Received: 15 June 2022 | Revised: 7 July 2022 | Accepted: 15 July 2022

DOI: 10.1111/all.15450

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

LETTER

Evidence of epithelial remodelling but not epithelial–mesenchymal transition by transcriptome profiling in vernal keratoconjunctivitis

To the Editor,

Vernal keratoconjunctivitis (VKC) is a severe type 2 ocular disease associated with corneal involvement, tissue remodelling, potential complications and visual impairment.[1] The tarsal (T-VKC), limbal (L-VKC) and mixed (M-VKC) phenotypes are characterized by either the presence of tarsal giant papillae, infiltrates/papillae/nodules at the limbus or by the coexistence of both, respectively. The clinical research has been focused on the identification of inflammatory mediators to untangle the immune mechanisms involved and to identify potential targets for therapeutic interventions. Recently, we showed different gene expression between the three different phenotypes, with modulations of genes involved in innate and adaptive immunity, antigen presentation, Th2- and Th17-priming.[2] Furthermore, our data suggest that epithelial barrier dysfunction and the epithelial-to-mesenchymal transition (EMT) may play a role in the VKC pathogenesis.[3] Aiming to identify differences in gene expression between VKC and normal subject (CT) with a particular focus to epithelial and EMT-related genes, we obtained impression cytology samples (Eyeprim™ device, OPIA Technologies SAS) from 15 active VKC patients (Table S1) and five healthy age-matched CT. The VKC severity was evaluated using three different clinical scores (Appendix S1). The study complied with the tenets of the Declaration of Helsinki and was approved by the IRB of our Institution. Informed consent was obtained from the subjects (or patients), who were informed of the nature of the study.

The GeneChip™ WT Pico Kit (Thermofisher Scientific) has been used to analyse the modulation of the transcriptome based on 21,448 probes, revealing 325 DEGs comparing VKC to CT: 92 upregulated and 241 downregulated (Figure 1A). Considering the three different phenotypes, 17 differently expressed genes (DEGs)

