Author’s response to reviews

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

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1. Consent to participate

The statement “Written informed consents were obtained from all participants in this study. Written informed consents were obtained from the parents of all participants under the age of 16.” Was moved from ‘Consent for publication’ to the “Ethics approval and consent to participate” section.

2. Consent for publication

The following sentence “Moreover, written informed consents for publication of identifying images or other personal or clinical details were obtained from the parents or legal guardians of any participant under the age of 18.” Was added to the “Consent for Publication” section.

3. Availability of data and materials

The paragraphs below were added to the “Availability of data and materials” section.
The highlighted variants in association with keratoconus in this article are available in Leiden Open Variation Database LOVD 3.0 (https://databases.lovd.nl/) according to reference genome GRCh37.73 used in this study.
KC003 (MYOF): https://databases.lovd.nl/shared/individuals/00307910
KC003 (STX2):https://databases.lovd.nl/shared/individuals/00307911
KC007 (ZNF676):https://databases.lovd.nl/shared/individuals/00307912
KC008 (COL6A5):https://databases.lovd.nl/shared/individuals/00307914
4. Overlap

The “Molecular Genetic analysis” section and the “Variant validation” section of the Methods were modified by removing the paragraphs below and replaced with “Total genomic DNA was extracted with FlexiGene DNA kit. WES was conducted to 28 individuals (17 keratoconic and 11 healthy). Exome sequencing, bioinformatics analyses and variants validation by sanger sequencing were done as described previously (32). “

“In order to capture exome, SureSelectXT V.6 Library Prep Kit was used (Agilent Technologies, USA). The Illumina NOVASEQ6000 platform (Illumina Inc., San Diego, CA, USA) was employed for the exome sequencing. The sequencing reads (150 bp pair end) were mapped to the reference genome (UCSC hg19, University of California Sana Cruz human genome 19) using the Burrows-Wheeler Aligner software(33). Polymerase chain reaction duplicates were removed using samblaster(34). Single-nucleotide variants and small insertions/deletions (indels) were called using freebays(35) and annotated using SnpEff-3.3 (Ensembl-GRCh37.73)(36). Sequencing was conducted by Macrogen (Seoul, Republic of Korea) and the pipeline megSAP was used(37).”

“Variants validation
Sanger sequencing was performed to segregate and confirm the identified variants. The primers were designed using primer3 version 4.1.0(41, 42). Polymerase chain reaction (PCR) was done using Taq polymerase (Invitrogen), PCR products were purified using ExoSAP-IT (Affymetrix Inc.). Clean PCR products were sequenced using BigDyeTM Terminator V.3.1 cycle sequencing kit and ABI PRISM 3730XL sequencer (Applied Biosystems Inc., USA). Sanger sequence files were edited and analysed using chromas Lite 2.1.1 (Australia Technelsium Pty Ltd).”