PEER REVIEW HISTORY

This paper was submitted to the BJO but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Open. The paper was subsequently accepted for publication at BMJ Open.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Targeted Exome Capture and Sequencing Identifies Novel PRPF31 Mutations in Autosomal Dominant Retinitis Pigmentosa in Chinese Families
AUTHORS	Yang, Liping; Yin, Xiaobei; Wu, Lemeng; Chen, Ningning; Zhang, Huirong; Li, Genlin; Ma, Zhizhong

VERSION 1 - REVIEW

REVIEWER	Rivolta, Carlo University of Lausanne, Medical Genetics
REVIEW RETURNED	15-Jul-2013

GENERAL COMMENTS	The manuscript by Yang et al. reports the identification of two novel mutations in PRPF31, associated with autosomal dominant retinitis pigmentosa with reduced penetrance in two Chinese families, via a gene panel / NGS approach. The authors perform as well some preliminary functional characterization of one of the two mutant alleles, via the analysis of RNA extracted from patients' blood samples. The report is well written, the results are solid, and the conclusions are fully justified by the experimental data. The information provided on the capture and sequencing procedures is rather poor. However, since the focus of this work is mostly on PRPF31 molecular genetics, rather than on NGS, this is perfectly acceptable. We have only minor comments, which are: 1) Please carefully check the nomenclature of the mutations described and provide the accession number of the mRNA reference sequence that has been used. A good program to perform this operation is mutalyzer (https://mutalyzer.nl/). 2) Methods. It would be useful to better describe the procedure used to isolate and sequence RT-PCR fragments. The current text seems to imply that RT-PCR products were sequenced directly, whereas Supplementary Figure 1 report the use of M13F and M13R primers, suggesting that an intermediate cloning procedure was in fact followed. Please also provide a citation after "as described previously." (Page 9). 3) Results. An image depicting the migration of the 590 and 517 bp bands should be shown. This is particularly relevant since the short

	RT-PCR product is predicted to undergo NMD, as the authors rightfully state. 4) Figure 1. Is the genotype of patient IV:11 from adRP61 +/+ or M/+? 5) Figure 3. Please replace the word "Normal" with "Control", as this adjective refers to human beings. 6) Figure 3. Please provide a color version of the chromatograms. 7) Please provide a real legend to Supplementary Figure 1, detailing the nature of the sequences that are depicted, the individuals they belong to, etc. 8) Please scan the text for typos. For instance, 'Supplementary' is misspelled (page11), 'spliceosome' is misspelled (page 12), etc.

- The manuscript received two reviews at The BJO but the other reviewer have declined to make the reviews public. Please contact BMJ Open editorial office for any further information.

VERSION 1 – AUTHOR RESPONSE

The manuscript by Yang et al. reports the identification of two novel mutations in PRPF31, associated with autosomal dominant retinitis pigmentosa with reduced penetrance in two Chinese families, via a gene panel / NGS approach. The authors perform as well some preliminary functional characterization of one of the two mutant alleles, via the analysis of RNA extracted from patients' blood samples. The report is well written, the results are solid, and the conclusions are fully justified by the experimental data. The information provided on the capture and sequencing procedures is rather poor. However, since the focus of this work is mostly on PRPF31 molecular genetics, rather than on NGS, this is perfectly acceptable.
We have only minor comments, which are:
Comments 1: Please carefully check the nomenclature of the mutations described and provide the accession number of the mRNA reference sequence that has been used. A good program to perform this operation is mutalyzer (https://mutalyzer.nl/).
Response to 1：The accession number of the mRNA reference sequence that has been used was added on page 8 line 18 as follows: “mRNA reference number NM_015629”.
Comments 2: Methods. It would be useful to better describe the procedure used to isolate and sequence RT-PCR fragments. The current text seems to imply that RT-PCR products were sequenced directly, whereas Supplementary Figure 1 report the use of M13F and M13R primers, suggesting that an intermediate cloning procedure was in fact followed. Please also provide a citation after "as described previously." (Page 9).
Response to 2：The RT-PCR fragments were not sequenced directly, they were further cloned into plasmid for sequencing. This information has been added in the Methods on page 8 line 20 as follows: “The RT-PCR products were further cloned into plasmid for sequence analysis.”
Comments 3: Results. An image depicting the migration of the 590 and 517 bp bands should be shown. This is particularly relevant since the short RT-PCR product is predicted to undergo NMD, as the authors rightfully state.
Response to 3：The RT-PCR products were not much enough to be sequenced directly and to be taken picture on the agrose gel. But it can be successfully cloned into the plasmid for sequencing, which were shown in the Supplementary Figure 1. These information have been presented in the Results on page 12 line 3 as follows: “The RT-PCR products were further cloned into plasmid for sequence analysis. It yielded a 590bp product from normal control samples as expected with normal

splicing. One 590bp fragment and another 517bp product were amplified from the sample of affected individuals. Sequence analysis showed that the 517bp product skipped Exon11, resulting in frameshift i.e. p.Y359SfsX29 leading to premature termination with 28 new amino acids downstream. The sequence comparison details between wild type and mutated is listed in the Supplementary Figure 1. ”

Comments 4: Figure 1. Is the genotype of patient IV:11 from adRP61 +/+ or M/+?

Response to 4：The genotype of patient IV:11 from adRP61 is M/+, this mistake has been corrected in Figure 1.

Comments 5: Figure 3. Please replace the word "Normal" with "Control", as this adjective refers to human beings.

Response to 5：This has been corrected in Figure 3.

Comments 6: Figure 3. Please provide a color version of the chromatograms.

Response to 6：The sequencing results in Figure 3 have been changed to a color version of the chromatograms.

Comments 7: Please provide a real legend to Supplementary Figure 1, detailing the nature of the sequences that are depicted, the individuals they belong to, etc.

Response to 7：This information has been added on page 22 line 2 as follows: “Supplementary Figure 1. The sequence comparison details between wild type and mutated is listed. RP19-2_134 from patient Ⅳ3; RP19-2_146 from patient Ⅴ3; RP19-2_136 from unaffected family member Ⅳ15; RP19-2_145 and 147 from normal controls not related to the family. It yielded a 590bp product from unaffected family member and control samples, One 590bp fragment and another 517bp product were amplified from the affected individuals.”

Comments 8: Please scan the text for typos. For instance, 'Supplementary' is misspelled (page11), 'spliceosome' is misspelled (page 12), etc.

Response to 8：These mistakes have been corrected, we also scanned the whole text to correct the misspelling.