Author's response to reviews

Title: Targeted next-generation sequencing in anophthalmia and microphthalmia patients confirms <i>SOX2</i>, <i>OTX2</i> and <i>FOXE3</i> mutations

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Version: 2 Date: 8 December 2011

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Thank you very much for providing the comments of the two reviewers. We could find no specific points to respond to in their reviews. However, please let me know if there is anything specific required.

For the Editorial requirements:

We have provided contextual information in the background section of the abstract and rewritten the abstract to avoid going over the word count as follows:

“Background: Anophthalmia/microphthalmia (A/M) is caused by mutations in several different transcription factors, but mutations in each causative gene are relatively rare, emphasizing the need for a testing approach that screens multiple genes simultaneously. We used next-generation sequencing to screen 15 A/M patients for mutations in 9 pathogenic genes to evaluate this technology for screening in A/M.

Methods: We used a pooled sequencing design, together with custom single nucleotide polymorphism (SNP) calling software. We verified predicted sequence alterations using Sanger sequencing.

Results: We verified three mutations – c.542delC in SOX2, resulting in p.Pro181Argfsx22, p.Glu105X in OTX2 and p.Cys240X in FOXE3. The SOX2 mutation has not been published, although it was detected on clinical testing and served as a positive control. We found several novel sequence alterations and SNPs that were likely to be non-pathogenic - p.Glu42Lys in CRYBA4, p.Val201Met in FOXE3 and p.Asp291Asn in VSX2. Our analysis methodology gave one false positive result comprising a mutation in PAX6 (c.1268A>T, predicting p.X423LeuextX*15) that was not verified by Sanger sequencing. We also failed to detect one 20 base pair (bp) deletion and one 3 bp duplication in
SOX2.

Conclusions: Our results demonstrated the power of next-generation sequencing with pooled sample groups for the rapid screening of candidate genes for A/M as we were correctly able to identify disease-causing mutations. However, next-generation sequencing was less useful for small, intragenic deletions and duplications. We did not find mutations in 10/15 patients and conclude that there is a need for further gene discovery in A/M.

2. We have included a conclusion as suggested:

“Conclusion

We used next-generation sequencing with a pooled approach to sequence 9 known causative genes in 15 A/M patients. We were successful in identifying three mutations – c.542delC in SOX2, resulting in p.Pro181Argfsx22, p.Glu105X in OTX2 and p.Cys240X in FOXE3. Our analysis methodology resulted in one false positive PAX6 mutation that was not verified by Sanger sequencing; we were also unable to detect a small deletion of 20 bp and a duplication of 3 bp, both in SOX2. Next-generation sequencing with pooled samples enabled rapid screening of candidate genes for A/M and efficient detection of missense mutations; however, we were less successful in finding small intragenic deletions and duplications.”

3. We have revised the Tables so that there are no vertical lines or text within the tables. Please let us know if any further formatting changes are required.