Author’s response to reviews

Title: A novel mutation in the OAR domain of PITX3 associated with congenital posterior subcapsular cataract

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Author’s response to reviews:

Editorial Board
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Dear Sir/Madam,

Thank you very much for your letter and for the reviewers’ comments concerning our manuscript entitled “A novel mutation in the OAR domain of PITX3 associated with congenital posterior subcapsular cataract” (MGTC-D-18-00300).
We greatly appreciate both your help and that of the reviewers concerning improvement to this paper. We cherish this opportunity to revise the manuscript and have tried our best to make the revisions.

Based on the comments, we have made careful modifications on the original manuscript. All changes are marked in red in the revised manuscript. We hope that the revised manuscript will meet your journal’s standard.

Please find enclosed the full-length article, entitled “A novel mutation in the OAR domain of PITX3 associated with congenital posterior subcapsular cataract”, for consideration for publication in BMC Medical Genetics.

We thank you again for considering our manuscript. We sincerely hope that this revised manuscript is now suitable for publication.

Yours sincerely,

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Response letter:

To Reviewer #1's comments:

General comments
1. Throughout the manuscript, the others state that this is the first mutation in the OAR domain. While this is accurate, they should also note that the majority of previously reported mutations also result in loss of the OAR domain as they cause frameshift prior to the OAR domain.

Response: Thank you very much for kindly pointing this out. We apologize for the inaccurate conclusion, and this statement in the original manuscript has been revised to “The majority of reported mutations in PITX3 result in a loss of the OAR domain, as they cause frameshifts prior to the OAR domain, while the mutation identified in this paper was located within the OAR domain-encoding region.” (Revised manuscript: page 16, line 59-60 and page 17, line 1-2).

2. Both protein and DNA nomenclature should be given for the mutations (ie, c.797_814del, p.(Ser266_Ala271del))

Response: Thank you for your kindly suggestion. Both protein and DNA nomenclature have been given for the mutations in the revised manuscript.

3. Co-segregation in two individuals in the family could occur by chance. It would be helpful is a more distant family member could be recruited and tested for co-segregation (ie, II-3, II-5, or II-7).

Response: Thank you for your valuable suggestion. As you suggested, the results would be markedly convinced if additional affected family members could be examined and assessed for the PITX3 mutation. We managed to contact some affected family members (III:2, III:4 and IV:1). But they were not willing to undergo DNA sequencing and publish the results. Other affected family members (II:3, II:5, II:7 and III:8), for the limited interpersonal contact with the proband’s family (IV:3), we cannot get in touch with them. Hence, it is a limitation of this study that more distant family members could not be recruited and tested. We have enclosed this limitation at the discussion part in the revised manuscript (Revised manuscript: page 17, line 2-4).
Background

4. The authors report that there are four groups of genes that cause cataract, but not all genes fit into one of these 4 groups, so this should be re-phrased to 'four major groups' or something similar

Response: Thank you very much for kindly pointing this out. “These genes may be arbitrarily divided into four groups” in the original manuscript is now revised to “These genes may be arbitrarily divided into four major groups” (Revised manuscript: page 11, line 29-30).

5. 'Knockdown of PITX3 induced small eyes without lenses': this should be specified to be in mouse and the mouse gene nomenclature, Pitx3, should be used

Response: Thank you very much for kindly pointing this out. “Knockdown of PITX3 induced small eyes without lenses” in the original manuscript is now revised to “Knockdown of Pitx3 in mouse induced small eyes without lenses” (Revised manuscript: page 15, line 35-36).

6. References need to be given for each of these mutations. I could not find a reference for the c.543delG mutation noted by the authors. In addition, the c.38G>A, p.S13N mutation is left off of the list and another one of the variants included, c.94G>A is a questionable variant only reported in Parkinson's disease, not in cataract as suggested by the introduction and figure. In addition, the c.657_673dup17 nomenclature is incorrect- it should be c.640-656dup17)

Response: Special thanks for your careful reviewing. We have removed the c.543delG and c.94G>A mutation and included the c.38G>A mutation, c.657_673dup17 nomenclature is revised to c.640-656dup17. And references have been given for all the included mutations (Revised manuscript: page 15, line 46-54).

7. It would also be useful to note that the c.640_656del mutation is recessive, while the rest are dominant

Response: Thanks for your kindly suggestions. We have enclosed the statement that “The c.640_656del mutation is recessive, while the rest are dominant.” (Revised manuscript: page 15, line 58-60).
Methods

8. The authors should clarify from the start (and throughout the manuscript) that only two members were enrolled. The statement ‘a four-generation Chinese family...was enrolled in this study’ is misleading. Perhaps the authors could state ‘two members of a four-generation Chinese family...’ or something similar

Response: Thank you very much for kindly pointing this out. “a four-generation Chinese family...was enrolled in this study” in the original manuscript is now revised to “we present two members of a four-generation family with isolated autosomal dominant posterior subcapsular cataract” (Revised manuscript: page 11, line 58-60) or “Three members of a four-generation Chinese family were enrolled in this study” (Revised manuscript: page 12, line 19).

9. Since the cataracts are inherited from the father, nothing enrollment of III-6 is irrelevant

Response: Thanks for your kindly suggestions. Genetic analysis indicated that the cataracts are inherited from the father. We believe that the enrollment of the mother (III-6) would help provide the genetic background of the proband. Hence, the III-6 was enrolled in the gene panel assay and Sanger sequencing.

Results

10. gnomAD would represent a better database to cite for absence of the mutation in the general population since this database includes an East Asian subpopulation

Response: Thank you for your valuable suggestion. GnomAD database had been used to cite for the absence of the mutation in the general population in the revised version (Revised manuscript: page 14, line 33-37).

11. the authors state that the mutation is expected to disrupt the protein structure, but it is not clear what this is based on. In silico or functional analysis would be beneficial, especially because the mutation is an in-frame deletion

Response: Thank you for your valuable suggestion. We have applied the standards of the American College of Medical Genetics (ACMG) to the variant to determine its likelihood of pathogenicity. Variants were classified as benign, likely benign, pathogenic, likely pathogenic, and novel variants of uncertain clinical significance according to ACMG (Revised manuscript: page 13, line 15-21). The deletion mutation we identified in this paper was classified as likely pathogenic (Revised manuscript: page 14, line 38-40). We hypothesize that this mutation may be
able to disrupt the protein structure, or alter the OAR domain DNA-binding profile and/or transactivation activities. But exact mechanisms are unclear. We intend to conduct further functional analysis and DNA-protein binding assay to confirm the pathogenicity of the mutation (Revised manuscript: page 16, line 50-52).

Discussion

12. The authors state that PITX3 plays a major role in cataract, but this gene does not explain a large proportion of cases, so this seems to be overstatement.

Response: Thank you very much for kindly pointing this out. “PITX3 plays a major role in cataract” in the original manuscript is now been deleted in the revised manuscript.

13. Further comparison of this mutation and other previously reported mutations should be added, along with noting that the majority of the prior mutations also disrupt the OAR domain. Especially of interest is the fact that the majority of dominant alleles result in erroneous protein extension, while this in-frame deletion would not be expected to do so.

Response: Thank you for your valuable suggestion. Further comparison of the mutation and other previously reported mutations had been added in the revised version (Revised manuscript: page 16, line 33-52).

The deletion mutation in this study was an in-frame deletion mutation. It resulted in a deletion of 6 amino acid residues at the C-terminus that altered the OAR domain. Interestingly, the majority of previously reported-PITX3 mutations were out-of-frame deletions that lead to the disruption of the OAR domain, while this in-frame deletion would not be expected to do so. For the downstream codons to be translated properly, folding of the OAR domain cannot be disrupted. Perhaps its DNA-binding profile and/or transactivation activities may have been altered. However, whether the OAR domain folding or the DNA-binding profile and/or transactivation activities were altered was not clear. Further functional analyses and DNA-protein binding assays are required to confirm the molecular mechanism.

Figures

14. Figure 4 is very low quality as provided and difficult to read. Figure 2 would also benefit from increased resolution.

Response: Thanks for your kindly suggestions. We have increased resolution of Figure 2 and Figure 4 to 600 dpi. We hope that the figures are now clearly enough for read.
To Reviewer #2’s comments:

Introduction

1. Page 1 Line 19: The 30 genes listed by reference 6 (Shiels and Hetjmancik) are those identified for isolated cataract. Many more (over 100) have been linked to cataract more broadly, including many with only minor additional features (eg microcornea). Please clarify this, particularly given that mutations in PITX3 are often associated with more complex phenotypes such as ASMD, but the family described in this paper doesn't appear to have any additional features, or they are not reported.

