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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Version: 1 Date: 03 Oct 2019

Author’s response to reviews:

Dear Editor,

Thanks for your letter dated September 10. We are pleased to know that our work is rated as potentially acceptable for publication in “BMC Medical Genetics”. We appreciate you and two reviewers for the time and efforts that you have put into reviewing the previous version of the manuscript. Your suggestions have enabled us to improve our work.

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

A series of corrections made our conclusions more scientific and rigorous. As for the language, extensive modification by a native English speaker was performed, which we think improved the quality of written English. We hope that the revised manuscript is acceptable for publication in “BMC Medical Genetics”. Thank you again!
Should you have any question, please feel free to contact me.

Yours sincerely,

Qing-Lin Kang

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Reviewer reports:

Sadaf Naz (Reviewer 1): The authors report the identification of two new variants in COL10A1 associated with MCDS.

I have the following comments:

Major comments:

1. There are 54 COL10A1 variants reported as of today (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=COL10A1) and current literature. At least two are in the signal peptide, another in the triple helical domain. This should be corrected in the manuscript and supplement. All, except one variant, are reported to be dominantly inherited. The authors need to propose genotype-phenotype correlations after considering genetic and phenotypic descriptions of all reported patients with COL10A1 variants with their comparison with the phenotype in the two families presented here.

Response: Thank you for your helpful suggestions. Now we have checked the above database and all relevant literature, and updated information about the reported COL10A1 variants was presented in sections of "Background" (Line 74 to 78, Page 4), "Discussion" (Line 254 to 261, Page 12) and "Supplementary material. 1". To our knowledge, over fifty kinds of mutations in COL10A1 have been reported, and 50 variants of those are directly associated with MCDS. In addition, we further expounded the genotype-phenotype correlations of the present two families combined with previous studies, which was added to the "Discussion" (Line 261 to 268, Page 12 to 13).
2. Please remove "Despite the significant clinical and radiological features found in MCDS patients, there is still no systematic description in terms of the phenotype and heredity characteristics of MCDS nowadays." This is not correct.

Response: Thanks for your correction. According to the comment, this sentence in the "Background" section has been removed (Line 60 to 62, Page 3). Now we also think that it is not rigorous enough. Thanks again.

3. The authors need to define atavism and what they are specifically labeling as such.

Response: We are sorry for our unclear explanation of the meaning of "atavism". Atavism is a phenomenon of the reappearance of an ancestral character in a descendant individual whose immediate ancestors lacked this character. We have added the general definition of it in the first paragraph of the "Discussion" section. Afterwards, we further explained in more detail what it means in the present study based on the genetic characteristics of family 1. (Line 215 to 224, Page 10 to 11)

4. The convention in naming individuals in pedigrees is I:1 or I-1 and not I1. This should be corrected in the manuscript.

Response: Thanks for your kind suggestion. All naming throughout the manuscript has been modified according to the comment. For instance, "I1" has been replaced by "I:1", and so has the others.

5. The authors should indicate in materials and methods and pedigree legend or figure, all individuals who participated in the study.

Response: It is a really helpful suggestion. All individuals who participated in the study have been labeled with "*" in the pedigrees (Figure 1) and described in manuscript (Line 97 to 98, Page 5) according to the comment, which would be more conducive to the readers' understanding.
6. The manuscript requires editing for writing the manuscript in scientific style and removing grammatical errors.

Response: We are sorry for the not specially good English expression, particularly a few grammatical errors. Extensive modification by a professional native English speaker had been performed. We think that the quality of the manuscript has been improved.

7. The conservation of amino acids in figure 3E should include diverse vertebrate species arranged in order (at least one each from mammals, birds, reptiles, amphibians and fish).

Response: Thanks for your kind suggestion. We have rearranged the order of the species. In turn, they are mammals (human, mouse), birds (chicken), reptiles (lizard), amphibians (frog) and fish (zebrafish) as shown in revised Figure. 3E. This modification not only made the figure look clearer, but also improved the scientific and representative nature of this study.

