Differential epithelial and stromal protein profiles in cone and non-cone regions of keratoconus corneas

Gary Hin-Fai Yam, Matthias Fuest, Lei Zhou, Yu-Chi Liu, Lu Deng, Anita Sook-Yee Chan, Hon Shing Ong, Wei-Boon Khor, Marcus Ang, Jodhbir S Mehta

Supplemental Information

Experimental Procedures

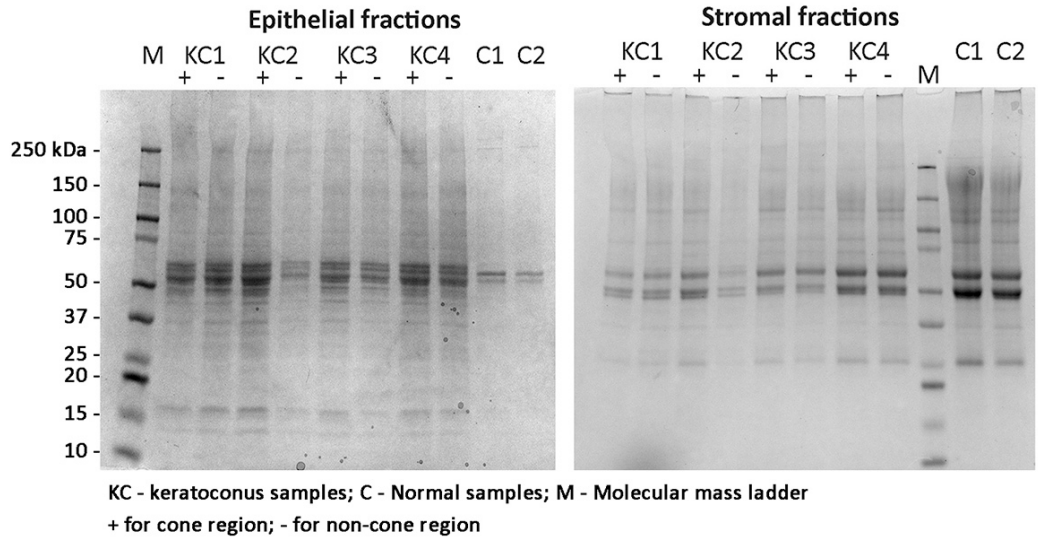
Quantitative proteomics (SWATH-MS analysis)

Sample processing - Protein samples (at constant 100 μg protein loads, to eliminate quantitative changes due to the corneal volume variation in the thinned cone region compared to the relatively thicker non-cone region) were acetone-precipitated followed by dissolution in lysis buffer (200 mM Tris-HCl pH 8.5, 10 mM EDTA, 4% SDS). Proteins in 5 mM TCEP (Tris 2-carboxyethyl phosphine hydrochloride, Sigma) were denatured at 60°C and passed through 3 kDa cut-off membrane. Retained proteins were washed with 75% urea/Tris-HCl and alkylated in 15 mM iodoacetamide (Sigma). Trypsin-digested peptides were then desalted prior to LC-MS/MS analysis.

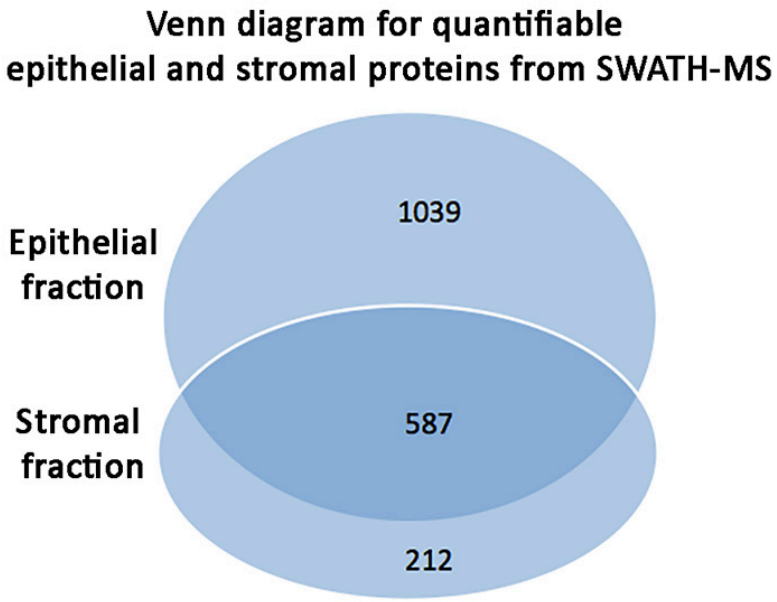
LC-MS/MS – peptide samples reconstituted in loading buffer (ACN/water with 0.1% formic acid, 2/98 vol/vol) were applied to SGE Reprosil C18-AQ trap column at 10 μl/min and washed for 5 min with loading buffer. The system was switched in line to C18 analytical capillary column connected to a spray tip (New Objectives, Woburn, MA), which was directly coupled with the nano-spray interface into AB Sciex 6600 tripleTOF mass spectrometer. The mobile phases were: solvent A: loading buffer, and solvent B: water/ACN, 2/98 vol/vol, with 0.1% formic acid. A step linear gradient (in total of 2 hr LC run) of mobile phase (solvent B) from 5 to 10% over 0.5 minute, 10 to 20% for 89.5 minutes, 20 to 28% for 30 minutes, 28 to 45% for 10 minutes and lastly, 45 to 80% over 5 min at flow rate of 400 nl/min was utilized.

Information dependent acquisition (IDA) analysis for building ion library **-** The instrumentation settings were: Ionspray Voltage Floating = 2100 V, curtain gas = 30, ion source gas 1 = 8, interface heater temperature = 125, declustering potential = 80 V and Nebuliser current = 3 for nitrogen gas. All data were acquired using IDA mode (high sensitivity) with Analyst TF1.7 (AB Sciex, Singapore). For IDA parameters, 0.25 sec

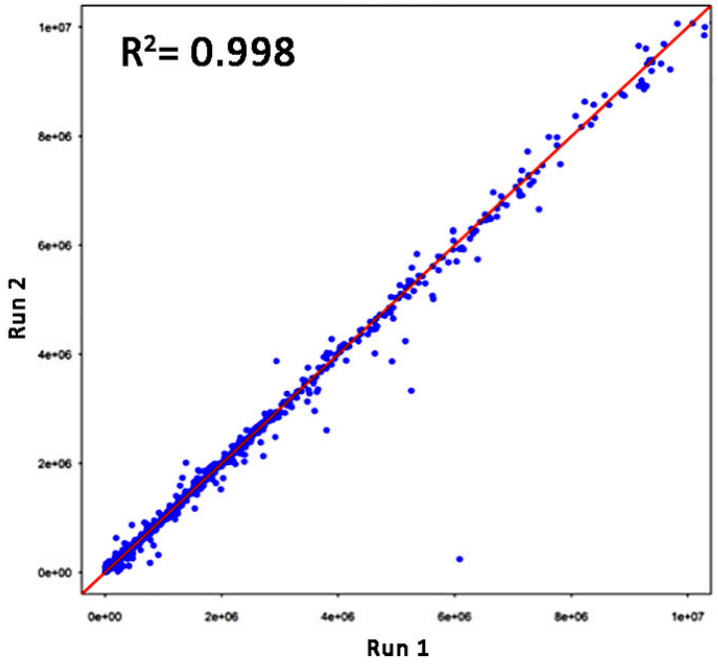
TOF MS survey scan in mass range of 350-1800 were followed by product ion scan of 0.05 sec in the mass range of 100-1800. “Rolling collision energy” was used in IDA collision energy setting. Switching criteria were set to ions *m*/*z* >350 and <1800 with charge state of 2-5, maximum number of candidate ions to monitor per cycle was 50 spectra and an abundance threshold of >125 counts. For SWATH acquisition, 100 variable windows between m/z 350-1800 at 30 msec per SWATH window were used.



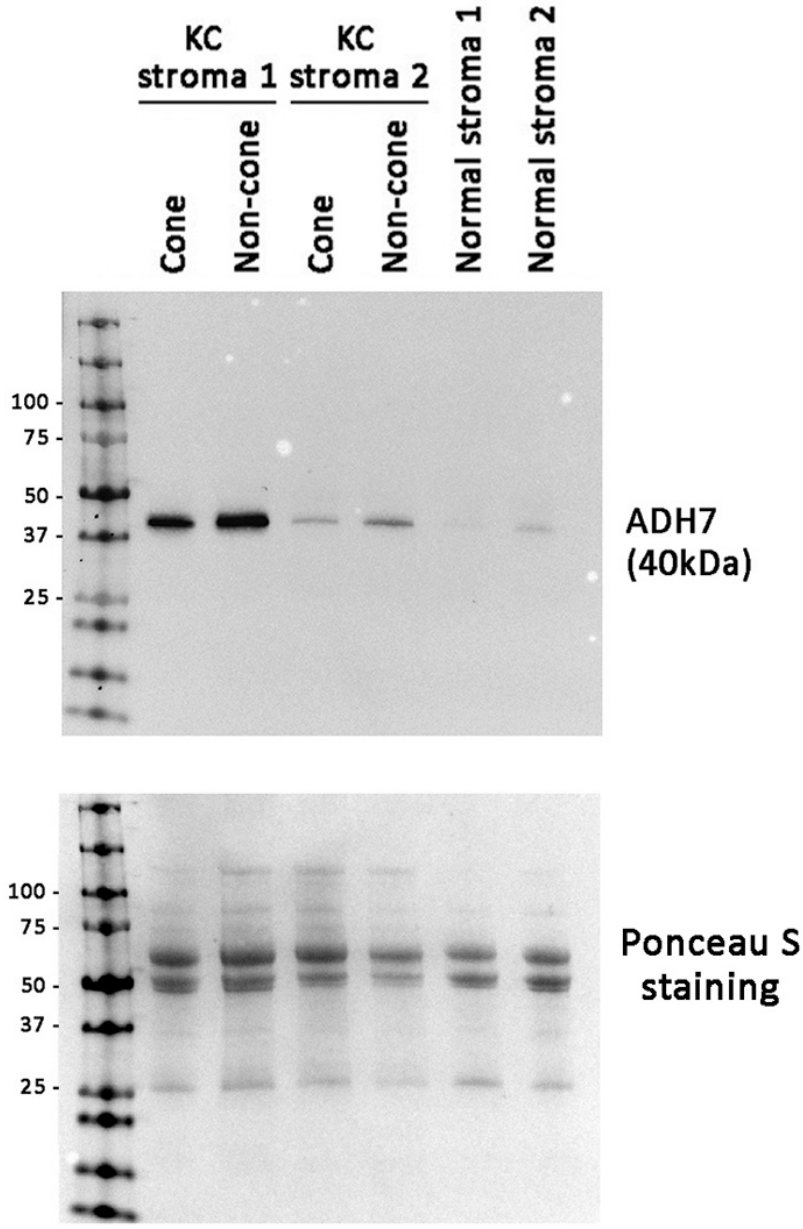
Supplemental Figure S1. SDS-soluble proteins extracted from KC and normal corneal samples. Epithelial and stromal fractions from 4 KC samples with separated cone (+) and non-cone (-) regions. The proteins (20 μg) were resolved by 4-20% SDS-PAGE followed by Coomassie Brilliant Blue R250 visualization.



Supplemental Figure S2. Venn diagram for quantifiable epithelial and stromal proteins by SWATH-MS.



Supplemental Figure S3. Reproducibility of quantitation between two technical runs in SWATH-MS using a standard yeast digest sample. Excellent correlation was achieved between two technical runs (correlation efficiency $R^2 = 0.998$).



Supplemental Figure S4. Full length gel images of western blot data for ADH7 and overall gel pattern revealed by Ponceau S staining as shown in Fig. 6C.

Supplemental Table S1. Enriched Gene Ontology terms and KEGG pathways identified for differentially expressed proteins between KC cone and normal corneal epithelial samples. The biological events were ranked using enrichment scores. *Adjusted Benjamini $p<0.05$ represents statistical significance.

	Enrichment scores	Biological events	Proteins (UniPro Accession no.)	*p*
(A) Enriched GO terms (DAVID) (non-cone versus normal CE)				
1	20.1	GO:0098609~Cell-cell adhesion	Q99439, Q9UGI8, Q13596, Q7L1Q6, Q9UHD8, Q9HC38, Q9BR76, P37802, Q9Y6E2, P31946, P43487, O14618, Q9C0C2, Q9UH65, P30041, P55196, P40121, P07737, Q7KZF4, O95372, P31949, Q15907, P61313, P50402, Q9H3U1, Q99497, Q06830, Q07157, Q9P0L0, P35613, Q9H2G2, Q14677, O75369, Q92522, P28072, O95292, P47756, Q96HC4, O60664, Q02878, Q9NQC3, P53367, Q15365	5E-16*
2	4.27	GO:0006120~ Mitochondrial electron transport, NADH to ubiquinone	O43674, O14561, O75489, Q9NX14, O00217, P56556, P09622, O43678, O75380	0.013*
3	3.87	GO:0006412~ Translation	P42766, P46779, P39019, P26639, Q969Q0, P62979, P62899, Q9NSD9, P62917, P62266, Q02878, P42677, P18077, P61313, P62851, P46782, P23381	0.194
4	3.87	GO:0000184~ Nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	Q15287, P42766, P46779, P39019, P62979, P62899, P67775, P62917, P62266, Q02878, P42677, P18077, P61313, Q92900, P62851, P46782	5.1E-04*
5	3.34	GO:0045454~Cell redox homeostasis	P02788, P07203, P09622, Q13162, P30041, P13667, O95831, P35754, P30044, P10599, Q16881, O76003, Q9BQE4, Q06830, Q13423	1.9E-05*
6	3.11	GO:0000398~ mRNA splicing, via spliceosome	Q15287, P52597, P26368, P51991, Q8IYB3, O75533, P62312, Q13435, Q9H2H8, P35637, O43143, P09012, P08621, Q6P2Q9, Q9Y3C6, Q15459, Q15365, Q9Y4Z0	0.032*
7	2.96	GO:0006094~ Gluconeogenesis	P40925, P60174, P09467, P04406, P40926, P18669, P00558, P06744, O00757	0.006*
8	2.81	GO:0055114~ Oxidation-reduction process	P00441, O14618, Q53TN4, Q13162, P16152, P30838, O95831, P30041, P30044, Q06830, Q13423, O75911, P40394, P00352, P56556, Q6IAN0, Q9Y2Q3, P99999, P35754, P04179,	0.395

9	2.587	GO:0005525~GTP binding	P10599, P52209, Q16881, P07919, Q7Z4W1, P14550, Q9UBQ7, O76003 P68104, P07437, Q9NR31, P61204, Q9UHD8, Q6IQ22, P36405, P61020, P68371, P51148, P11233, P20339, P11234, P21980, O00429, Q96I99, Q9BQE3, Q92930, P57735, Q13885, Q15907, Q9NRW1	0.137
10	2.203	GO:0010951~ Negative regulation of endopeptidase activity	P04080, P30740, P12111, P31025, P30086, P05067, P01011, O43278, Q92530, P36955, P01008, P36952, P01024	0.028*
(B) KEGG pathways (DAVID)				
1	5.324	hsa00190: Oxidative phosphorylation	O43674, O14561, P14406, P14854, P08574, O75489, Q9NX14, P56385, O00217, P56556, O43678, Q15181, Q93050, P13073, P10606, P30049, P07919, O75380	0.002*
2	5.324	hsa05012: Parkinson's disease	O43674, O14561, P14406, P68036, P14854, P08574, O75489, Q9NX14, O00217, P56556, O43678, P99999, P13073, P10606, P30049, P07919, P42574, O75380, Q99497	0.002*
3	3.486	hsa03010: Ribosome	P42766, P46779, P39019, Q969Q0, P62979, P62899, P62917, P62266, Q02878, P42677, P18077, P61313, P62851, P46782	0.082
4	3.113	hsa03040: Spliceosome	P26368, O14776, P51991, O75533, Q13435, P62312, O43143, P09012, P08621, Q6P2Q9, Q9Y3C6, Q15459, Q15365, Q9Y4Z0	0.072
5	2.067	hsa00630: Glycosylate and decarboxylase metabolism	P40925, P40926, Q9BWD1, P34896, Q9UBQ7, P23434, P09622	0.015*

Supplemental Table S2. Enriched Gene Ontology terms and KEGG pathways identified for differentially expressed proteins between KC non-cone and normal corneal epithelial samples. The biological events were ranked using enrichment scores. *Adjusted Benjamini $p<0.05$ represents statistical significance.

	Enrichment scores	Biological events	Proteins (UniPro As session no.)	*p*
(A) Enriched GO terms (DAVID) (non-cone versus normal CE)				
1	17.54	GO:0098609~ Cell-cell adhesion	Q9UGI8, O00151, Q13596, Q9UHD8, Q9HC38, Q9BR76, P37802, O14618, Q9UH65, P30041, P07737, P40121, Q7KZF4, O95372, O95817, P47914, P62826, Q9UNF0, P61313, Q9H3U1, Q99497, Q06830, P53990, Q9P0L0, Q7Z2W4, Q92616, P00338, Q92817, P31939, O00567, O75369, O95292, Q96HC4, O60664, P63244, P14618, Q9NQC3	1.2E-14*
2	5.32	GO:0006120~ Mitochondrial electron transport, NADH to ubiquinone	O43674, O14561, O75489, Q9P0J0, Q9NX14, P56556, O43678, O75380, Q16795	0.003*
3	3.71	GO:0006094~ Gluconeogenesis	P40925, P60174, P09467, P04406, P40926, P53007, P18669, P00558, P06744	0.003*
4	3.23	GO:0006412~ Translation	P60866, P53007, P42766, P46779, Q92616, Q969Q0, P62979, P62899, P62861, E9PAV3, Q9NSD9, P62841, P47914, P42677, P49591, P61313, P46782	0.003*
5	3.13	GO:0051287~ NAD binding	O43865, P40925, P04406, P00338, Q9UBQ7, P07195	0.083
6	3.04	GO:0051082~ Unfolded protein binding	P62937, Q16543, Q9UHV9, Q99471, O60925, Q9UBS4, Q9NQP4, P68371	0.319
7	2.72	GO:0000302~Response to ROS	P99999, P30041, P30044, P09211, P04179, P10599, Q16881, O14618, P05090, Q06830	1.6E-04*
8	2.07	GO:0015629~Actin cytoskeleton	P20339, P61160, Q9UHD8, O15231, O14776, Q9ULV4, Q96HC4, P07305, Q9BR76, O43707, P23528, O75369, P60981	0.046*
(B) KEGG pathways (DAVID)				
1	5.32	hsa00190: Oxidative phosphorylation	O43674, O14561, Q9H2U2, P14854, P08574, O75489, Q9P0J0, Q9NX14, P56385, P56556, O43678, P21283, Q15181, Q93050, P13073, P10606, P30049, P07919, P00403, P36543, O75380, Q16795	1.4E-07*

2	5.32	hsa05012: Parkinson's disease	O43674, O14561, P68036, P14854, P08574, O75489, Q9P0J0, Q9NX14, P56556, O43678, P99999, P13073, P10606, P30049, P07919, P00403, Q16795, O75380, Q99497	4.6E-05*
3	3.71	hsa00010: Glycolysis / Gluconeogenesis	P60174, P09467, P04406, P40394, P18669, P00338, P06744, P07195, P30838, P00558, P08237, P14550, P14618	6.9E-05*
4	3.23	hsa03010: Ribosome	P60866, P62979, P62861, P62899, P42766, P46779, P62841, P47914, P42677, P61313, Q969Q0, P46782	0.069
5	3.13	hsa00270: Cysteine and methionine metabolism	O43865, P25325, P40925, P40926, P31153, P00338, P07195	0.027*
6	3.13	hsa00620: Pyruvate metabolism	P40925, P40926, P00338, Q04760, Q9UBQ7, P14618, P07195	0.033*

Supplemental Table S3. **Enriched Gene Ontology terms and KEGG pathways identified for differentially expressed proteins between KC cone and normal corneal stromal samples**. The biological events were ranked using enrichment scores. All had adjusted Benjamini $p<0.05$ representing statistical significance.

	Enrichment scores	Biological events	Proteins (UniPro As session no.)	*p*
(A) Enriched GO terms (DAVID) (non-cone versus normal CE)				
1	10.72	GO:0098609~Cell-cell adhesion	Q7L1Q6, P31947, P25685, P37802, Q00341, P30041, P52907, P40121, P15311, O95372, P50990, Q9Y265, P63104, P42167, Q9Y490, Q02487, P21333, Q14134, P14923, Q9NTK5, Q15417, Q05682, Q6IBS0, Q15691, P23229, Q14247	3.97E-11
2	7.88	GO:0005576~ Extracellular region	P01031, Q14574, Q06828, P01714, P14780, P16035, P68366, P18428, P06309, P08697, P01593, P02787, P40394, Q9Y490, P02748, P02747, P21333, Q9Y240, P02743, Q6UWY5, P08123, P02649, P01742, P29622, P00746, P35443, P01781, P00441, P27797, P09871, P41222, P36980, P84243, P52907, O43866, P20774, Q14116, P01776, P01773, Q92743, P10745, P02652, P02760, P02671, P01861, P02768, P02766, P02452, Q9UBX5, P31025, P23083, P51888, P05154, Q12805, P21810, Q08380, P09429, P06316, P0C0L4, P01763	1.8E-15
3	5.37	GO:0006457~ Protein folding	P40227, P78371, Q8NBS9, P27797, O95302, P25685, Q9Y230, P02743, P49368, P10599, P48643, P50990, P50991, P26885	3.2E-11
4	5.11	GO:0006958~ Complement activation, classical pathway	P01593, P01781, P01031, P01861, P01871, P02748, P02747, P01714, Q07021, P09871, P06309, P01776, P01773, P06316, P01880, P01742, P0C0L4, P01763	5.1E-04
5	5.11	GO:0038095~Fc ε– receptor signaling pathway	P01781, P01593, P25789, P62837, Q06323, P01714, P63000, P62158, P06309, P01776, P01773, P01742, P06316, P01763	1.8E-04
6	3.02	GO:0005525~GTP binding	P68104, P36405, P30520, Q9NVA2, Q9NTK5, P61019, P68371, P63000, P18085, P21980, P68366, P61586, Q9BQE3, Q13885, Q9BUF5	0.035
7	2.84	GO:0005882~ Intermediate filament	P02545, P22392, P15924, P13645, P12035, P02533, P35908, P14923	0.011
8	2.69	GO:0010951~	P01031, P16035, P05154, O95428,	6.8E-04

