Reviewer's report

Title: Two novel missense substitutions in the VSX1 gene: Clinical and Genetic analysis of families with Keratoconus from India

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Reviewer: James Hejtmancik

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In this manuscript the authors describe a probably causative mutation and a probably benign sequence variant in VSX1 found by screening 8 families with keratoconus. While the results are generally believable, some additions to the analysis would help increase the significance of the findings. Specific comments follow:

1. Abstract, Results "In silico analysis revealed that L268H is a pathogenic variant affecting the protein coded by VSX1, whereas S251T showed a neural effect on functional properties of VSX1.": In silico analysis cannot reveal the pathogenic nature of a sequence change, but only suggest it. Also, what is a 'neural effect'?

2. Results, mUTATION SCREENING..., P. 6, "This mutation was not present in 50 normal controls and the unaffected family members.": The comments about the various databases (1,000 Genomes, NHLBI ESP, BGI Complete Genomics, etc.) should probably be given here rather than below, with a little additional description. Similarly, were the sequence changes present in dbSNP or the Biobase web site?

3. Results and Discussion, "we screened 20 patients of eight unrelated families with KC...": It would be informative to see whether the two families with the L268H mutation were not actually related. One way to accomplish this would be to genotype intragenic VSX1 SNPs in both families and compare the haplotypes.

4. Discussion, "One of the variants is a missense mutation and the other a missense substitution.": It is unclear just what the difference between these two is. The authors should be careful about calling the sequence changes a 'mutation' based on bioinformatic analysis--maybe a caveat should be added.

5. Figure 2C: While inclusion of zebrafish helps, it would be good to add more diverse species to the alignment (maybe chicken, Xenopus, etc.) to the alignment to increase evidence of conservation rather than the mostly mammals currently shown.

6. Discussion, "However, leucine 268 amino acid residue was located in the C-terminal region of the CVC CVC (Chx10/Vsx-1 and ceh-10) domain of VSX1 and has been previously reported in familial KC patients [16, 24].": The meaning
here is unclear. Are the authors saying that the L268H variant is not novel?

7. General: While the English in this article is generally understandable, there are a number of usage and phrasing difficulties that make the manuscript difficult to read and impede the meaning in some instances.