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Short Communication

A Missense Mutation in *COL10A1* Gene in a Pakistani Consanguineous Family with Schmid Type Metaphyseal Chondrodysplasia

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Authors' Contribution
FI had designed, supervised the study and revised the manuscript. ML, FB and MNA had located the families, collected the blood sample, epidemiological and clinical data, MA, MA and MI extracted the DNA, SM had performed lab experiments, drawn the pedigree, analyzed the results and prepared the manuscript.

Key words
Schmid-type metaphyseal chondrodysplasia, WES, Sanger sequencing

ABSTRACT

Schmid-type metaphyseal chondrodysplasia (MCDS) is an autosomal dominant disorder caused by *COL10A1* mutations and is characterized by short stature, waddling gait, coxa vara and bowing of the long bones. A large family from Southern Punjab in Pakistan suffering from MCDS following autosomal dominant mode of inheritance were enrolled in present study. Whole exome sequencing (WES) approach was adopted to identify causative agent of dwarfism that reveled a previously reported a missense mutation (c.2011A>G, p.Ser 671Pro) in exon 3 of *COL10A1* gene. Sanger sequencing confirmed these mutations in all enrolled subjects and mutation followed Mendalian pattern of inheritance. Multiple sequence alignment by Clustal Omega revealed that domain of *COL10A1* containing mutations is highly conserved. In conclusion, we are reporting a previously reported a missense mutation in *COL10A1* gene that is causing MCDS in a large consanguineous Pakistani family.

The metaphyseal chondrodysplasias are clinically and etiologically heterogeneous (Hall, 2002). Among them, metaphyseal chondrodysplasia Schmid type (SMCD; MIM# 156500) has been identified as the most common type of metaphyseal chondrodysplasia (Bateman *et al*., 2005). The radiographic features of SMCD are metaphyseal abnormality, including widening, sclerosis and irregularity. The distal femoral and proximal tibial metaphyses are the most consistently and severely affected. Coxa vara frequently in association with mild femoral bowing is also observed in the majority of cases and is an important radiographic sign in differentiating SMCD from other forms of metaphyseal chondrodysplasia (Hall, 2002).

We enrolled a consanguineous dwarf family with typical MCDS syndromes from Pakistan and used whole exome sequencing (WES) approach to identify the variant(s) associated with MCDS. Here we report that MCDS in this family was due to a known missense point mutation in *COL10A1* gene.

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Materials and methods

A dwarf family was enrolled from Ranjanpur District in Punjab (Pakistan) having multiple siblings suffering from short stature. Subjects were diagnosed to be suffering from metaphyseal chondrodysplasia Schmid type (MCDS) by a medical specialist. The ethical committee at Bahauddin Zakariya University, Multan approved this research. Written informed consents were obtained for all participants. Ten blood samples were collected from family including eight patients and two controls (Fig. 1A). Blood was sampled from median cubital vein and preserved in 0.5M EDTA containing blood collection tube. Basic clinical data including age, gender, body weight and height was recorded at sampling site. X-ray radiography was performed to verify the symptom of dwarfism with lower limbs for an affected individual IV- 13 and a normal individual V-13 (Fig. 1A). DNA extraction was carried out by using commercial kit (Qiagen, Germany) following the instructions of the manufacturer.

Whole exome sequencing (WES) was performed in two affected individuals (V-8, V-10). WES and data analysis were performed as described previously by Zhou *et al*. (2014). Variants were annotated by ANNOVAR. Candidate variants were filtered to select those that were

non-synonymous or they were in splice sites within 6 base pairs of an exon and had less than 1% mutant allele frequency in the gnom AD, Kaviar and in house database and they were co-segregated with the phenotype.

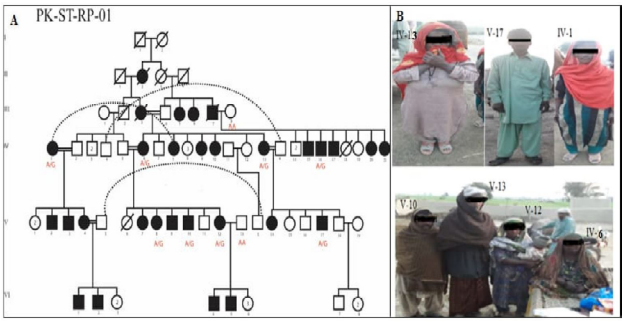

Fig. 1. Pedigree and clinical manifestations. **A**, Pedigree of a consanguineous Pakistani family segregating autosomal dominant form of metaphyseal chondrodysplasia Schmid type. Double lines are indicative of consanguineous union. Clear symbols represent unaffected individuals while filled symbols represent affected individuals. The diagonal line through a symbol is indicative of a deceased family member. **B**, affected individual IV-13, V-17, IV-1, V-10, V-13, V-12, IV-6 from enrolled family showing disproportionate short stature with bowed legs.