Minor Comments:

Title

1. Please change the title of the manuscript. It is too long and grammatically incorrect.

Response: Thank you for the title suggested. The previous title has been replaced, and the revised one is "Identification of two novel COL10A1 heterozygous mutations in two Chinese pedigrees with Schmid-type metaphyseal chondrodysplasia". We think it may be better. (Line 1 to 3, Page 1)

Abstract

1. Please remove "However, the research about unusual phenotype features of MCDS is rare". The authors do not present unusual phenotype features of MCDS in the abstract or point out what they are anywhere else in the manuscript.

Response: As you said, we did not describe the unusual phenotype features of MCDS in the "Abstract" and manuscript. To be exact, what we aim to express here is the differential expressivity of the disease rather than unusual phenotype features. Now we have replaced the sentence "However, the research about unusual phenotype features of MCDS is rare" with "However, descriptions of the expressivity of MCDS are rare", which may be more appropriate. Thanks for this helpful comment. (Line 22 to 24, Page 1 to 2)
2. Remove "Then" from "then the spatial model of type X collagen (α1) C-terminal noncollagen…"

Response: We have removed "Then" from the previous sentence according to the comment. The new version is "A spatial model of the type X collagen ..." (Line 29, Page 2)

3. Remove "members exhibited evident irregular dominance." Replace with "the phenotype exhibited irregular dominance"

Response: Thanks for your kind correction. The sentence "the affected family members exhibited evident irregular dominance" have been replaced with "the phenotype of affected family members exhibited irregular dominance." (Line 31 to 32, Page 2)

4. Here and elsewhere in the manuscript remove words such as "remarkably", "extremely" when presenting data.

Response: Thank you for this comment. We have removed words such as "remarkably", "extremely" and "evidently" here (Line 36, Page 2) and elsewhere when presenting data throughout the manuscript.

Background

1. Remove "Besides" from "Besides, the distinctive clinical features of MCDS…"

Response: "Besides" have been removed from the sentence "Besides, the distinctive clinical features of MCDS…" (Line 53, Page 3)

2. Please revise "confirmed pathogenic gene". It is not the gene but variants which are pathogenic.

Response: Thanks for your kind correction. This inappropriate expression has been modified. The phrase "... as the only confirmed pathogenic gene" has been replaced by "... when pathogenic variants occur in this gene." (Line 64, Page 3)
3. Please revise "…and elaborates the rules of the disease." Please write it scientifically, and only after thorough review of literature with genotype-phenotype correlations.

Response: Thanks for the comment. For more scientific writing, the sentence "The present study describes unique phenotype features of two unrelated Chinese pedigrees with MCDS and elaborates the rules of the disease" have been changed to "The present study describes the phenomenon of irregular dominance and summarizes several potential genetic patterns in two unrelated Chinese pedigrees with MCDS." (Line 84 to 86, Page 4)

Methods

1. Please remove "Then" from "Then we sequenced the whole-exome of all available affected individuals.."

Response: "Then" have been removed from the sentence "Then we sequenced the whole-exome of all available affected individuals…". (Line 106, Page 5)

2. Please indicate here what frequency criteria were used for identifying common SNP. "…which rejected common single nucleotide polymorphism (SNP) sites".

Response: It is a very nice comment. As we indicated in "Results", the variants in which minor allele frequency (MAF) are greater than 1% were identified as SNP sites. We have added this information to the "Methods" section (Line 121, Page 6). In addition, the workflow of bioinformatics and variant filtration process was illustrated in "supplementary material. 3" in details.

Results

1. Please rephrase "…and there was a trend that male patients were significantly more severe than females." to "male patients were significantly more severely affected than females." Please also point to data and numbers which supports this.

