Author’s response to reviews

Title: The mutation spectrum in familial versus sporadic congenital cataract based on next-generation sequencing

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Dear Editor Dafne Solera,

Thank you very much for giving us an opportunity to revise our manuscript, "The mutation spectrum in familial versus sporadic congenital cataract based on next-generation sequencing" (BOPH-D-20-00148R1). We have provided point-by-point responses to the comments below. All changes to the manuscript are indicated by the red text. We hope that our improved manuscript now meets the criteria for publication in BMC Ophthalmology.

We look forward to receiving your decision.

Yours sincerely,

Yi Luo
Response to the Reviewers’ comments:

Dorota Monies (Reviewer 1):
The paper entitled "The mutation spectrum in familial versus sporadic congenital cataract based on next-generation sequencing" the authors have analyzed a cohort of 54 patients who were clinically diagnosed with congenital cataract (CC). These data will improve the knowledge on the mutation spectrum of genes responsible for CC. However, the paper could be improved in several aspects.

Dear Professor Dorota Monies,

Thank you very much for your thorough review and insightful comments, which have helped us improve the paper and make the research more rigorous. We learned a great deal from your suggestions and are truly grateful.

Results
Participant characteristics

The authors should provide more information how they have defined familial cases, who and how many members are affected in tested families.

Reply: Thank you for your suggestion. It is of great importance to provide the information you have mentioned. We added the definition in the Methods section (Page 3, lines 87-92), and refined the Results (Page 3, lines 116-121) according to your suggestion.

In our study, the probands for whom at least one immediate family member had a history of CC were defined as familial cases. A total of 155 subjects of 54 families were recruited in this study, including 16 familial cases (49 subjects in total) and 38 sporadic cases (106 subjects in total). Parental samples in 1 familial case and 6 sporadic cases were not completely obtained for some reasons beyond control. All the familial cases had at least one affected parent (11 mothers and 5 fathers). In addition, the available affected immediate relatives, the brother (7#), the paternal grandfather (6#) and the maternal grandmother (no positive results), in 3 familial cases also participated in the test.

Variants identified

1) Authors should differentiate variants (30) between pathogenic/likely pathogenic mutations and variants of unknown significance (VUS). It should be explained how the identified variants have been validated.

Reply: According to the ACMG mutation guidelines, 17 of 30 variants were classified as pathogenetic, 5 were likely pathogenic, and 8 were uncertain significance (VUS). Sanger sequencing was performed to confirm the candidate variants. Revisions have been made to the Results (Page 4, lines 133-135) and Methods (Page 3, lines 109-112) based on your guidance.
2) The authors say that 54 patients have been recruited along with their parents whereas in most of the sporadic cases (Table 2) segregation is not known. Confirmation of "De novo/sporadic" status of variant requires parental segregation.

Reply: Most of the identified heterozygous variants listed in Table 2 also presented in an unaffected parent. According to typical Mendelian inheritance, if the heterozygous gene also exists in a parent with a normal phenotype, then the pathogenic gene is completely excluded. After testing, the definition of "sporadic" (defined before testing) in these cases cannot be completely determined in the genetic sense. There are several possibilities and explanations. Owing to incomplete and various penetrance, the identified cataract or syndromic associated heterozygous variants are also present in unaffected parents. Those identified non-cataract related genes might indicate other inherited eye disorders or syndromes, in which a cataractous phenotype may not present in every carrier. Another possibility was that the exact cataractous causative genes were located in regions that have not yet been detected, or even that the cataractous phenotype was not caused by genetic factors at all or may involve epigenetic factors. We have deepened this point in the Discussion. (Page 5-6, lines 202-213)

3) It is not clear how the authors have classified "pathogenic and likely pathogenic" variants.

Reply: We apologize for the missing information and incomplete description in the Methods. Variants were classified in accordance with the American College of Medical Genetics (ACMG) and genomics guidelines (attached jpeg file below) as pathogenic, likely pathogenic, uncertain significance (VUS), likely benign, or benign. (Page 3, lines 109-112)

4) Can the authors explain how they have defined causative variants in sporadic cases if identified heterozygous variants are also present in unaffected parent, e.g. families: 3,4,5,6,7,8,9,11 (Table2)