To confirm the sequence change identified by the WES, exon 3 of *COL10A1* and its flanking intronic sequences were amplified by polymerase chain reaction (PCR) from genomic DNA by using 5'TCAGGGGGAAGGTTTGTTG3' as forward primer and 5'ATGACCCAAGGACTGGAATCT3'as reverse primer. PCR was carried out in a total volume of 50 µl. PCR reaction mixture consisted of 0.4 µM deoxynucleotide triphosphate (dNTPs), 0.3 µM ul of each primer, 2X of buffer, 5ul of DNA template at a concentration of 40 ng/µl, 1 µl of KOD FX taq polymerase (Toyobo, Japan). Amplification of DNA was processed in a DNA thermo cycler (Applied Biosystems, USA). Thermal profile conditions were initial denaturation at 94°C for 2 min followed by 35 cycles of denaturation at 94°C for 10 seconds, annealing at 55°C for 30 seconds and extension at 68°C for 45 seconds. Final extension was carried out 4°C for 1 min PCR products were keep at 4° C till their electrophoresis on 3% Agarose gel.

Candidate variant was validated on DNA of all available family members by Sanger sequencing. PCR products were sequenced using Big Dye Terminator v.3.1 (ABI Thermo Fisher) on ABI 3730. The shortlisted variant allele frequency was checked in 50 ethnically matched controls and all public databases.

Sequence of COL10A1 protein for different species was downloaded from ensemble (https://asia.ensembl.org/index.html). Multiple sequence analysis was performed by Clustal Omega.

Result and discussion

Affected individuals in this family exhibited features of autosomal dominant form of metaphyseal chondrodysplasia Schmid type. Clinical features were short trunk, short stature, bowed legs, normal intellect and normal facial features (Fig. 1B). Basic clinical data of enrolled subjects is presented in Supplementary Table I.

Radiographic analysis revealed that all affected individuals had bowed lower limbs, coxa valga, metaphyseal widening and sclerosis. These changes were not observed in control subject (Fig. 2).

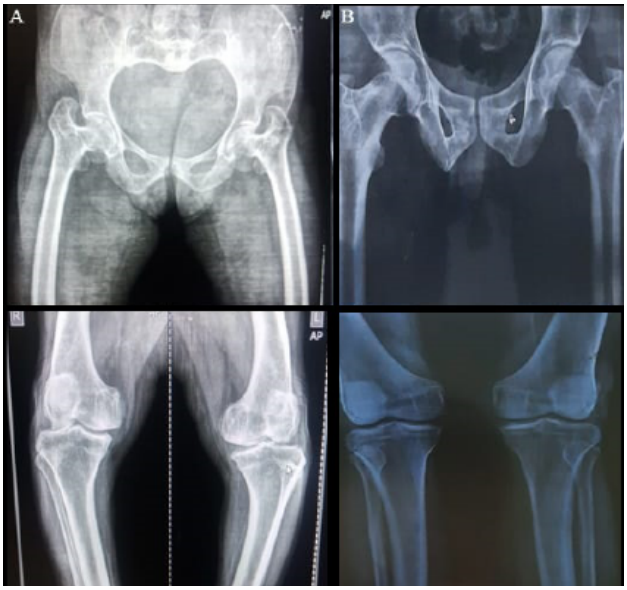

Fig. 2. A, Radiographic features from lower limbs of a metaphyseal chondrodysplasia, Schmid type (MCDS) patient (IV-13 shown in 1B). B, Radiographic features from lower limbs of a normal subject (V-13 shown in 1B). MCDS patient had bowed lower legs, coxa valga, metaphyseal widening and sclerosis while these changes were not observed in control subject.

Analysis of WES data performed in two affected siblings (V-8, V-10) and their father (III-2) identified 4880 variants in each trio [after filtering for novel and rare variants (allele frequency <1%)]. All suspected variants were confirmed by Sanger sequencing. Sequence analysis of the gene *COL10A1* detected a previously reported heterozygous A to G transition at nucleotide position 2011 (c.2011A>G, p.Ser671Pro) (*rs111033552)* in third exon in all affected individuals of enrolled family. While the

normal siblings were homozygous AA at position 2011 in exon 3 (Fig. 3A). This mutation caused an amino acid change from Serine to Proline at position 671.

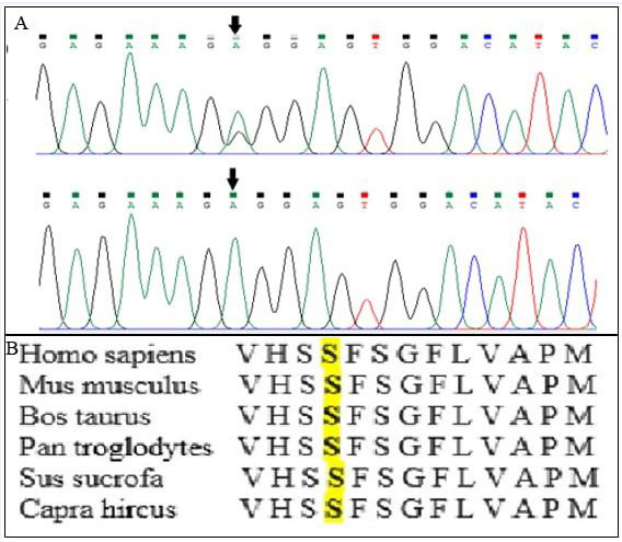

Fig. 3. Chromatogram and Clustal Omega alignment of COL10A1. **A**, Chromatogram for *COL10A1* selected region showing c.2011A>G transition. B **B**, Multiple sequence alignment of COL10A1 from six different organisms performed with Clustal Omega showing p.Ser671Pro (shown in bold) conservation in diverse vertebral species.

Clustal omega analysis revealed that Serine and other amino acids of this domain are highly conserved in vertebrates (Fig. 3B) and any mutation in this region may have profound effects on the phenotype of the individuals.

This heterozygous mutation in *COL0A1* may cause autosomal dominant form of MCDS. Zhang *et al*. (2018) had reported same variant *rs111033552* (c.2011T>C [p.Ser671Pro]) in *COL10A1* in another large dwarf family from the same region in Pakistan. They reported that the mutation was absent in 69,985 individuals representing >150 global populations. They also examined other variant forms, including copy number variation and insertion/deletion, but failed to identify such variants enriched in the affected individuals and concluded that rs111033552 had priority for linkage with dwarfism. Zhang *et al*. (2018) had reported that affected individuals had bowed lower legs, coxa valga, metaphyseal widening and sclerosis while these abnormalities were not observed in normal subjects. In present study, we have observed exactly the same skeletal abnormalities in the enrolled subjects confirming the findings of Zhang *et al*. (2018). These identifiable features of lower limb that are observed in affected individuals are also in accordance with the MCDC phenotype reported by Bateman *et al*. (2005).

The type X collagen gene (*COL10A1*) is specifically expressed by hypertrophic chondrocytes during bone development. Type X collagen influences deposition of other matrix molecules to hypertrophic zone and thereby provides a proper environment for hematopoiesis, mineralization and modeling that are essential for endochondral ossification (Grskovic *et al*., 2012). Mutations and abnormal expression of *COL10A1* are closely linked to abnormal chondrocyte hypertrophy that has been seen in multiple skeletal dysplasia and osteoarthritis (Ikegawa *et al*., 1998; Drissi *et al*., 2005).

Kong *et al*. (2019) had investigated two independent non-consanguineous five-generation Chinese families containing 19 MCDS patients and identified 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.

A significant contribution on this topic was done by Makitie *et al*. (2005) who had sequenced 10 patients with MCDS and identified that All 10 patients were found to have a mutation in *COL10A1*. Eight of the mutations were single nucleotide substitution and two patients had a deletion of a nucleotide.

Park *et al*. (2014) had performed mutational analysis of the *COL10A1* genes in 4 unrelated Korean patients with diagnosed MCDS. Mutational analysis of *COL10A1* identified c.1904_1915delinsT (p.Gln635LeufsX10) and c.1969dupG (p.Ala657GlyfsX10) and 2 novel frame shift mutations, c.2030T>A (p.Val677Glu) and c.862G>C (p.Gly288Arg) at unusual mutational sites.

Conclusion

In conclusion, we have reported a missense mutation c.2011T>C [p.Ser671Pro]) in exon 3 of *COL10A1* gene in a consanguineous Pakistani family. Review of literature has revealed a series of missense mutation in *COL10A1* gene from different ethnic backgrounds suffering from autosomal dominant form of MCDS confirming that mutations in this gene can lead to autosomal dominant metaphyseal chondrodysplasia Schmid type in local population.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20200624130642

Statement of conflict of interest

The authors have declared no conflict of interest.

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