		Negative regulation of endopeptidase activity	P36952, P08697, P31025, P35237, P0C0L4, P29622, P02760	
9	2.43	GO:0030574~ Collagen catabolic process	P12110, P12111, P12109, P02452, P14780, P08123	0.003
10	2.37	GO:0000302~ Response to reactive oxygen species	P30041, P02649, P30044, P09211, P00441, P10599	0.012
(B) KEGG pathways (DAVID)				
1	2.9	hsa04610: Complement and coagulation cascades	P01031, P02671, P05154, P02748, P02747, P08697, P09871, P0C0L4, P00746	0.006
2	2.02	hsa00010: Glycolysis / Gluconeogenesis	P09972, P09467, P40394, P18669, P49189, P00325, P17858	0.046

Supplemental Table S4. **Enriched Gene Ontology terms and KEGG pathways identified for differentially expressed proteins between KC non-cone and normal corneal stromal samples**. The biological events were ranked using enrichment scores. All had adjusted Benjamini $p<0.05$ representing statistical significance.

	Enrichment scores	Biological events	Proteins (UniPro As session no.)	*p*
(A) Enriched GO terms (DAVID) (non-cone versus normal CE)				
1	9.32	GO:0098609~Cell-cell adhesion	Q7L1Q6, P61026, P31947, Q14134, P37802, P31946, P43487, P40121, P52907, O60763, Q15417, Q05682, O95372, P50990, P14618, P09758, Q9Y265, P63104	2.9E-05
2	6.51	GO:0006958~ Complement activation, classical pathway	P01593, P01861, P01871, P02748, P01714, Q07021, P01611, P01601, P09871, P04433, P04220, P01857, P01623, P06309, P01764, P01773, P01880, P01742, P0C0L4, P06681	7.6E-13
3	6.51	GO:0038096~Fc-γ receptor signaling pathway involved in phagocytosis	P01593, P01861, O15145, P01714, P01611, P01601, O15511, P04433, P01857, P01623, P06309, P01764, P01773, P01742	5.4E-06
4	6.51	GO:0006955~ Immune response	P01593, P02748, P01714, Q07021, P01611, P01601, P23083, P04433, P22413, P01623, P06309, P01764, P18510, Q14116, P01773, P01880, P01742	0.01
5	5.55	GO:0005576~ Extracellular region	Q06828, P01714, P01611, P14780, Q9ULZ3, P16035, P12830, P01857, P18428, P68366, P06309, P08697, P55058, P06681, P01593, Q13751, P40394, P02788, P12111, P02748, P01601, P21333, Q9Y240, P02743, Q6UWY5, P08123, P04433, P02649, P24592, Q99969, P01742, P29622, P00746, P00441, P27797, P09871, P68871, P36980, P52907, O43866, Q14116, P01773, Q92743, P02760, P02671, P01861, P12110, P02766, P02452, P31025, P23083, P51888, P05154, P12109, P01623, P01764, Q12805, P21810, P09429, P0C0L4	1.8E-12
6	3.44	GO:0016491~ Oxidoreductase activity	Q06828, P01714, P01611, P14780, Q9ULZ3, P16035, P12830, P01857, P18428, P68366, P06309, P08697, P55058, P06681, P01593, Q13751, P40394, P02788, P12111, P02748, P01601, P21333, Q9Y240, P02743, Q6UWY5, P08123, P04433, P02649, P24592, Q99969, P01742, P29622,	1.8E-12

			P00746, P00441, P27797, P09871, P68871, P36980, P52907, O43866, Q14116, P01773, Q92743, P02760, P02671, P01861, P12110, P02766, P02452, P31025, P23083, P51888, P05154, P12109, P01623, P01764, Q12805, P21810, P09429, P0C0L4	
7	3.11	GO:0034987~ Immunoglobulin receptor binding	P01861, P04220, P01871, P01857, P01764, P01601, P01880	0.001
(B) KEGG pathways (DAVID)				
1	2.46	hsa00010: Glycolysis / Gluconeogenesis	P09972, P09467, P40394, P18669, P00325, P09104, P14618, P17858	0.007
2	2.43	hsa04512: ECM-receptor interaction	P12110, Q13751, P12111, P05556, P12109, P02452, P23229, P08123	0.023

Supplemental Table S5. Patient information for archived KC and non-KC specimens

(A) Archived KC specimens				
Patient	**Age**	**Sex**	**Diagnosis**	**Surgery**
1	21	M	Advanced KC	DALK
2	27	M	Advanced KC	DALK
3	21	M	Advanced KC	DALK
4	54	F	Advanced KC	DALK
(B) Archived non-KC specimens				
1	87	F	Corneal scar	PK
2	42	F	Phytencular inflammatory corneal scar	DALK
3	60	M	HSV keratitis–related corneal scaring	DALK
4	44	M	Previous fungal keratitis with scaring and inflammation	DALK
(C) Normal corneal specimens				
Patient	**Age**	**Sex**	**Cause of Death**	
1	37	F	Acute cardiac crisis	
2	59	M	Coronary artery disease	

M – male; F – female; DALK - deep anterior lamellar keratoplasty; PK – penetrating keratoplasty

Supplemental Table S6. Primary antibody information

Antibody (clone)	Company (Catalogue No.)	Applications	Working concentration
NDUFB11 (G-4)	Santa Cruz (sc-374369)	Immunohistochemistry	1.5 μg/ml
ADH7	Proteintech (23425)	Immunohistochemistry Western blotting	0.5 μg/ml
STEAP4	ThermoFisher (PA5-62054)	Immunohistochemistry	2 μg/ml
IL1RN	Sigma-Aldrich (HPA001482)	Immunohistochemistry	2 μg/ml
β-Actin-horseradish peroxidase conjugate	Sigma (A3854)	Western blotting	0.5 μg/ml