Response: Thanks for your suggestion. The sentence "… and there was a trend that male patients were significantly more severe than females" has been replaced with "male patients were significantly more severely affected than females" according to the comment (Line 169 to 171, Page 8). In addition, we have added the detailed data about the morbidity rates and statistical analysis (Fisher exact test) to this part for supporting this point (Line 153 to 155, Page 7 to 8), and we further discuss it in the "Discussion" section (Line 244 to 249, Page 12).
2. Please remove "Interestingly" from "…interestingly, the two novel mutations were not found in ExAC and gnomAD database,"

Response: "Interestingly" have been removed from the sentence "Interestingly, the two novel mutations were not found in ExAC and gnomAD database" according to the comment. (Line 180, Page 9)

3. Please write wild type instead of wild in the following statement "… hydrophobicity of wild residue". Please make this change in other places in the manuscript as well.

Response: Thanks for your kind correction. "wild" has been replaced by "wild type" here (Line 202, Page 10) and elsewhere throughout the manuscript.

Discussion

1. Please rephrase the following so it is grammatically correct: "Moreover, the presentations of II1 in family 1 was normal absolutely including relatively moderate stature without any deformity,…"

Response: This sentence has been modified to "The presentation of II:1 in family 1 was absolutely normal without any deformity except moderate stature …" (Line 217 to 219, Page 10)

2. Please rephrase the following "…which was similar with a mutation-attacking person who exhibited normal phenotype in a previous report". The word "mutation-attacking" is not correct.

Response: This uncorrected word has been modified and the revised sentence is "…which was similar to a person who carried a COL10A1 variant, but exhibited normal phenotype in a previous report". (Line 222 to 224, Page 11)

3. Please rephrase "..irregular dominance of these MCDS patients." Dominance is of traits, not patients.

Response: Thanks for your kind correction. The phrase "…irregular dominance of these MCDS patients" has been replaced with "… the associated mechanism of the irregular dominance phenomenon" (Line 231, Page 11)
4. Please rephrase "….patients that onset age was only around 6 months old, but conversely there was only short stature in those abnormal development occurred in 10 months old or later." This is grammatically incorrect.

Response: Thanks for your kind correction. This sentence has been replaced with "... decreased quality of life, including unstable standing or waddling gait was observed in patients whose onset ages were only around 6 months old. Conversely, only short stature without other deformities was exhibited in those whose onset ages were 10 months old or later". (Line 240 to 244, Page 11 to 12)

5. Please reword "...and 15 patients of 28 males (54%) and 5 patients of 16 females (31%) were.." to "...and 15 of 28 male patients (54%) and 5 of 16 female patients (31%) were.."

Response: Thanks for this comment. As for the description about the morbidity of this disease, we have added the detailed data to the "Results" section (Line 153 to 155, Page 7 to 8). So this sentence in "Discussion" has been replaced with "we found that there were possible, but not significant, potential differences in gender susceptibility in MCDS" (Line 244 to 247, Page 12).

6. Please revise, "Moreover, despite the trend that male patients were more severe than females was presented in family 2, we still could not draw a firm conclusion due to the rather small sample size." This is grammatically incorrect.

Response: Thanks for your kind correction. This sentence has been modified to "Moreover, despite the trend that male patients were more severely affected than females, as observed in family 2, we still could not draw a firm conclusion due to the rather small sample size" (Line 247 to 249, Page 12).

7. Please remove "as for" and "it" from the following "As for the molecular structure of type X collagen, it is a homotrimer…"

Response: Thanks for your kind correction. The previous version has been replaced with "The molecular structure of type X collagen is a homotrimer of …" (Line 250, Page 12)
8. Please remove "Obviously" from "Obviously, all of the identified mutation sites of MCDS associated with COL10A1 including mutations in present study were located in NC1 domain…"

Response: Thanks for your kind correction. The word “Obviously” has been removed from this sentence. Revised one is "All of the identified mutation sites of COL10A1 associated with MCDS, including mutations in the present study, are located in the NC1 domain, except for two missense mutations in the signal peptide and one in the triple helical domain". (Line 257 to 261, Page 12)

9. Please revise "On the other hand, the content of correctly folded homotrimer of collagen X reduced accordingly, and functional haploinsufficiency became the most probable cause of the clinical phenotype in MCDS."

