Author’s response to reviews

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

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Author’s response to reviews:

Response to Reviewer
29 April 2020

Reviewer reports:
Petra Liskova, M.D., Ph.D. (Reviewer 1): 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.

Response: We updated the manuscript according to the comments below where applicable.

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.

Response: The term of consanguineous was modified to “consanguineous marriages and/or multiple keratoconic individuals”

The study design would be more solid if homozygosity mapping is be applied in the consanguineous families first.

Response: we do not find need for homozygosity mapping because we have performed trio and even quad whole exome sequencing (WES). Homozygosity mapping is done if WES is done for the index patient only.
OMIM abbreviation is widely used but has not been introduced. Response: We added this sentence at the end of the manuscript “Abbreviations: Gene names used in this article are used in abbreviation as mentioned in OMIM”

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.

Response: The sentence above is changed to “these genes were revealed in the genome aggregation database (gnomAD; https://gnomad.broadinstitute.org)”

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.

Response: gnomAD citation is added

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.

Response: you are right that there are several examples in the literature in which mutations in the same gene are reported to cause both syndromic and non-syndromic ocular diseases. And the same for many other mendelian rare diseases, however our assumption and hypothesis in this study is to identify the rare variants in non-syndromic ocular diseases and we preserve our hypothesis.

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),
Response: we have fixed the issue, thanks.

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

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.
Response: adjusted to “No previous studies were conducted in Jordan to identify aetiology of KC. However, in one study homozygous frameshift variant in the gene GALNT14 is identified to be in association with KC”

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.
Response: adjusted, thanks

Comment 8
Belin Ambrosio' method was used to confirm KC.
Correct is Belin / Ambrósio Enhanced Ectasia Display
Response: Adjusted, thanks

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.
Response: Adjusted, thanks

Comment 10
Lab work
Molecular genetic analysis would be more appropriate to be used- sounds more professional.
Response: Adjusted, thanks

Comment 11
Whole exome sequence (WES)
Abbreviation has been introduced previously.
Response: Adjusted, thanks
Comment 12
The illumina
Ilumina - capital I.
Response: Adjusted, thanks

Comment 13
sites (according to Ensembl database v68)
Please use genome build
Response: Adjusted, thanks

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.
Response: citations are added

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

Comment 14
No detail information is available
Correct- no detailed
Response: adjusted, thanks

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

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