Response: Thanks for your kindly suggestions. We have detailed that many more genes have been linked to cataract more broadly, including many with only minor additional features (Revised manuscript: page 11, line 17-25), especially enclosed the statement that mutations in PITX3 are often associated with more complex phenotypes such as ASMD (Revised manuscript: page 15, line 18-23).

2. The abstract and the end of the introduction say the aim of the study is to report the mutation in OAR domain of PITX3. I believe the aim of the study was to identify a causative mutation in the proband and her family and the result is that the mutation is in the OAR domain of PITX3. When reading further it becomes clear that a panel of genes was assessed in the patient, but the introduction is focused only on PITX3 and ultimately the OAR domain, giving the impression that only this gene is going to be assessed. Please reframe the introduction to this paper to give an overview of cataract genetics that justifies the testing of the 790 genes in the panel with the aim of identifying a mutation likely to cause cataract. The discussion of the paper should then focus on PITX3 as the main result and highlight and discuss the novelty of the mutation in the OAR domain.

Response: Thank you for your valuable suggestion. The aim of the study was revised to “The aim of this study was to identify causative mutations in a Chinese family with isolated autosomal dominant posterior subcapsular cataract” (Revised manuscript: page 10, line 5-7). And the introduction was reframed to give an overview of cataract genetics (Revised manuscript: page 11, line 17-31) and the use of a gene panel including 790 genes (Revised manuscript: page 11, line 39-56), while the discussion was focused on PITX3 mutation (Revised manuscript: page 15, line 25-61 and page 16, line 1-9).
Methods

3. Please describe the bioinformatics for the analysis for the NGS data in more detail. Which version of the human reference genome was used? How variants were called and prioritised from the NGS panel? What filtering criteria were used? Were all genes considered equally, or analysis limited to genes known to cause cataract?

Response: Thank you for your valuable suggestion. We have enclosed the bioinformatics for the analysis for the NGS data in the revised version (Revised manuscript: page 12, line 58-60 and page 13, line 1-21). The output sequence data were aligned to the reference human genome UCSC hg38 using Burrows-Wheeler Aligner, version 0.7.10. The variants were filtered to eliminate benign variants with MAF (minor allele frequency) > 0.1% in the 1000 Genomes dataset, and the dbSNP, EXAC, Tumor Heart Freq_Hom, ESP6500, and internal databases. All 790 genes were considered equally. Finally, variant prioritization was performed by combining the total depth, quality score, MAF, potential deleterious effect and the existence of mutation reports in common databases, such as The Human Gene Mutation Database (HGMD), The Retinal Information Network (RetNet), ClinVar, and Online Mendelian Inheritance in Man (OMIM).

4. How was the affection status of all the family members shown in the pedigree determined if only the proband and her parents were examined?

Response: Thank you very much for kindly pointing this out. Other family members affected status was obtained from the medical records of the local hospital from previous ophthalmological examinations. We have detailed this in the revised version (Revised manuscript: page 12, line 33-37).

Results

5. Please include individual number labels for every person on the pedigree (eg. '3' under the proband, '5' under her father).

Response: Thank you for your valuable suggestion. We have included individual number labels for every person on the pedigree in Figure 1.
6. For the proband, what age is the first BCVA recorded at? Birth?

Response: Thank you for your valuable suggestion. We have detailed that the proband’s BCVA was recorded when her first referred to our hospital at the age of 10 (Revised manuscript: page 13, line 54).

7. Various units are used for the BCVA measurements, even within the one patient and its not clear if its logMAR or decimal VA that is given. Please choose one measurement scale and make it clear which one. Its currently difficult to know if there was an improvement after surgery (if the scale is decimal) or if remained similar (if it is logMAR).

Response: Thank you for your valuable suggestion. We have chosen logMAR as measurement scale in the revised version.

8. How many variants were found in total in the 790 genes? How many meeting filtering criteria? If additional variants met filtering criteria, they should be listed and an argument made for focusing on the PITX3 deletion.

Response: We have enclosed the full results of the genetic testing panel as Supplementary Table 1 (raw variants) and Supplementary Table 2 (filtering variants) in the revised manuscript. After the data acquisition and analysis, a total of 14076 raw variants were found in the 790 genes, and 211 variants met the filtering criteria. Finally, according to the inheritance mode and literature retrieval, we excluded the genes that were independent of isolated congenital cataract, and one deletion mutation (c.797_814del, p.(Ser266_Ala271del)) in PITX3 was identified as a potentially pathogenic mutation (Revised manuscript: page 14, line 19-29).

9. Were all three available family members subjected to the panel sequencing, or just the proband?

Response: All three family members were subjected to the panel sequencing and Sanger sequencing in the study.

10. When reading the text it sounds like the deletion of 6 amino acids is right at the end of the protein (c-terminus) but looking at Figure 4, it is with in the C-terminal domain (also the OAR domain). Please clarify the description of the location of the mutation.
Response: Thank you very much for kindly pointing this out. We have clarified the description of the location of the mutation as “at the C-terminus of the protein (266-271, from a total of 302)” (Revised manuscript: page 14, line 46-48).

11. Please provide the appropriate nomenclature for the mutation at protein level.

Response: Thank you for your kindly suggestion. Both protein and DNA nomenclature have been given for the mutations in the revised version.

12. Please consider applying the standards of the American College of Medical Genetics (ACMG) to the variant to determine its likelihood of pathogenicity. It is difficult to interpret this variant when only two closely related family members are available, and the mutation is in frame. While the authors speculate it may affect protein folding or DNA binding ability, there is no direct evidence for this presented. All other reported mutations except the missense variant near the N-terminus are frameshift mutations severely disrupting protein sequence. It appears that there are many rare missense mutations in the OAR domain region (residue 258 onwards) listed in gnomAD (http://gnomad.broadinstitute.org/gene/ENSG00000107859). This also makes it difficult to be sure the reported novel mutation is linked to cataract.

Response: Thank you for your valuable suggestion. According to your suggestion, we applied the standards of the American College of Medical Genetics (ACMG) and genomics guidelines to the variant to determine its likelihood of pathogenicity. Variants were classified as benign, likely benign, pathogenic, likely pathogenic, and novel variants of uncertain clinical significance according to the ACMG (Revised manuscript: page 13, line 15-21). The deletion mutation we identified in this paper was classified as likely pathogenic (Revised manuscript: page 14, line 38-40).

The mutation this study identified was an in-frame deletion mutation that resulted in a deletion of 6 amino acid residues. The majority of previously reported dominant alleles cause frameshift prior to the OAR domain, which lead to disruption of the OAR domain and result in erroneous protein extension, while this in-frame deletion would not be expected to do so. For the downstream codons can be translated properly, folding of the OAR domain may not be disrupted. Perhaps, its DNA-binding profile and/or transactivation activities may be altered. However, whether the OAR domain folding or the DNA-binding profile and/or transactivation activities were altered was not clear. Further assays were required to confirm the molecular mechanism. Hence, we intend to undertake further functional analysis and DNA-protein binding assay to confirm the pathogenicity of the mutation (Revised manuscript: page 16, line 33-52).
13. Resolution of text on figure 4 is not high enough to read, even when zoomed in.

Response: Thanks for your kindly suggestions. We have increased resolution of Figure 4 to 600 dpi. We hope that the figures are now clearly enough for read.

Discussion

14. Please include a discussion of the limitations of this project, including that only 2 members were available for clinical and genetic analysis. Discuss the use of the gene panel and any limitations with it.

Response: Thank you for your valuable suggestion. The uses of the gene panel and the limitation with it have been discussed in the revised version (Revised manuscript: page 14, line 56-60 and page 15, line 1-11). We also have enclosed the limitations of this project in discussion part, including that only 2 members were enrolled in genetic analysis (Revised manuscript: page 17, line 3-11).

15. The uncertainties around whether this is really the right variant need to be addressed. This would be simpler if the full results of the genetic testing panel were included as well.

Response: Thanks for your kindly suggestions. We have enclosed the full results of the genetic testing panel as Supplementary Table 1 (raw variants) and Supplementary Table 2 (filtering variants) in the revised manuscript (Revised manuscript: page 14, line 19-23).

16. This paper would be markedly improved if additional (affected) family members could be examined and assessed for the PITX3 mutation, although this reviewer acknowledges that this is not always possible.

Response: Thank you for your valuable suggestion. As you suggested, the results would be markedly convinced if additional affected family members could be examined and assessed for the PITX3 mutation. We managed to contact some affected family members (III:2, III:4 and IV:1). But they were not willing to undergo DNA sequencing and publish the results. Other affected family members (II:3, II:5, II:7 and III:8), for the limited interpersonal contact with the proband’s family (IV:3), we cannot get in touch with them. Hence, it is a limitation of this study that more distant family members could not be recruited and tested. We have enclosed this limitation at the discussion part in the revised version (Revised manuscript: page 17, line 2-4).