Reviewer’s report

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

Version: 1 Date: 3 September 2014

Reviewer: Kathryn Burdon

Reviewer’s report:

This manuscript describes two novel mutations in the VSX1 gene in 3 Indian families with keratoconus. Mutations in the VSX1 gene have been previously implicated in keratoconus with rare non-synonymous variants identified in a small percentage of keratoconus patients. It does not appear that VSX1 accounts for a high proportion of keratoconus, although highly penetrant rare variants may make a contribution.

In this paper the sequencing of the VXS1 gene in 20 keratoconus patients from 8 families from India was undertaken in order to determine if VSX1 mutations could explain disease in any of the patients. Two novel variants were detected. One of these was found in 2 apparently unrelated families and is predicted to be pathogenic by a number of algorithms. The other was found in a single family and is predicted to be a neutral variant.

The study described is relatively straightforward and appears to have been conducted appropriately. The new knowledge gained is incremental and only a small number of families were assessed. It does add to the mutation spectrum observed in keratoconus patients.

Minor essential revisions

Abstract: Change “Till date” to “To date” and “neural” to “neutral”

Methods: Were the sequence chromatograms visualized to identify the mutations? The chromatograms are presented, but the methods only discuss the use of Blast alignment on the sequence to find the mutations.

Results: Were any other previously reported polymorphisms found?

Table 1: Please define the abbreviations

Table 2: SIFT classification is usually notated as Deleterious (not damaging).

Table 3: Please indicate how the pathogenicity classifications in this table were determined. From the original report, or from modern bioinformatics predictions? Perhaps the evidence for pathogenicity or not could be summarized? How can the same mutation be both pathogenic and not pathogenic? Is this a case of it first being reported one way, then a later paper having a different conclusion? Does this table only include mutations in KC patients, or also in PPCD patients as implied in the text?
Major compulsory revisions

Methods:
Which individuals were sequenced? All 20 affecteds? 1 affected from each of the 8 families? All family members? Were controls sequenced?
There is no description of how the mutations were assessed in the 50 controls. Were they sequenced for the whole gene, or was a targeted assay used?
Which PolyPhen2 algorithm was used? HumDiv or HumVar?
The AAMSPSM results should be noted in the results section and the tool should be described in the methods section.
The rate of variants in this study is 3 out of 8 families, although probably only 2 out of 8 have a potentially pathogenic change. This seems quite high compared to other studies which typically find VSX1 mutations are quite rare. Please discuss this mutation rate and how it compares to other studies.
It is stated that the two families with the L268H mutation are not related. How was this determined? Are they from the same geographical region or ethnic group? Was any genetics (haplotype analysis) done to confirm the same mutation has arisen twice? It seems improbably that two unrelated families in the same research study would have the same novel rare variant.
The last sentence of the conclusion (also re-iterated in the abstract) is perhaps overstated and should be removed. There may be some utility for genetic counselling in the 2 families segregating the pathogenic variant, but this study does not shed much light on the role of VSX1 in keratoconus. In fact, it adds to the general confusion over this gene with a likely neutral polymorphism segregating, probably by chance.

Discretionary revisions

Results: The first paragraph is difficult to follow. It refers to S251T being identified in “both patients and their family members”. Which patients? It becomes clearer later on that this is a third family, but it could be clarified here.
Discussion: First paragraph; “Among these, eight patients from three families had two coding variants…” is ambiguous. It sounds like all 8 patients had the same 2 variants.

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