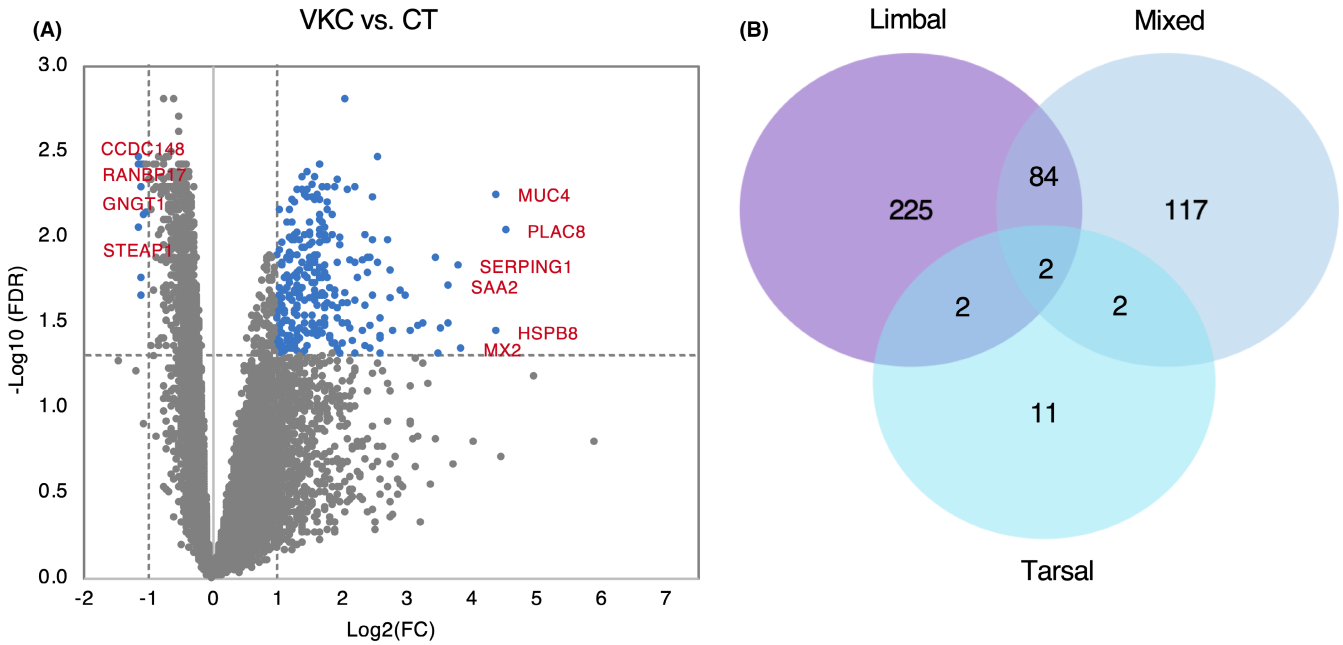

FIGURE 1 (A) Volcano plot comparative analysis of transcripts in VKC patients. A total of 92 genes were upregulated and 241 downregulated with a >2-fold-change increase and a significant p value, compared with controls. (B) Venn Diagram of DEGs in the three different VKC phenotypes, tarsal, limbal and mixed, shows relatively few DEGs in common.

TABLE 1 Significantly over-expressed and downregulated genes (alphabetical order) associated with the epithelial barrier in VKC compared with CT

Gene symbol	Gene name	FC	*p* Value	Adjusted *p* Value
AKAP17A	A kinase (PRKA) anchor protein 17A	2.11	.0001	.007[a]
AKAP7	A kinase (PRKA) anchor protein 7	−3.08	.0004	.015[a]
CAV1	Caveolin 1	−3.63	.0066	.078
CAV2	Caveolin 2	−2.33	.0007	.021[a]
CDH1	Cadherin 1, type 1	2.08	.0279	.179
CDH3	Cadherin 3, type 1, P-cadherin	1.43	.0311	.191
CDH22	Transcript Identified by AceView, Entrez Gene ID(s) 64405	−1.76	.0032	.051
CDH26	Cadherin 26	2.91	.0057	.072
CLDN8	Claudin-8	−5.19	.0007	.021[a]
CLDN34	Claudin-34	−1.61	.0225	.158
CGN	Cingulin	2.68	.0106	.102
CLDND1	Claudin domain containing 1	−2.28	.0013	.031[a]
CTNNAL1	Catenin-1	−2.59	.0034	.053
CTNNBIP1	Catenin, beta interacting protein 1	−1.69	.0066	.078
DSG1	Desmoglein 1	−10.52	.0156	.128
EDIL3	EGF-like repeats and discoidin I-like domains 3	−5.08	.0025	.044[a]
GNG10	Guanine nucleotide binding protein (G protein), gamma 10	−1.64	.0024	.044[a]
GNG11	Guanine nucleotide binding protein (G protein), gamma 11	−11.80	.0000	.003[a]
GNGT1	Guanine nucleotide binding protein (G protein), gamma Transducing activity polypeptide 1	−12.35	.0000	.005[a]
GJB2	Gap junction protein beta	6.12	.0021	.040[a]
GJC1	Gap junction protein gamma 1	−1.38	.0150	.126
KRT6A	Keratin 6A, type II	11.04	.0220	.157
KRT78	Keratin 78, type II	8.53	.0152	.126
KRT23	Keratin 23 type 1	11.32	.0029	.048[a]
LAGAL3BP	Lectin, galactoside-binding, soluble, 3 binding-protein	3.07	.0018	.037[a]
LGALS9	Lectin, galactoside-binding, soluble, 9	2.25	.0298	.186
LGALS9B	Lectin, galactoside-binding, soluble, 9B	2.18	.0090	.092
LIN7C	In-7 homolog C (C. elegans)	−1.67	.0059	.073
LUM	Lumican	−4.58	.0043	.060
MARVELD3	MARVEL domain containing 3	1.63	.0352	.206
MUC1	Mucin 1, cell surface associated	3.52	.0002	.010[a]
MUC2	Mucin 2, oligomeric mucus	2.67	.0476	.245
MUC3A	Mucin 3A, cell surface associated	3.13	.0018	.036[a]
MUC4	Mucin 4, cell surface associated	21.18	.0000	.005[a]
MUC5AC	Mucin 5 AC, oligomeric mucus	6.71	.0144	.122
MUC16	Mucin 16, cell surface associated	9.32	.0015	.033[a]
MUC20	Mucin 20, cell surface associated	−4.83	.0275	.177
OCLN	Occludin	1.89	.0470	.243
PARD6A	Par-6 family cell polarity regulator alpha	−1.51	.0172	.136
PARD6B	Par-6 family cell polarity regulator beta	2.45	.0541	.263
PCDH1	Protocaderin 1	2.97	.0018	.037[a]
RHOB	As homolog family member B	1.77	.0095	.096
RHOBTB3	Rho-related BTB domain containing 3	−3.17	.0090	.092
SPINK1	serine peptidase inhibitor, Kazal type 1	−1.55	.0147	.124
SPINK2	Serine peptidase inhibitor, Kazal type 2	−7.62	.0000	.005[a]
SPINK8	Serine peptidase inhibitor, Kazal type 8	−2.33	.0000	.003[a]
TJP1	Tight junction protein	3.18	.0007	.022[a]

[a]Statistically significant adjusted *p* value.

were identified in T-VKC, 232 in L-VKC and 166 in M-VKC, with few common DEGs (Figure 1B). The regression analysis based on clinical scores and performed to identify genes whose expression associates with disease severity, showed that 397, 461 and 297 DEGs were identified in patients with high Bonini's, CLEK and Oxford scores, respectively (Table S2).

Enrichment analyses showed that in T-VKC several gene ontology biological process (GOBP) were related to the tissue remodelling and B cells regulations and cytokine production (Table S3), while in L-VKC, the ribonucleoprotein complex biogenesis was the most represented GOBP. By Reactome analysis, comparing with CT, 169 out of 325 identifiers were found in all VKC cohort, and 128 out of 226 in L-VKC (Table S4). Many of the genes involved in these pathways belong to the ribosomal and mitochondrial ribosomal proteins families suggesting that further investigations are needed to relate environmental stress, typical of VKC, unfolded or misfolded proteins and ribosomal functions.

Since EyePrim collects mostly epithelial cells, we looked at the expression of genes related to the epithelial barrier function. Of the many genes, either up- or down-regulated (Table 1), TJP1, K23 (validated by IHC, as shown in Figure S1) and GJB2 were upregulated, CLDN8 and GNGT1 were highly downregulated similarly to what reported for atopic dermatitis[4] and Sjogren syndrome,[5] suggesting a possible *epithelial barrier dysfunction* and *remodelling* in VKC. Furthermore, because of the potential evolution in fibrosis, we found an upregulation of epithelial marker E-cadherin, a non-increased expression of genes encoding for EMT markers, and a downregulation of the transcription factors required for EMT (Table S5), suggesting that EMT is inhibited in VKC besides TGF-β, a potent inducer of both remodelling and EMT, is upregulated, as previously reported.[6]

In conclusion, different VKC phenotypes and severities reveal different trends of gene expression, highlighting the role of the epithelial barrier in the complexity of the disease pathogenesis. Further analyses, possibly based on whole transcriptome methods, will certainly expand our knowledge on molecular mechanisms during VKC and on possible endotypes that underline the different VKC phenotypes.

ACKNOWLEDGEMENT

None. Open Access Funding provided by Universita degli Studi di Padova within the CRUI-CARE Agreement. Open Access Funding provided by Universita degli Studi di Padova within the CRUI-CARE Agreement.

FUNDING INFORMATION

This study was supported by an unrestricted grant from Santen SAS.

CONFLICT OF INTEREST

The authors have no conflict of interest, only Philippe Daull and Jean-Sébastien Garrigue are the employees of Santen SAS.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.