Reply: This phenomenon may be explained by the incomplete and variable penetrance, the mechanisms underlying which remain largely unknown. According to the typical Mendelian inheritance, if the heterozygous gene also exists in a parent with a normal phenotype, then the pathogenic gene should be completely excluded. However, it is possible that parents with a disease-predisposing variant fail to express the corresponding disease phenotype. A recent study also provides strong evidence to support that variants associated with inherited eye disorders are frequently encountered in unaffected individuals, and 1 in 6 genes implicated in inherited eye disorders is possibly associated with variable penetrance (Ref: Green, D.J. et al. Variability in Gene Expression is Associated with Incomplete Penetrance in Inherited Eye Disorders. Genes 2020, 11, 179). Thus, we suppose that these causative variants provided clues regarding the possibility of complication with other inherited ocular disorders [e.g., optic dystrophy, BEST, WFS or Alport syndrome (ID 3,4,5,8,9,)] other than cataract. Combined with clinical observation during operations after the removal of cataracts, including settled subretinal exudates and dragging of the optic disc in both eyes, patient 11 clarified the diagnosis of FEVR with regard to the TSPAN12 mutation. We also observed dental, facial and mental anomalies at 2 years after the first CC operation in patient 6 and made a new diagnosis of NHS syndrome regarding an identified NHS mutation. These findings are of great significance for clinical decision-making before cataract surgery.

Thank you for your suggestion. We explained this issue in the Discussion. (Page 5-6, lines 192-213)
5) Can the authors explain how homozygous variant is possible in child if the parents are not carriers of this variant, family 10 (Table 2).

Reply: We sometimes have the same question arise in our work. One possibility is that it is a de novo biallelic mutation in spite of an extremely low probability. The other more likely situation is that either parent is non-biological, although we confirm this information repeatedly before testing. As the de novo status was full of doubt and uncertainly, we deleted the conclusion regarding FYCO1 variants from the Results and Abstract.

Juan Carlos Zenteno (Reviewer 2):
This is an interesting paper which illustrated the power of NGS for the identification of genetic causes of congenital cataract. The sample size is robust and the results are interesting. However, several points need to be clarified by the authors, specially those related with the identification of variants in non-classical cataract genes (see below).

Dear Professor Juan Carlos Zenteno,

We are grateful for your review and positive comments on our paper. Thank you very much for your valuable comments, which have improved our article and from which we learned a great deal. Many thanks.

Abstract: Please change the term "X-linked syndromic proteins" to "proteins associated with X-linked syndromic conditions"

Reply: The term has been corrected as suggested in the Abstract. (Page 1, line 26)

Abstract: Please indicate the NGS approach used for genetic testing (exome, panel, etc)

Reply: Panel-based NGS was performed on all subjects in this study. We have indicated this in the Abstract and Methods. We designed the Target_Eye_792_V2 chip with exon-capture and UTRs of 792 genes involved in common inherited eye diseases (Supplementary Table S1), in collaboration with BGI-Shenzhen (Shenzhen, Guangdong, China). (Page 1, line 19 and Page 3, lines 94-98)

Page 6, line 130 "also identified a monoallelic mutation in BMP4, which has been associated with isolated hypospadias, a disorder of sexual development.(40)". Please note that BMP4 pathogenic variants are mostly associated with microphthalmia and/or facial clefts. (see https://www.omim.org/entry/112262?search=bmp4&highlight=bmp4)

Reply: Thank you for your reference. We have modified this in the text as you suggested and quoted the study containing the same nucleotide change (c.751C>T) in the table. (Page 4, lines 145-146 and Table 3, No. 6)

In my opinion, the analysis of genotype-prognosis correlation is not appropriate. No methods for statistical analysis for this correlation are mentioned nor how a "poor prognosis" vs "good prognosis" classification was considered. In addition, groups of genetically diagnosed vs non-genetically diagnosed are too small for a proper comparison (4 vs 5 cases). I suggest to delete this section.
Reply: Indeed, the result was not sufficiently supported. We agree with your suggestion. This section has been deleted from the Results and Discussion.

Caution must be taken for ascribing a cataractogenic effect to some identified variants. For example, BEST1 p.Ser7Asn is present in 9 Asian individuals in Gnomad (https://gnomad.broadinstitute.org/variant/11-61719298-G-A?dataset=gnomad_r2_1). This data in conjunction with the fact that BEST1 has not been previously associated with congenital cataract but with a macular disease indicated that most probably this is not the causative mutation. The same applies for p.Leu107Val in CYP1B1. These and other variants in non-classical cataract genes must be carefully reviewed.

Reply: We fully agree with you that some identified variants, such as BEST1 and CYP1B1, may not be the causative mutation for cataract. However, they provided clues regarding the possibility of complication with inherited ocular or systemic diseases other than cataract, such as BEST disease and glaucoma. All genes detected have been reviewed in the table Note (other reported phenotypes and references) column. As we stated in the Discussion, these loci provided additional ophthalmological diagnostic information. These findings are of great significance for clinical decision-making before cataract surgery. We have modified and reclassified the cataract causative variants in the Results after a careful review of the text, as you suggested. (Page 4, lines 136-142)

As previously suggested, conclusions on a potential genotype-visual prognosis correlations are not supported and could be eliminated from the discussion.

Reply: Paragraphs related to prognosis have all been deleted from the Results and Discussion.