Response: The previous version has been replaced with "Meanwhile, the quantity of correctly-folded collagen X is reduced, and therefore functional haploinsufficiency was the most likely cause of the MCDS". (Line 274 to 277, Page 13)

10. Please remove "definitely" from "…pathogenic molecular mechanism of nonsense mutations in MCDS definitely"

Response: Thanks for your kind correction. The word "definitely" has been removed from this sentence. (Line 281, Page 13)

11. Revise "Nevertheless, it was completely different that the process of COL10A1 missense mutations results in MCDS" so that it is grammatically correct.

Response: We are sorry for the grammatical mistake. This sentence has been corrected, becoming "Nevertheless, the process via which COL10A1 missense mutations result in MCDS is completely different". (Line 281 to 283, Page 13)

12. Please change appeared to appears in the following "…appeared to be a disruption of collagen."

Response: Thanks for your kind correction. The word "appeared" in this sentence has been changed to "appears". (Line 284, Page 13)
13. Please change "In present study, one of the novel substitutions (p.Phe589Ile) locate on hydrophobic area and the other (p.Lys616Glu) on the surface of assembled trimer." To "In the present study, one of the substitutions (p.Phe589Ile) affects a hydrophobic area and the other (p.Lys616Glu) is predicted to affect the surface of assembled trimer."

Response: Thanks for your kind correction. This sentence has been modified according to the comment. (Line 285 to 288, Page 13 to 14)

14. Please change "…which seriously impacts the assembly and stability of hydrophobic channel of collagen X trimerization possibly." To … which may seriously impact the assembly and stability of hydrophobic channel of collagen X trimerization."

Response: Thanks for your kind correction. The sentence has been replaced with "... which is likely to seriously impact the assembly and stability of the hydrophobic channel and thus hinder collagen X trimerization". (Line 289 to 291, Page 14)

15. Please change "And the other substitution (p.Lys616Glu) transfers the alkaline residue site to acidity,…". One, sentence should not start with "and". Two, "transfers" should be replaced by "changes" . Three, acidity should be replaced by "acidic residue".

Response: Thank you very much for your kind correction. These mistakes has been corrected in the revised manuscript. This sentence has been replaced by "The other substitution (p.Lys616Glu) changes the residue site from alkaline to acidic, potentially destroying …" (Line 291 to 292, Page 14)

16. Please shorten the following "To our knowledge, the present study is the first report about COL10A1 missense mutations in Chinese pedigrees with MCDS, which lays the foundation for genetic research of MCDS in Chinese and further elaborates phenotype features and heredity characteristics of MCDS. In addition, dominant negative effect may be playing an important role in development of MCDS [16,28], but it has not been fully confirmed and still needs further experimental verification." Language editing is also required here.

Response: For the sake of conciseness, this paragraph has been shortened and modified. Professional language editing was also performed here and elsewhere throughout the manuscript. The revised version is "To our knowledge, the present study is the first report of COL10A1 missense mutations in Chinese pedigrees with MCDS. In addition, the dominant negative effect may be playing an important role in the development of MCDS [17, 33], but this undetermined mechanism will need further experimental verification". (Line 297 to 303, Page 14)
17. Please revise "Besides, our research preliminarily elaborated several rules on occurrence and development of the disease based on the two MCDS…". This statement needs to be grammatically correct and should also supported by data presented elsewhere after contrasting with other studies.

Response: Thanks for this comment. After repeated thoughts and discussions, we thought that there was not enough evidence to conclude common rules of this disease based on only two pedigrees due to the lack of extensive data. The previous version has been replaced with the summaries of this two families in the manuscript. So the revised conclusion is "In addition, our research revealed the phenomenon of irregular dominance and summarized several potential genetic patterns in the two Chinese pedigrees with MCDS" (Line 310 to 312, Page 15). Accordingly, the conclusion in "Abstract" was also corrected (Line 42 to 43, Page 2).

