Author’s response to reviews

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

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Author’s response to reviews:

Dear Editor,

Thanks for your letter dated October 22. We were pleased to receive several comments from editor and reviewer, which enabled us to further improve our work. We appreciate you and reviewers for the time and efforts that you have put into reviewing the previous version of the manuscript.

Appended to this letter are our point-by-point responses to the comments raised by editor and reviewer. The comments were reproduced and our responses were given directly afterward. Based on the instructions provided in your letter, we uploaded the files of the revised manuscript, table and figures.

We hope that this version is acceptable for publication in “BMC Medical Genetics”. Should you have any question, please feel free to contact me. Thank you!

Yours sincerely,

Qing-Lin Kang

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Reviewer reports:

Thank you very much for the improved version of the manuscript. As it has the potential of shedding more light on clinical features and genotype-phenotype correlation, these points should be deepened:

1. For genealogies, please give details about severity of every carrier individual, not only black/white fillings. This could be done using grayscale or similar scale, for instance.

Response: Thanks for this kind suggestion. The details about disease severity of each affected individual were exhibited in Table 1, and we further gave this information in revised Figure 1 using grayscale according to your suggestion. We think it is better to show the severity of all patients.

2. Both reviewers suggested to improve clinical depiction of cases, and to do a genotype-phenotype correlation. This was not comprehensively done. Supplementary table is only a compilation of variants, not a correlation. For example, it would be good to group patients with protein-altering variants (PAVs; e.g. missense variants, in-frame deletions or duplications) versus those with protein-truncating variants (PTVs; e.g. nonsense, frameshift, splicing, etc.) and to compare the frequencies of their clinical findings. This should be commented in the discussion section.

Response: Thanks for your comment. We are sorry for the insufficient clinical descriptions, especially genotype-phenotype correlation. As we know, the highlight of clinical findings in the present families was irregular dominance instead of some unique features. Even so we still added more details about clinical depiction of the current cases as possible to the revised manuscript (Line 143 to 146, Page 7; Line 275 to 279, Page 13). In terms of the analysis of genotype-phenotype correlation, we have tried our best to collect the clinical features of those from previous reports, which was further analyzed and summarized in the “Discussion” section according to your suggestion (Line 249 to 274, Page 12 to 13).

3. The severity grading in Table 1 is nice, but it can be improved. Unless you have carried out a quality of life questionnaire to all affected individuals and the asymptomatic carrier, this concern cannot be included as it is not standardised. Instead, you may add radiographic findings, age of onset, or to determine stature ranges, but using standard deviations as there are several children and it is not possible to know their final stature.
Response: Thanks for this very helpful suggestion. The quality of life has been excluded from grading criteria due to the lack of standardization. In addition, we added radiographic findings, stature ranges and specific clinical presentations to the grading criteria. The revised version was updated according to the new grading criteria (as shown below and in revised Table 1).

“Mild” indicates that the patients exhibit i) short stature (mean - 1.5 SD < Height < mean - 0.5 SD) without evident abnormal clinical or radiographic manifestations, or ii) mild genu varum was involved. “Moderate” patients represent that i) mean - 2.5 SD < Height ≤ mean - 1.5 SD, or ii) similar typical radiographic manifestation as illustrated in supplementary material.

“Severe” patients show that i) short stature (Height ≤ mean - 2.5 SD), ii) similar radiographic findings to severe manifestation shown in supplementary material, or iii) unbearable clinical symptoms, such as arthralgia and restricted motion of the joints.

Notably, we previously proposed that there was a phenomenon “atavism” in family 1, but no radiographic examination was performed for him due to individual’s reluctance. However, for optimizing grading criteria and updating clinical findings we had further tried to invite II:1 in family 1 to perform radiographic examination. Fortunately, we got the radiographic findings of him, which showed that there was a mild genu varum (the left radiograph shown in supplementary materials. 4), so we failed to continue our previous view that he was absolutely healthy. According to updating grading criteria, II:1 in family 1 was a mild case. Previous descriptions about II:1 in family 1 in the “Results” and “Discussion” section have been modified (Line 154 to 155, Page 7 to 8; Line 162 to 163, Page 8; Line 183, Page 9; Line 207 to 215, Page 10).

4. It would be helpful to add a figure showing radiographs of a mild, moderate and severe case in order to compare them.

Response: Thanks for this comment. According to your suggestion, we have added the radiographs showing mild, moderate and severe cases to supplementary material (Supplementary Materials. 4), which will be helpful to provide the reference for severity grading and other comparisons.

5. Also, there is a current report that bi-allelic variants in COL10A1 can cause a severe form of chondrodysplasia (PMID: 28830906). Is there any clinical overlapping between those severe cases reported by you and those reported in that work? If so, full COL10A1 sequencing for severe cases is mandatory.
Response: Thanks for this comment. We had ever carefully read that interesting article, and we thought that the authors just found a unique phenomenon that bi-allelic variants of COL10A1 leaded to more severe phenotype of chondrodysplasia than heterozygous mutations. That severe phenotype, such as short stature was even more severe than the severe cases in our study. However, whether the disease in that study is MCDS still needs further exploration due to insufficient evidence. The clinical manifestations of that disease are indeed very similar to MCDS, and both of the pathogenic genes are COL10A1, but notably, the pathogenic mechanism that is critical to identify a genetic disease is very likely to be different from MCDS. So far, all identified MCDS patients were caused by COL10A1 heterozygous mutations, most of which were located in NC1 domain (The mutation site in that study was located in NC2 domain, which was the first report about mutation in this area), and there is no doubt that MCDS is an autosomal dominant disease (That study was not suitable for this well-known view). Therefore, the severe cases they described may be a new type of chondrodysplasia. We made sure that our cases were caused by heterozygous mutations, which had been validated by Sanger sequencing. In addition, grading of disease severity in our study was based on the completely identical genotype, so it was more likely that extra reasons rather than single genetic factor leaded to irregular dominance, which has been discussed in the “Discussion” section. In short, there is no evidence to link our study to that study, but this interesting point attracts our attention to further exploring the relation of its mechanism to MCDS.

6. Albeit the work included several different in-silico approaches to interpret the variants, it is mandatory to depict what ACMG 2015 criteria these variants meet. In-silico analyses are only a supportive criterion, that is, it is one of the weakest supports of pathogenicity, and c.1765T>A variant may meet benign criteria as well, as it is present in an asymptomatic carrier.

Response: Thanks for this comment. The pathogenic evidences of the two variants include: i) The variant p.Phe589Ile or p.Lys616Glu was identified in all affected individuals in family 1 or 2, respectively, and all available unaffected family members and 250 healthy donors did not carry any COL10A1 variants. Therefore, the relative risk was significantly increased with COL10A1 variants. ii) The COL10A1 heterozygous mutations had been identified to result in MCDS as an independent genetic risk factor, and the typical clinical features of the two families showed that the disease they suffered was MCDS. So we have another evidence to believe that COL10A1 heterozygous variants in this study were pathogenic factors. iii) As illustrated in protein model, the two variants were located in NC1 domain which is mainly to assist the folding of the peptide chain and homotrimer formation. Importantly, all of the other variants located in this area had been reported to lead to MCDS. Therefore, the variants in the present study was also likely to be pathogenic. iv) The changed amino acid residues were highly conserved among several species. The in-silico analyses, such as Polyphen-2 and PROVEAN indicated that the variants were deleterious.
We have checked the “ACMG 2015 criteria”, and the two novel variants in this study are suitable for PS4 (The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls), PM1 (Located in a mutational hot spot and/or critical and well-established functional domain without benign variation), PP1 (Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease), PP2 (Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease), PP3 (Multiple lines of computational evidence support a deleterious effect on the gene or gene product), PP4 (Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology), respectively. Therefore, both of the variants meet the criteria of pathogenic variants, which has been added to the “Results” section (Line 185 to 187, Page 9).

On the other hand, all individuals carrying COL10A1 variants in this study showed varying degrees of MCDS after optimizing the grading criteria and updating the findings. Therefore, the variants only meet the item, BP1 (missense variant in a gene for which primarily truncating variants are known to cause disease) in the criteria for classifying benign variants, and consequently they do not meet the criteria of benign or likely benign variants. In summary, the two novel COL10A1 variants are pathogenic in MCDS according to the “ACMG 2015 criteria”.

Other minor changes:

1. Description of individuals in table 1, in terms of genealogy location, should follow the same rules that for the figure (e.g. I:1)

Response: Thanks for the kind comment. We are sorry for this point, and the descriptions of them in revised Table 1 have been modified following the same rules as the figures and manuscript.

2. Not all the figures are available in this new version of the manuscript.

Response: We are sorry for this mistake. Only modified figures were included in previous version of revised manuscript, and we neglected the other figures. Now in the new version, all of the figures are available.
Franco Cammarata-Scalisi (Reviewer 2):

1. Article that provides the data of two new mutations in the COL10A1 gene without any novel contribution from the clinical point of view. Even the description is poor in terms of perinatal history, among others. The positive is that it highlights the variable expressiveness of the entity.

Response: Thanks for this kind comment. As you said, the clinical features of patients in this study were common, and no unique clinical presentation was exhibited in them. In addition, we checked the perinatal histories of these affected and unaffected individuals according to your suggestion, which showed no abnormal situation. This updated detail has been added to revised manuscript (Line 143 to 144, Page 7). Despite these common clinical features, the novelty of this study is that it highlights a new potential genetic pattern of MCDS, irregular dominance, which we believe sheds more light on this disease. All of these indicates that there may be a key regulatory mechanism affecting the disease severity in the pathogenesis of MCDS that needs to be further explored. In summary, it is the description of irregular dominance and several other rules that provide insight into understanding of this disease from a new perspective, so we firmly believe this work is valuable for MCDS. Thank you again.

2. Although databases can be perfectly used to describe the number of mutations, they are not updated so it is recommended to review recent articles.

Response: Thanks for this kind suggestion. We have checked all recent relevant articles, and a recent variant (p.Val603fsX609) was added to the supplementary table. Now we made sure that all mutations associated with MCDS were included in this manuscript and supplementary material. This information has been modified in the “Introduction” and “Discussion” section (Line 67 to 68, Page 4; Line 243, Page 12).

3. Finally review the writing of the article.

Response: Thanks for this comment. In terms of language editing, the previous revised version of the manuscript had been thoroughly modified by professional editor, and we further invited native English speaker to check it with us again. Therefore, we believe that the writing of this manuscript is acceptable.