Reviewer’s report

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

Version: 0 Date: 14 Oct 2018

Reviewer: Kathryn Burdon

Reviewer's report:

Introduction

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.

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.

Methods

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?

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

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

For the proband, what age is the first BCVA recorded at? Birth?

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).

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.

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

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.

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

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.

Resolution of text on figure 4 is not high enough to read, even when zoomed in.

Discussion

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.
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.

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.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

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Please indicate the quality of language in the manuscript:

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