Supplemental Table S7. Reported functions of dysregulated proteins between KC cone and non-cone epithelial and stromal samples and potential link to KC pathogenesis

	Protein, UniPro No.	Cone vs Non-cone (fold change)	Reported functions and potential link to keratoconus pathogenesis	Ref
(A) Deregulated epithelial proteins in cone versus non-cone regions				
1	NADH: Ubiquinone oxidoreductase subunit B11, NDUFB11, Q9NX14	Up (24±17)	An integral inner mitochondrial membrane protein transfers electron from NADH to ubiquinone. Reported mutations link to microophthalmia with epithelial defects	(1, 2)
2	Glucosidase a, acid, GAA, P10253	Up (6.7±3)	Glycogen degradation	(3)
3	Nicotinamide nucleotide transhydrogen ase, NTT, Q13423	Up (2.5±1)	An integral inner mitochondrial membrane protein transfers hydrides between NAD(H) and NADP(+) to proton translocation	(1)
4	DNA fragmentation factor subunit a, DFFA, O00273	Up (3±1.5)	A substrate of caspase-3 to trigger DNA fragmentation	(4)
5	Ribosomal protein L28, RPL28, P46779	Up (3±1.5)	A component of 60S subunit to regulate translation	(5)
6	Insulin-like growth factor binding protein 6, IGFBP6, P24592	Down (0.3±0.03)	IGF metabolism to regulates cell growth, survival and apoptosis	(6)
7	Fumarate hydratase, FH, P07954	Down (0.2±0.1)	Involves in enzymatic reaction in tricarboxylic acid cycle	(7)
8	Glutaminase, GLS, O94925	Down (0.3±0.1)	Regulates energy production and antioxidant defense	(8)
9	Translocase of outer mitochondrial membrane 22,	Down (0.5±0.2)	Imports cytosolically synthesized mitochondrial preproteins for mitochondria homeostasis	(9)

	TOMM22, Q9NS69			
10	Eukaryotic translation initiation factor 3 subunit G, EIF3G, O75821	Down (0.5±0.1)	Translation initiation	(10)
11	Eukaryotic translation initiation factor 2β, EIF2S2, P20042	Down (0.5±0.2)	Forms ternary complex with GTP and initiator tRNA in early steps of protein synthesis	
12	ADP ribosylation factor interacting protein 1, ARFIP1, P53367	Down (0.4±0.1)	Regulates post-translational modifications	(11)
13	Syntaxin 4, STX4, Q12846	Down (0.4±0.2)	Vesicle transport to plasma membrane and cytokine signaling in immune system	(12)
14	Platelet activating factor acetylhydrolase 1b regulatory subunit 1, PAFAH1B1, P40394	Down (0.5±0.1)	Involves in microtubule polymerization for maintaining intracellular organelle integrity, intracellular trafficking, nucleokinesis and cell locomotion	(13)
15	Niemann-Pick type C2, NPC2, P61916	Down (0.4±0.2)	Regulates cholesterol transport through the late endosomal/lysosomal system and proper clearance of autophagosomes. Dysregulated autophagy due to oxidative stress has been reported in KC epithelia.	(14, 15)
16	Prefoldin subunit 4, PFDN4, Q9NQP4	Down (0.4±0.1)	A component of molecular chaperone complex involved in protein folding and prevention of protein aggregation, which is associated to ER stress and oxidative stress responses	(16, 17)
17	ADAM metallopeptida	Down (0.5±0.1)	A cell surface protein with protease activity in cleaving various extracellular proteins	(18)

	se domain 10, ADAM10, O14672		like TNFa and E-cadherin and has been implicated in EGFR and IL6 signaling, the latter is an inflammatory cytokine detected in KC epithelia.	
(B) Deregulated stromal proteins in cone versus non-cone regions				
1	Threonyl-tRNA synthetase, SYTC, P26639	Up (5±3)	tRNA aminoacylation for protein translation; immune response and angiogenesis	
2	RuvB like AAA-ATPase2, RUVBL2, Q9Y230	Up (2.4±1)	Acts as DNA helicase for homologous recombination and DNA break repairs; involves in ER-associated degradation pathway in regulating ER stress response; links to protein folding in stromal repair and ROS-related activities in KC	(19)
3	Secretion-associated Ras-related GTPase 1A, SAR1A, Q9NR31	Down (0.4±0.2)	Involves in endoplasmic reticulum (ER)-Golgi transport for protein post-translational processing and modifications	(20)
4	Tumor associated calcium signal transducer 2, TACSTD2, P09758	Down (0.3±0.1)	Reported for familial gelatinous drop-like corneal dystrophy with amyloid deposits. Its association to secondary corneal amyloidosis and inflammatory pathways that occur in keratoconus needs to be verified.	(21-23)
5	Vitrin, VIT, Q6UXI7	Down (0.4±0.2)	Involves in glycosaminoglycan binding during cell adhesion and migration; morpholino knockdown increased apoptosis	(24)
6	STEAP4 metalloreductase, Q687X5	Down (0.5±0.3)	A mitochondrial iron reductase participating in systemic metabolic homeostasis, integrating inflammatory and metabolic responses; relates to mitochondrial damages and oxidative stress	(25)
7	Phosphoglycerate dehydrogenase, PHDGH, O43175	Down (0.5±0.3)	Involves in NAD reduction for electron transfer and oxidoreductase activities. Reported variants in association with corneal epithelial and stromal thickness	(26, 27)
8	ATP binding cassette subfamily B11, ABCB11, O95342	Down (0.4±0.1)	Contains transporter and ATPase activities involved in multidrug resistance and bile salt secretion	
9	Splicing factor 3b subunit 3, SF3B3, Q15393	Down (0.5±0.3)	mRNA splicing	(28)
10	Basic leucine zipper and W2 domain 1,	Down (0.5±0.2)	Regulates histone H4 gene transcription	(29)

	BZW1, Q7L1Q6			
11	H2A histone family member J, H2AJ, Q9BTM1	Down (0.4±0.2)	Epigenetic regulation	(29)
12	Macrophage migrating inhibitory factor, MIF, P14174	Down (0.5±0.2)	An inflammation-regulatory cytokine released upon corneal injury	(30)
13	(NAD(P)H quinone dehydrogenase 1, NQO1, P15559	Down (0.4±0.2)	A downstream cytoprotective effector of NOX4 and ROS signaling against oxidative stress in corneal diseases	(31)
14	Alcohol dehydrogenase 7, ADH7, P40394	Down (0.5±0.1)	Possesses 11-cis-retinol dehydrogenase activity in photo-pigment regeneration; a defense role against oxidative damage from alcohols and aldehydes (by reducing 4-hydrozynonenal, a lipid peroxidation cytotoxic product)	(32)

References for Supplemental Information

1. Meimaridou, E., Kowalczyk, J., Guasti, L., Hughes, C. R., Wagner, F., Frommolt, P., Nurnberg, P., Mann, N. P., Banerjee, R., Saka, H. N., Chapple, J. P., King, P. J., Clark, A. J., and Metherell, L. A. (2012) Mutations in NNT encoding nicotinamide nucleotide transhydrogenase cause familial glucocorticoid deficiency. *Nature Genet* **44**, 740-742

2. van Rahden, V. A., Fernandez-Vizarra, E., Alawi, M., Brand, K., Fellmann, F., Horn, D., Zeviani, M., and Kutsche, K. (2015) Mutations in NDUFB11, encoding a complex I component of the mitochondrial respiratory chain, cause microphthalmia with linear skin defects syndrome. *Am J Human Genet* **96**, 640-650

3. Wan, L., Lee, C. C., Hsu, C. M., Hwu, W. L., Yang, C. C., Tsai, C. H., and Tsai, F. J. (2008) Identification of eight novel mutations of the acid alpha-glucosidase gene causing the infantile or juvenile form of glycogen storage disease type II. *J Neurol* **255**, 831-838

4. Liu, X., Zou, H., Slaughter, C., and Wang, X. (1997) DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. *Cell* **89**, 175-184

5. Frigerio, J. M., Dagorn, J. C., and Iovanna, J. L. (1995) Cloning, sequencing and expression of the L5, L21, L27a, L28, S5, S9, S10 and S29 human ribosomal protein mRNAs. *Biochim Biophys Acta* **1262**, 64-68

6. Neumann, G. M., Marinaro, J. A., and Bach, L. A. (1998) Identification of O-glycosylation sites and partial characterization of carbohydrate structure and disulfide linkages of human insulin-like growth factor binding protein 6. *Biochemistry* **37**, 6572-6585

7. Toro, J. R., Nickerson, M. L., Wei, M. H., Warren, M. B., Glenn, G. M., Turner, M. L., Stewart, L., Duray, P., Tourre, O., Sharma, N., Choyke, P., Stratton, P., Merino, M., Walther, M. M., Linehan, W. M., Schmidt, L. S., and Zbar, B. (2003) Mutations in the fumarate hydratase gene cause hereditary leiomyomatosis and renal cell cancer in families in North America. *Am J Human Genet* **73**, 95-106

8. Hu, W., Zhang, C., Wu, R., Sun, Y., Levine, A., and Feng, Z. (2010) Glutaminase 2, a novel p53 target gene regulating energy metabolism and antioxidant function. *Proc Nat Acad Sci USA* **107**, 7455-7460

9. Yano, M., Hoogenraad, N., Terada, K., and Mori, M. (2000) Identification and functional analysis of human Tom22 for protein import into mitochondria. *Mol Cell Biol* **20**, 7205-7213

10. Lee, A. S., Kranzusch, P. J., Doudna, J. A., and Cate, J. H. (2016) eIF3d is an mRNA cap-binding protein that is required for specialized translation initiation. *Nature* **536**, 96-99

11. Liu, C., and Yu, X. (2015) ADP-ribosyltransferases and poly ADP-ribosylation. *Current protein & peptide science* **16**, 491-501

12. Low, S. H., Vasanji, A., Nanduri, J., He, M., Sharma, N., Koo, M., Drazba, J., and Weimbs, T. (2006) Syntaxins 3 and 4 are concentrated in separate clusters on the plasma membrane before the establishment of cell polarity. *Mol Biol Cell* **17**, 977-989

13. Tanaka, M., Kim, Y. M., Lee, G., Junn, E., Iwatsubo, T., and Mouradian, M. M. (2004) Aggresomes formed by alpha-synuclein and synphilin-1 are cytoprotective. *J Biol Chem* **279**, 4625-4631

14. Elrick, M. J., Yu, T., Chung, C., and Lieberman, A. P. (2012) Impaired proteolysis underlies autophagic dysfunction in Niemann-Pick type C disease. *Human Mol Genet* **21**, 4876-4887

15. Shetty, R., Sharma, A., Pahuja, N., Chevour, P., Padmajan, N., Dhamodaran, K., Jayadev, Ghosh, A., and Nallathambi, J. (2017) Oxidative stress induces dysregulated autophagy in corneal epithelium of keratoconus patients. *PloS One* **12**, e0184628

16. Vainberg, I. E., Lewis, S. A., Rommelaere, H., Ampe, C., Vandekerckhove, J., Klein, H. L., and Cowan, N. J. (1998) Prefoldin, a chaperone that delivers unfolded proteins to cytosolic chaperonin. *Cell* **93**, 863-873

17. Abe, A., Takahashi-Niki, K., Takekoshi, Y., Shimizu, T., Kitaura, H., Maita, H., Iguchi-Ariga, S. M., and Ariga, H. (2013) Prefoldin plays a role as a clearance factor in preventing proteasome inhibitor-induced protein aggregation. *J Biol Chem* **288**, 27764-27776

18. Shetty, R., Ghosh, A., Lim, R. R., Subramani, M., Mihir, K., Reshma, A. R., Ranganath, A., Nagaraj, S., Nuijts, R. M., Beuerman, R., Shetty, R., Das, D., Chaurasia, S. S., Sinha-Roy, A., and Ghosh, A. (2015) Elevated expression of matrix metalloproteinase-9 and inflammatory cytokines in keratoconus patients is inhibited by cyclosporine A. *Invest Ophthalmol Vis Sci* **56**, 738-750

19. Cheng, H. M., Sun, H. Y., Lin, D. P., Chang, H. H., Chen, S. T., Yeh, S. M., Peng, M. L., Tseng, J. K., Su, K. C., Tseng, K. W., Chen, B. Y., Hsiao, C. J., Huang, S. Y., and Cheng, C. Y. (2012) Characterising visual deficits in children of an urban elementary school in Taiwan. *Clin Exp Optom* **95**, 531-537

20. Petrosyan, A., Cheng, P. W., Clemens, D. L., and Casey, C. A. (2015) Downregulation of the small GTPase SAR1A: a key event underlying alcohol-induced Golgi fragmentation in hepatocytes. *Sci Rep* **5**, 17127

21. Jongkhajornpong, P., Lekhanont, K., Ueta, M., Kitazawa, K., Kawasaki, S., and Kinoshita, S. (2015) Novel TACSTD2 mutation in gelatinous drop-like corneal dystrophy. *Human Genome Var* **2**, 15047

22. Cabral-Macias, J., Zenteno, J. C., Ramirez-Miranda, A., Navas, A., Bermudez-Magner, J. A., Boullosa-Grana, V. M., Graue-Hernandez, E. O., and Buentello-Volante, B. (2016) Familial gelatinous drop-Like corneal dystrophy caused by a novel nonsense TACSTD2 mutation. *Cornea* **35**, 987-990

23. Alehabib, E., Jamshidi, J., Ghaedi, H., Emamalizadeh, B., Andarva, M., Daftarian, N., Rezaei Kanavi, M., Mohammadi Torbati, P., Espandar, G., Alinaghi, S., Johari, A. H., Saghally, M., Mohajerani, F., and Darvish, H. (2017) Novel mutations in TACSTD2 gene in families with gelatinous drop-like corneal dystrophy (GDLD). *Int J Mol Cell Med* **6**, 204-211

24. Wang, M. S., Zhang, R. W., Su, L. Y., Li, Y., Peng, M. S., Liu, H. Q., Zeng, L., Irwin, D. M., Du, J. L., Yao, Y. G., Wu, D. D., and Zhang, Y. P. (2016) Positive selection rather than relaxation of functional constraint drives the evolution of vision during chicken domestication. *Cell Res* **26**, 556-573

25. Gordon-Shaag, A., Millodot, M., Shneor, E., and Liu, Y. (2015) The genetic and environmental factors for keratoconus. *BioMed Res Int* **2015**, 795738

26. Mullarky, E., Lucki, N. C., Beheshti Zavareh, R., Anglin, J. L., Gomes, A. P., Nicolay, B. N., Wong, J. C., Christen, S., Takahashi, H., Singh, P. K., Blenis, J., Warren, J. D., Fendt, S. M., Asara, J. M., DeNicola, G. M., Lyssiotis, C. A., Lairson, L. L., and Cantley, L. C. (2016) Identification of a small molecule

inhibitor of 3-phosphoglycerate dehydrogenase to target serine biosynthesis in cancers. *Proc Nat Acad Sci USA* **113**, 1778-1783

27. Koehn, D. R., Meyer, K. J., and Anderson, M. G. (2015) Genetic Evidence for Differential Regulation of Corneal Epithelial and Stromal Thickness. *Invest Ophthalmol Vis Sci* **56**, 5599-5607

28. Chen, K., Xiao, H., Zeng, J., Yu, G., Zhou, H., Huang, C., Yao, W., Xiao, W., Hu, J., Guan, W., Wu, L., Huang, J., Huang, Q., Xu, H., and Ye, Z. (2017) Alternative Splicing of EZH2 pre-mRNA by SF3B3 Contributes to the Tumorigenic Potential of Renal Cancer. *Clin Cancer Res* **23**, 3428-3441

29. Nielsen, K., Hjortdal, J., Pihlmann, M., and Corydon, T. J. (2013) Update on the keratoconus genetics. *Acta Ophthalmol* **91**, 106-113

30. Matsuda, A., Tagawa, Y., Matsuda, H., and Nishihira, J. (1997) Expression of macrophage migration inhibitory factor in corneal wound healing in rats. *Invest Ophthalmol Vis Sci* **38**, 1555-1562

31. Gorrini, C., Harris, I. S., and Mak, T. W. (2013) Modulation of oxidative stress as an anticancer strategy. *Nature Rev Drug Discovery* **12**, 931-947

32. Alvarez, R., Vaz, B., Gronemeyer, H., and de Lera, A. R. (2014) Functions, therapeutic applications, and synthesis of retinoids and carotenoids. *Chem Rev* **114**, 1-125

Supplemental Data File S1:

Lists of quantifiable epithelial and stromal proteins from KC samples by SWATH-MS.

Supplemental Data File S2:

Lists of up- and down-regulated epithelial and stromal proteins compared among KC cone, non-cone and normal corneal samples. Mean fold changes, SD and P-values are listed for each candidate proteins.