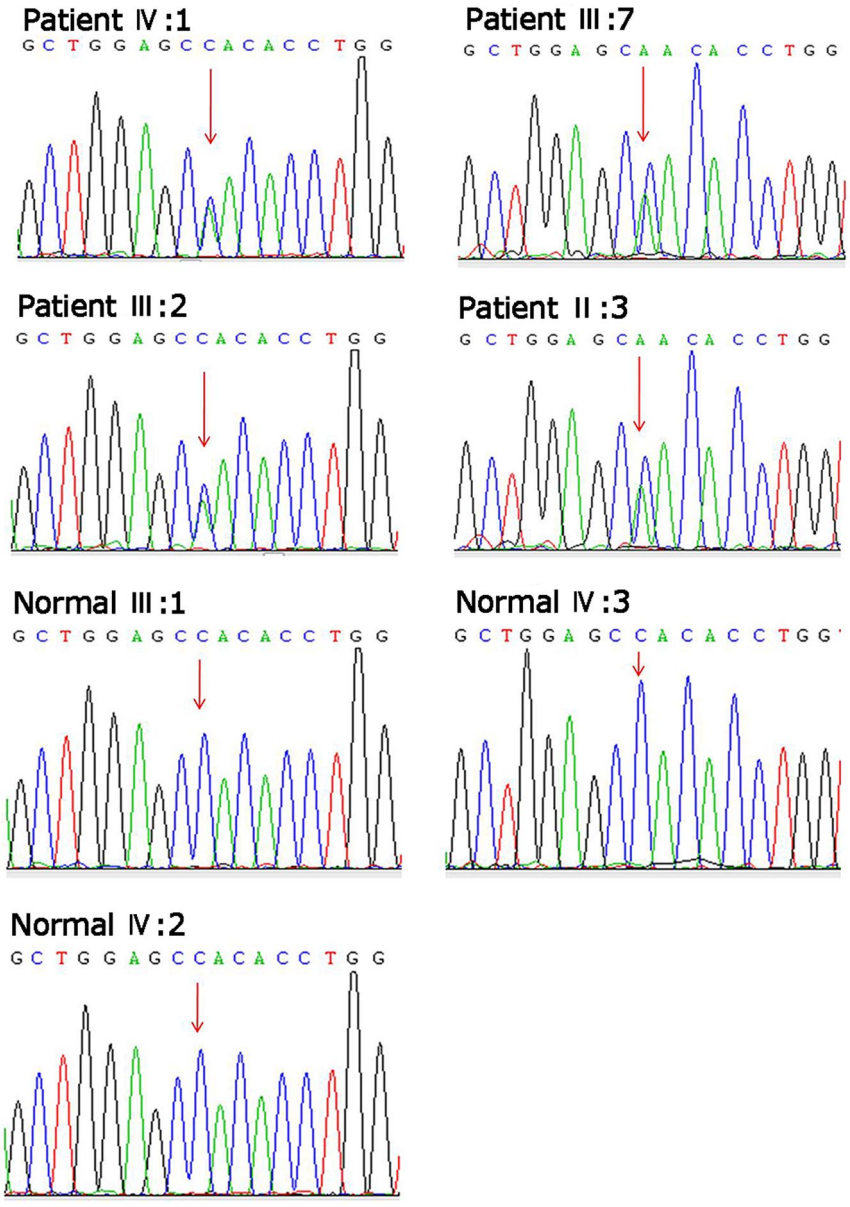

FIGURE 3 Sequence analysis of *COL10A1* gene for the family affected by SMCD. The heterozygous mutation c.1952 G>T was identified in all patients with SMCD. Normans exhibited wild type

which spans ~100 kb and encodes a 680 amino acid membrane glycoprotein, is located on chromosome 6q22 (Chan et al., 1998). The alpha chain of type X collagen, encoded by *COL10A1*, is a short-chain collagen present in the extracellular matrix and expressed exclusively by hypertrophic chondrocytes in the cartilage growth plates of growing bones during endochondral ossification (Chan et al., 1998; Wilson et al., 2005). Pathogenic variants in *COL10A1* are clustered in the C-terminal non-collagenous (NC1) domain, which contains motifs required for normal assembly of the collagen trimer (Ain et al., 2018). Both missense and truncating (frameshift and nonsense) variants in *COL10A1* cause collagen X protein misfolding during its synthesis, resulting in failure of trimerization and aggregation within the endoplasmic reticulum of hypertrophic chondrocytes. Resultant endoplasmic reticulum stress, activation of the unfolded protein response, and reduced levels of functional type X collagen in the growth plate cause chondrodysplasia and development of the SMCD phenotype (Richmond & Savarirayan, 1993).

To date, the Human Gene Variants Database (http://www. hgmd.cf.ac.uk) has described 130 variants in *COL10A1* that are reportedly responsible for SMCD (Stenson et al., 2017). The present study identified and confirmed a novel *COL10A1* gene variant through Trio-WES and Sanger sequencing. This variant was later verified to be absent in 141 000 healthy individuals without SMCD who did not have any family history of the disease as well (https://gnomad.broadinstitute.org/) (Karczewski et al., 2020). *COL10A1*(NM_000493.4) variants c.1951T>C(p.Trp651Arg), c.1952 G>A(p.Trp651Ter), and c.1953 G>A(p.Trp651Ter) in the affected family with SMCD were reported in Human Gene Variant database and ClinVar database (Landrum et al., 2014; Makitie et al., 2005; McIntosh et al., 1995). Based on these results, we speculated that the c.1952 G>T (p.Trp651Leu) variant might affect protein function. Therefore, we identified the novel missense variant *COL10A1*:c.1952 G>T (p.Trp651Leu) as a likely pathogenic variant according to the American College of Medical Genetics and Genomics (ACMG) guidelines for interpretation of genetic variants with the pathogenic criteria PM2, PM5, PP1, and PP3 (Green et al., 2013; Richards et al., 2015).

SMCD is characterized by progressive short stature that develops by two years of age (Elliott et al., 2005). As the clinical and radiographic features characteristic of SMCD are usually not present at birth, prenatal molecular testing is a good method for its accurate diagnosis (Al Kaissi et al., 2018). Moreover, a noninvasive prenatal testing early during pregnancy would be preferred (Papasavva et al., 2013). In this study, we attempted to develop a method for noninvasive prenatal testing of *COL10A1* variant in the fetal offspring of the proband early in the pregnancy. We demonstrated targeted cfDNA capture combined with high-depth next-generation sequencing for the detection of paternal variant in the fetus (Ryan & Martin, 2017). In theory, as the pregnant woman did not carry *COL10A1*:c.1952 G>T (p.Trp651Leu) variant, the variant should not be detected in her plasma (Cheng et al., 2015). The T base identified in the maternal blood plasma can be explained by the presence of cfDNA, indicating that the fetus inherited *COL10A1*:c.1952 G>T (p.Trp651Leu) variant from the father. The result of noninvasive prenatal testing was identical to that of the invasive amniotic fluid molecular diagnosis; both tests identified the presence of *COL10A1*: c.1952 G>T variant in the fetus. This study provided evidence that sequencing cfDNA obtained from maternal peripheral blood can be used as rapid and non-invasive prenatal diagnostic method to detect the pathogenic or likely pathogenic variant.

In conclusion, the present study identified a novel *COL10A1*:c.1952 G>T (p.Trp651Leu) variant in a Chinese family with SMCD. Our findings expanded the variant spectrum of *COL10A1*, thereby supporting future genetic diagnosis and counselling for SMCD. The routine clinical methods for prenatal diagnosis of SMCD include amniocentesis or villus puncture. However, these invasive procedures may increase the risk of miscarriage and/or cause anxiety in pregnant women. This study demonstrated a noninvasive prenatal testing method using cfDNA in the plasma of pregnant women involving target capture and high-depth next-generation sequencing.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

JZ and TL designed the study. YCY, WHL, and JWL collected the samples. LSZ and JWL performed the molecular diagnosis and family analysis. YCY and GW designed the NIPD assay and developed the experimental protocol. LSZ and WHL performed the NIPT assay. GW analyzed the NIPT data. YCY, TL, JZ, and WHL drafted the manuscript and all authors contributed to editorial changes.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the Medical Ethics Committee of *The Third Affiliated Hospital of Sun Yat-sen University* (*Guangzhou*, China).

PATIENT CONSENT FOR PUBLICATION

Not applicable.

DATA AVAILABILITY STATEMENT

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

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