18. Please delete "We also summarized the underlying pathogenic mechanisms of COL10A1 mutations."

Response: Thanks for your suggestion. This sentence in the "Conclusion" has been removed. (Line 315 to 316, Page 15)

Table 1:

1. Please change "have extra performance" to "have additional phenotypes"

Response: Thanks for your kind correction. This phrase has been changed in Table 1 according to the comment.

Legends

Figure 2

1. Please change were to are in the following, "…irregularity of the growth plates of distal femur (white arrows) were shown in X-ray image…”

Response: Thanks for your kind correction. This word has been changed in the legend (Figure 2) according to the comment. (Line 473, Page 22)
2. This should be re-worded to be scientifically and grammatically correct "plates and even hip (white arrows) (C). In family 2, the boy, proband, showed a deformity of the lower extremities (D) and similar radiological presentations (white arrows) (E) with proband in family 1. However, proband's mother in family 2 exhibited almost normal appearance in X-ray image…"

Response: Thanks for this comment. A series of scientific and grammatical mistakes in this paragraph has been removed. The revised version is "Moreover, the X-ray image of III:1 in family 1 also presented bowing of the femurs, deformity of growth plates and hips (white arrows) (C). In family 2, the proband showed deformity of the lower extremities (D) and similar radiological presentation (white arrows) (E) to proband in family 1. However, the proband's mother in family 2 exhibited normal appearance in X-ray image (F)". (Line 474 to 478, Page 22)

Figure 4

1. "The two substitutions p.Phe589Ile and p.Lys616Glu were illustrated in the ribbon protein modeling with two mutually perpendicular perspectives, which showed that two substitutions were located in different subunits". Please rephrase after consulting manuscript descriptions of ribbon diagrams.

Response: Thanks for this very helpful comment. For the consistency of the manuscript and legend, modification of this sentence has been performed and the revised legend is "As illustrated in the ribbon protein model, both of the novel mutations are located in the NC1 domain of type X collagen (α1). One of the substitutions (p.Phe589Ile) affects a hydrophobic area and the other (p.Lys616Glu) is predicted to affect the surface of the assembled trimer". (Line 488 to 491, Page 23)

2. Please change following "Furthermore, changes in present study and mostly previous mutations (supplementary data) were located in NC1 domain." To "Changes in the present study and most previous variants (supplementary data) are located in NC1 domain."

Response: Thanks for your kind correction. This sentence has been changed according to the comment. (Line 495 to 496, Page 23)

Franco Cammarata-Scalisi (Reviewer 2):

Always remember to mention a gene should be italicized.

Response: Thanks for this kind reminder. We have checked this point throughout the manuscript to make sure they are all italicized.
The strength of this work is the number of individuals studied, it would be more interesting to describe better through a table the clinical characteristics found.

Response: Thanks for your kind suggestion. In this regard, we have collected the clinical characteristics of all affected individuals in the two pedigrees. However, there was no unique features apart from those typical presentations such as short stature, waddling gait, coxa vara and bowing of the long bones. However, the unique point in this study is that affected individuals exhibited differential severity. We therefore graded these patients based on the disease severity of them according to the following criteria: "Mild" indicates that the patients only exhibit short stature without any additional evident abnormal manifestations; "Moderate" represents that patients have additional phenotypes such as coxa vara and bowing of the femur; "Severe" indicated that patients show decreased quality of life including unstable standing or waddling gait (Table 1). We presented the items of "Height" and "Severity of disease" in Table 1 to provide a concise and accurate description of these patients' clinical features. Thanks again!

References can use updated articles.

Response: Thanks for this comment. In addition to those classical references, we have added the following updated references, which we believe would improve the quality of this manuscript:


