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

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A B S T R A C T

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 revealed a previously reported a missense mutation (c.2011 A > 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.

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). 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. 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).

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) (rs1110333552) 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, 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 COL10A1 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 metafysal 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.

References


Supplementary Material

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Supplementary Table I. Clinical features and COL10A1 mutation in 8 patients with Schmid type of Metaphyseal Dysplasia and in 2 control subjects.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Position in pedigree</th>
<th>Pheno-type</th>
<th>Gender</th>
<th>Age (Years)</th>
<th>Height (cm)</th>
<th>Weight (Kg)</th>
<th>Mutation Other abnormalities (If any)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IV-13</td>
<td>Dwarf</td>
<td>Female</td>
<td>40</td>
<td>129</td>
<td>41</td>
<td>c.2011 A &gt; G Pain in pelvic region</td>
</tr>
<tr>
<td>2</td>
<td>IV-1</td>
<td>Dwarf</td>
<td>Female</td>
<td>40</td>
<td>132</td>
<td>34</td>
<td>c.2011 A &gt; G Restlessness</td>
</tr>
<tr>
<td>3</td>
<td>V-17</td>
<td>Dwarf</td>
<td>Male</td>
<td>25</td>
<td>132</td>
<td>59</td>
<td>c.2011 A &gt; G Not applicable</td>
</tr>
<tr>
<td>4</td>
<td>IV-6</td>
<td>Dwarf</td>
<td>Female</td>
<td>60</td>
<td>119</td>
<td>60</td>
<td>c.2011 A &gt; G Pelvic and joint pain</td>
</tr>
<tr>
<td>5</td>
<td>V-12</td>
<td>Dwarf</td>
<td>Female</td>
<td>35</td>
<td>132</td>
<td>40</td>
<td>c.2011 A &gt; G Asthma, miscarriage</td>
</tr>
<tr>
<td>6</td>
<td>V-10</td>
<td>Dwarf</td>
<td>Male</td>
<td>36</td>
<td>132</td>
<td>60</td>
<td>c.2011 A &gt; G Not applicable</td>
</tr>
<tr>
<td>7</td>
<td>V-13</td>
<td>Normal</td>
<td>Male</td>
<td>65</td>
<td>157</td>
<td>65</td>
<td>c.2011 A Not applicable</td>
</tr>
<tr>
<td>8</td>
<td>V-8</td>
<td>Dwarf</td>
<td>Female</td>
<td>25</td>
<td>121</td>
<td>50</td>
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</tr>
<tr>
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<td>III-8</td>
<td>Normal</td>
<td>Female</td>
<td>60</td>
<td>142</td>
<td>41</td>
<td>c.2011 A Not applicable</td>
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<tr>
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<td>Dwarf</td>
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<td>25</td>
<td>134</td>
<td>46</td>
<td>c.2011 A &gt; G Not applicable</td>
</tr>
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</table>

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