Reviewer's report

Title: Whole exome sequencing highlights variants in association with Keratoconus in Jordanian Families

Version: 1 Date: 11 Mar 2020

Reviewer: Petra Liskova

Reviewer's report:

General comments
Although the manuscript has been improved it is still difficult to be read. Some comments have not been addressed properly. Many typing errors are still present. In addition, the manuscript needs to be updated as listed below.
The variant filtering is based on the assumption that the families are consanguineous (or as the authors state in the reply "According to our hypothesis in this study: genetic factors causing KC in consanguineous families with multiple affected individuals"). yet already the first family "Family KC001 has 3 keratoconic siblings born to non-consanguineous parents." No known consanguinity is present in family 4 as well.

The study design would be more solid if homozygosity mapping is be applied in the consanguineous families first.
OMIM abbreviation is widely used but has not been introduced.

Comment 1
these genes were revealed in the Exome Aggregation Consortium (EXAC: Large-scale reference data sets for 60,706 individuals of diverse ancestries that are used for the efficient filtering of candidate disease-causing variants, and for the discovery of human 'knockout' variants in protein-coding genes; http://exac.broadinstitute.org/), suggesting that the mutations in VSX1 and SOD1 may not contribute to KC. Other studies have shown that mutations in MIR184 (the most abundant expressed microRNA in the corneal and lens epithelia) are candidates (but not consistent) causing KC and cataract (21, 22). This part as well as other parts with ExAC needs to be altered as the ExAC browser is no longer available.

Comment 2
In some parts references needs to be added, for example: Homozygous variants were filtered according to three criteria. Firstly, rare variants with minor allele frequency (MAF) less than 0.01 in the following databases: exome aggregation consortium (EXAC), genome aggregation consortium (gnomAD) 1000 gnome project and in the inhouse sequenced controls.
These are filtration criteria that were adopted is based on our hypothesis and thus no reference is needed.
References were meant to be added for the web resources used. GnomAD has on its pages information on how to cite. In addition, ExAC no longer exists.

Comment 3
Filtering of the variants seems too stringent, for example, why could not a variant contribute to the disease if reported in OMIM with non-ocular trait, especially when keratoconus has been reported to be associated with a number of other conditions? This is because the aim is to identify the variants in association with non-syndromic KC as presented in the manuscript. There are several examples in the literature in which mutations in the same gene cause both syndromic and non-syndromic ocular disease.

Comment 4
The authors should carefully check and be consistent when it comes to references and spaces, sometimes there are spaces sometimes there are not. For example:
Surgery is required in order to restore optimal visual acuity (2).
the prevalence (per 100,000) of KC is high in societies with high consanguinity such as India (2300)(3), Iran (2500)(4),

Comment 5
However, contradictory results were obtained regarding the pathogenicity of these genes. It is more correct to say pathogenic variants within these genes.

Comment 6
In Jordan, homozygous frameshift variant in the gene GALNT14 is a consanguineous family was identified in association with KC (23). This sentence does not make sense.

Comment 7
In this pilot study, Whole Exome Sequencinges (WES) is used to identify the rare variants associated with KC in eight Jordanian families. Not THE rare variants- just rare variants.

Comment 8
Belin Ambrosio' method was used to confirm KC. Correct is Belin / Ambrósio Enhanced Ectasia Display

Comment 9
The hypothesis in this study is the identification of genetic factors being in association with KC. This is no hypothesis- this is the goal.

Comment 10
Lab work Molecular genetic analysis would be more appropriate to be used- sounds more professional.
Comment 11
Whole exome sequence (WES)
Abbreviation has been introduced previously.

Comment 12
The illumina
Ilumina - capital I.

Comment 13
sites (according to Ensembl database v68)
Please use genome build

Comment 14
missense variants predicted to be possibly- or probably-damaging by Polyphen2 Humvar and predicted to be deleterious by SIFT.
References or web resourced used should be added.

Comment 13
because we aim to identify rare variants with non-syndromic KC (Figure 2).
associated to be added?

Comment 14
No detail information is available
Correct- no detailed

Comment 15
variants in these two zinc finger proteins might be in association with keratoconus
Abbreviation KC has been introduced previously.

Comment 16
In summary, whole exome sequencing and the validation
Abbreviation WES has been introduced previously.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

Quality of written English
Please indicate the quality of language in the manuscript:

Not suitable for publication unless extensively edited

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