Reviewer’s report

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

Version: 4 Date: 16 March 2015

Reviewer: Kathryn Burdon

Reviewer’s report:

This manuscript is much improved, however, some points of confusion still remain for me.

Major Compulsory Revisions

1. While the 3 families with segregating variants are clearly presented, there is no information on the remaining 5 families. Please include a summary table describing all 8 families. Information could include the family ID, the number of affected and unaffected individuals, the mean or range of age at diagnosis, any variants detected and if those variants segregate or not.

2. The manuscript would flow better if the mutation screening results were presented before the detailed clinical description of the 3 families. It would then be clear why only these 3 families are described in detail.

3. The haplotype analysis requires further consideration. The haplotype in families 1 and 2 is not shared at the SNP closest to the novel mutation (IVS-24C>T). This suggests that either the SNP has arisen later than the putative mutation, or the haplotypes are not shared identical-by-descent in the two families. Please provide additional information on the SNPs used for haplotype mapping such as the bp position of each SNP, or the distance between them as well as the population frequencies of each SNP. It would aid interpretation to know clearly if the alleles on the segregating haplotype were the common or rare alleles in this population.

4. Please re-visit the assertion in the discussion that mutations in 2 out of 8 families is not higher (end of discussion section). I am certain that the reported rate of VSX1 mutations is very much less than 25% of all sequenced probands.

5. The beginning of the second paragraph in the discussion says that onset of disease in these families is in the teens. This data is not presented in the paper, but could be included in the table suggested above.

Minor Essential Revisions

1. The chromatograms in Figure 3 are labelled with the IDs of multiple individuals. Please only label with the actual individual that gave rise to the presented chromatogram. It is evident from Figure 1 that additional individuals have the same genotype, but it is misleading to indicate that 3 individuals
contributed to 1 chromatogram.

2. Typo: Polypen-2, discussion

3. Please put the DNA and protein level descriptions of the 2 variants in the legend for Figure 3.

4. The heading for Table 2 could be improved to be more informative. Consider adding a sentence along the lines of "The functional classification and score for each variant is given from a number of predictive bioinformatics tools"

Discretionary revisions
1. At two different locations in the manuscript the authors use different references to illustrate the ocular expression pattern of VSX1. It would be preferable to collate information from the two references.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests