

RNA sequencing of corneas from two keratoconus patient groups identifies potential biomarkers and decreased NRF2-antioxidant responses

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Running Head: Keratoconus Transcriptomes of two distinct patient groups

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Supplemental Figure Legends –

Supplemental Fig S1: Fig.S1A: Technical replicates of RNA sequencing data are highly reproducible. Eight RNA samples from the Baltimore group were sequenced twice. The X and Y axes represent \log_2 transformed (FPKM+1) value of the first and the second sequence data, respectively, and shows high reproducibility between technical replicates. Fig. S1B: A Scree plot showing the fraction of total variance as explained by each Principal Component.

Supplemental Fig S2: Principal component analysis (PCA) of Keratoconus and controls. 4,787 genes at FPKM ≥ 5 in all samples were used. The major separation along PC1 was between KCN and controls. KC and KJ samples cluster together along PC1. African American donor (DN) and KC patient samples are labeled purple and red, respectively; Caucasian donors (LE) are in blue and Middle Eastern patients (KJ) in green. Euclidean distance between samples in a pairwise comparison of samples in PC1 and PC2 is shown in Supplemental Table S3.

Supplemental Fig S3:(A) Differentially expressed gene (DEG) classification based on protein localization. All DEGs (819 in KC and 993 in KJ) are categorized based on the Ingenuity Pathway Analysis (IPA) annotated location of encoded proteins. The percent of each category is the average of KC and KJ. **(B)** DEG classification by function using the DAVID Bioinformatics Resource 6.8, with additional manual annotation.

Supplemental Fig S4: (A) KEAP1 immunostaining in 2 individual DN and KCN corneas. **(B)** Immunostaining of NRF2 in DN and KCN corneas.

Supplemental Fig S5: The expression of selected genes in 4DN and 4KC human cornea was measured by qRTPCR using TaqMan™ primers. Relative expression ($2^{-\Delta\Delta CT}$) normalized to GAPDH was plotted using Graphpad Prism v7. Data are shown as means \pm S.E.M, the statistical significance was measured using unpaired t- test; *P < 0.05, **P < 0.01. The list of Taqman primer is provided in Supplemental Table S7.

Supplemental Fig S6 RXRA immunostaining in individual DN and KCN corneas.

Supplemental Table Titles –

Supplemental Table S1: Patient demographics and clinical characteristics.

Supplemental Table S2: Corneal samples RNA yield and quality.

Supplemental Table S3: Euclidean distance between samples in PCA

Supplemental Table S4 Differentially expressed genes (DEG) by Cuffdiff analysis

Supplemental Table S5: Differentially expressed genes (DEG) by DESeq2 analysis

Supplemental Table S6: The ten most increased and decreased DEG

Supplemental Table S7: List of TaqMan Primers