Author’s response to reviews

Title: The identification and characterization of the p.G91 deletion in CRYBA1 in a Chinese family with congenital cataracts

Authors:

Dan Li (lenslab@163.com)
Qinghe Jing (qinghe_jing0708@163.com)
Yongxiang Jiang (yongxiang_jiang@163.com)

Version: 2 Date: 23 Jul 2019

Author’s response to reviews:

Editor Comments:

Not many different mutations in the CRYBA1 gene have been reported in affected individuals with congenital cataract. However, the p.G91 in-frame deletion has been identified in multiple unrelated cases, which is very interesting. As we know, the nonsense mutation-induced mRNA degradation in many disease-causal genes has been widely studied. However, the underlying mechanism of an in-frame deletion of single amino acid remains unclear. Thoughtful comments and suggestions from the reviewers are provided. More work to add to this report will be very interesting.

BMC Medical Genetics operates a policy of open peer review, which means that you will be able to see the names of the reviewers who provided the reports via the online peer review system. We encourage you to also view the reports there, via the action links on the left-hand side of the page, to see the names of the reviewers.

Reviewer reports:

Zhang Yikui (Reviewer 1): This manuscript confirmed that small in-frame one amino acid deletion in the beta-crystalline gene CRYBA1 was the genetic cause of congenital cataract in a Chinese family by sequencing; the authors also performed several in vitro experiments to try to explain why this indel mutation in gene CRYBA1 would cause congenital cataract.

Here following are my comments on this manuscript:
1. Whether the esotropia and nystagmus of these patients with congenital cataract were caused directly by this indel mutation or were just as a consequence of congenital cataract? Is it possible that this indel mutation may also affect ocular muscular movement genetically?

Answer: Thank you for this point. Considering this case alone, it is possible that this indel may also be responsible for the malfunction of ocular muscular movement. However, in the studies on genetic screening of esotropia and/or nystagmus, there is only one report finding the CRYBA1 c.594G>A:p.(Trp198Ter) mutation in families with nystagmus [1]. In addition, other families with the p.G91del mutation did not show such phenotype (summarized in Table 1). Therefore, we believe this deletion may not be the causing mutation responsible for esotropia and nystagmus. I have added this content to the end the second paragraph in the Discussion section.

2. Line 49 of Abstract: "more aggregation" should be corrected as "more aggregate", because "aggregate" is an adjective while "aggregation" is not.

Answer: Thank you for pointing this out, the revision has been made accordingly.

3. Line 60 of Abstract: the authors didn't provide sufficient evidence proving that this mutation was able to "destabilize the protein". According to the results of this manuscript, it will be more appropriate that this mutation results in abnormal expression and distribution of CRYBA1 protein.

Answer: Thank you very much for the advice, it is more accurate in this way, I have changed the sentence accordingly.

4. Line 29 of Results: the authors should state clearly that whether both eyes of these patients developed cataract or not. If both eyes were opaque due to congenital cataract, the expression of "showed no other abnormalities in THE OTHER eye structures" is quitly confusing and misleading; if only one eye developed cataract while the other eye is fine, how to explain that the other eye could spare the pathogenetic indel mutation of the beta-crystalline gene CRYBA1?

Answer: Thank you for this point. I agree that the expression was confusing. All the affected patients have bilateral CC. What I actually want to express is that except for cataract, esotropia and nystagmus, no other phenotypes (for example fundus diseases) were detected. I have changed the sentence “The results of ophthalmic examinations, including fundus photochromy, OCT, and VEP, showed no other abnormalities in the other eye structures.” to “Fundus
photochromy, OCT, and VEP were performed on CC patients, the results showed no other ocular or systemic abnormalities”.

Yaqin Wang, M.D. (Reviewer 2): Manuscript number: MGTC-D-19-00143R1

Manuscript title: An example of the role of a small in-frame deletion in congenital cataracts: a genetic study in a Chinese family

Comments:

Although the CRYBA1 gene is not a new causal gene for congenital cataracts, in which 15 different mutations have been reported (HGMD). Interestingly, the CRYBA1 p.G91 in-frame deletion is frequently present in patients from different races, suggesting a critical role the residue G91 in the encoded protein. Using WES analysis, this study identified the same mutation of CRYBA1 in a two-generation Chinese family with cataracts. Further experiments showed a markedly decreased CRYBA1 mRNA and protein expression in cataract lens capsular tissues and also the mutant CRYBA1 cell lines, possibly due to an unstable transcript of CRYBA1 missing that three "specific" nucleotides. Overall, the manuscript is acceptable with a revision.

Here are my comments and suggestions:

1. In the results, both of the mRNA and protein levels of CRYBA1 are found to be significantly decreased in cataract lenses and mutant cells. However, it is not clear in the abstract why to conclude this mutation only destabilized the encoded protein. In fact, much lower mRNA levels are shown compared to the protein levels. Therefore, the reasons of significantly reduced mRNA level of the gene also has to be discussed.

Answer: Thank you for this point. We did detect significant decrease at both mRNA and protein levels in cataract lenses vs. normal controls (donor lenses). However, in the WT and mutant CRYBA1 plasmid transfected cells, both plasmids increased CRYBA1 mRNA to more than 10^6 times (\(\Delta\Delta CT > 20\)), and the mRNA amount between WT and mutant is comparable, (not shown in the previous manuscript, present in the current Fig. 4A). In contrast, the protein level of mutant CRYBA1 was dramatically decreased, we have added a new Western blot image using CRYBA1 antibody besides the FLAG antibody image in SRA cells (revised Fig. 4B). For this reason, we proposed that the dysfunction of mutant CRYBA1 is mainly at the protein level.
2. Genetic compensation in response to gene mutations can lead to the transcriptional upregulation of homologous genes. For example, the CRYBA4 gene is also highly expressed in lens. Mutations of CRYBA4 curated in HGMD database are also the causes of cataract. It would be interesting to check if the CRYBA4 expression is compensatorily increased or not.

Answer: This is quite an interesting point. As suggested, we checked CRYBA4 expression level in wildtype and mutated CRYBA1 over-expressed SRA cells. Interestingly, the protein level of CRYBA4 in the WT-CRYBA1 group is lower than that in mutant-CRYBA1 group and the non-transfected control, suggesting that the overload of CRYBA1 (more than 10^6 folds quantified by qPCR) would reduce the production of CRYBA4, while the mutant type of CRYBA1 was not able to induce such reduction. The Western blot results from two independent experiments are provided in supplementary Figure (Fig. S1). We have added the related text to the last paragraph in the Results section. However, we are not able to check the CRYBA4 level in the lens of the patients in this CRYBA1-mutated family due to the shortage of their lens capsule samples.

3. Phenotype-genotype correlation analysis is encouraged to look if any differences can be found in terms of the in-frame deletion versus missense mutations or LoF mutations.

Answer: Thank you for the advice. I’m trying to respond this and the 5th comment by literature study. I’ve added the following paragraph in the Discussion section:

Besides the p.G91del mutation we reported here, together with 8 other reports [2-9], the other main group of CRYBA1 mutations have been identified in congenital cataracts: the mutations in the first two bases at the donor splice site of intron 3 (IVS3+1 G>A, IVS3+1 G>T, IVS3+1 G>C and IVS3+2 T>G) [2, 10-16]. Unlike the p.G91del mutation as summarized in Table 1, the subtypes of cataract caused by splice mutations are more variable, including nuclear [12], suture [11, 13], posterior polar [14] and progressive nuclear and cortical [16]. Of note, even the affected members with the same "IVS3+1 G>A" mutation in one family pedigree would present different cataract phenotypes [15]. In addition, a 2-bp deletion (c.590-591delAG) in exon 6 of CRYBA1 was identified in five members with nuclear cataract in a Chinese family [17].

4. In terms of evolutionary conservation, MutationTaster program and/or other algorithms may show if the deleted amino acid is critical or not.

Answer: Thank you for the advice, this is an important issue. The G91 in CRYBA1 is conserved through human, rat, mouse, cow and chick [4], and also conserved in the βγ-crystallin family [9]. Using MutationTaster program, this deletion is predicted to be "disease causing". We have added this content to Results under the "Identification of the genetic mutations" subtitle.
5. In the clinic part, more detailed information is encouraged to provide, which will be important for phenotype-genotype correlation analysis across all the CRYBA1- mutation-related literature.

Answer: This point has been responded in the 3rd comment.

6. In background of the abstract, line 3, A small in-frame one amino acid deletion", small can be deleted.

Answer: Thank you for the advice, the sentence has been revised accordingly.

7. The study compared the CRYBA1 expression between the anterior capsule pieces from age-related cataract patients and lens capsular tissues form normal donors. The author may need to explain which site of lens capsular for normal donors is extract from, the anterior, posterior or whole tissues?

Answer: Thank you for the notice. The capsular piece of the normal donor lens was collected from the anterior part, the same site at which the capsule samples collected in the cataract surgery. We have added this sentence to the Methods.


