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Transcriptome profiling of refractory atopic keratoconjunctivitis by RNA sequencing

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Short summary: We found upregulation of 47 immunoglobulin genes and 22 *S. aureus* infection-related genes in refractory atopic keratoconjunctivitis tissue by RNA-seq analysis, suggesting that lymphoid neogenesis and stimuli from infection are essential components of the disorder.

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38 **To the Editor.**

39 Atopic keratoconjunctivitis (AKC) and vernal keratoconjunctivitis (VKC) are severe
40 chronic forms of allergic conjunctivitis that are often accompanied by the formation of
41 giant papillae in the tarsal conjunctivae (supplementary figure 1) with prominent Th2
42 cytokine profiles.¹ Recent studies have demonstrated the efficacy of tacrolimus for the
43 treatment of AKC/VKC.² However, there are refractory cases, especially of AKC,
44 despite intense treatments using immune suppressants and glucocorticoids.² To elucidate
45 the pathophysiological characteristics of refractory AKC, we investigated gene
46 expression profiles using tissues of giant papillae obtained from patients with AKC,
47 refractory to tacrolimus treatment for more than 8 weeks (clinical information of the
48 patients is shown in supplementary table 1). AKC was defined as bilateral chronic
49 inflammation of the conjunctiva and lids associated with atopic dermatitis, often
50 accompanied by corneal pannus formation (arrows, supplementary figure 1) and
51 papillary hypertrophy of the upper tarsal conjunctiva (supplementary figure 1B, 1D,
52 1F).¹ It is also associated with superficial keratitis.³ Giant papillae from three AKC
53 patients were obtained during resection surgeries as previously described.⁴ Upper bulbar
54 conjunctivae resected from 3 conjunctivochalasis patients were used as control

55 samples.⁴ Conjunctivochalasis was defined as a redundant conjunctiva without definite
56 inflammation typically located between the globe and lower eyelid. All of the
57 procedures were approved by the ethics committees of the Juntendo University School
58 of Medicine and Kyoto Prefectural University School of Medicine. The study was
59 conducted in accordance with the tenets of the Declaration of Helsinki. Total RNA was
60 extracted from the giant papillae and conjunctivochalasis tissues and RNA sequencing
61 (RNA-seq) was performed with an Illumina HiSeq 1500.

62 Between 23 and 51 million reads were obtained per samples (36, 24, 36 million for
63 AKC samples and 33, 36, 53 million for conjunctivochalasis samples) by RNA-seq.
64 After trimming and filtering using FastX-Toolkit, 1.9 to 3.2% of the reads were
65 excluded and 94.3 to 96.1% of the remaining reads were mapped to the human genome
66 using HISAT2. Fragments per kilobase of exon per million mapped reads (FPKM) and
67 FDR were calculated using Cufflinks. We defined differentially expressed genes
68 (DEGs) as those with a fold change >2 , FPKM >10 , and false discovery rate (FDR) <0.05 ,
69 and found 872 hyperexpressed transcripts in AKC samples and 674 hypoexpressed
70 transcripts in AKC samples (supplementary figure 2). We made several lists of DEGs by
71 sorting our results. We first made a DEG list sorted by the fold change of expression
72 intensity (including the DEGs above log fold >3 , which means at least 8-fold changes)

73 with FPKM values greater than 100 in the AKC samples (Table 1). One of the findings
74 was the abundance of immunoglobulin-encoded transcripts (among the 872
75 hyperexpressed-DEGs, 47 mapped to immunoglobulin genes). This result was
76 consistent with our previous report showing ectopic lymphoid organs in the giant
77 papillae obtained from AKC patients⁴, and a previous report showing immunoglobulin
78 accumulation in giant papillary conjunctivitis (GPC).⁵ Taken together, these results
79 suggested that local immunoglobulin production was a feature of chronic allergic
80 conjunctivitis like AKC and GPC. KEGG analysis showed that the ‘*S. Aureus* infection’
81 pathway was enriched in AKC samples (supplementary table 2). Hyperexpressed-DEGs
82 included genes within the ‘*S. Aureus* infection’ pathway; ICAM1,
83 complement-associated transcripts (C1QC, C1QB, and CFI), and formyl peptide
84 receptors and (FPR1 and FPR2), which are known to be associated with process host
85 defense against *S. Aureus*.⁶ These results seemed to be reasonable considering the fact
86 that microbial infection (especially *S. Aureus* infection) is one of the clinical features of
87 AKC.³

88 We also made another DEG list sorted by the order of expression intensity in AKC
89 tissue. Epithelial cell-derived genes, including damage-associated molecular pattern
90 (DAMP) genes (S100A2 and S100A8) and hyperproliferative state-related keratin genes

(KRT6A and KRT16), are listed in supplementary table 3. These results suggested the importance of epithelial damage in the pathophysiology of AKC. Finally, we made a list of DEGs including the genes with possible relevance to AKC pathophysiology (Table 2). Transcripts encoding cytokines and their receptors were highly expressed, including Th2 cytokines, epithelial cell-derived type 2 initiate cytokines, and eosinophil-, macrophage- and neutrophil-attracting chemokines (Table 2; cytokines and receptors). Signature genes of mast cells (TPSAB1, CPA3 and FCER1A), eosinophils (PRG2, encoding eosinophil major basic protein), T cells (CD4), dendritic cells (ITGAX encoding CD11c) and Langerhans cells (CD207) were included in the list of DEGs (Table 2; inflammatory cells). The roles of these inflammatory cells in the pathophysiology of AKC were reported previously¹, and these findings supported the reliability of our present study. Remodeling-associated transcripts and their receptors (adhesion molecules) were also activated in AKC samples (Table 2, remodeling associated/adhesion molecules), including matricellular proteins [periostin, tenascin-C, and SPARC], MMPs and their inhibitors, collagen genes and adhesion molecules (integrins). In agreement with these findings, GO and KEGG analyses showed enriched 'ECM-receptor interaction' and 'extracellular matrix organization' pathways, respectively (supplementary table 2). These results were consistent with previous

109 reports showing the roles of remodeling-associated genes⁷ in chronic atopic disorders.
110 We also found genes significantly decreased in AKC samples (supplementary table 4),
111 some of which had immunosuppressive roles, e.g., insulin-like growth factor 2 (which
112 enhances regulatory T cell functions), and dual-specificity phosphatase 1 (which
113 dephosphorylates mitogen-activated protein kinase). There have been some RNA-seq
114 studies concerning atopic dermatitis⁸, as well as chronic rhinosinusitis with nasal
115 polyps⁹; however, this is the first transcriptome analysis of tissues from giant papillae
116 obtained from patients with refractory AKC using the RNA-seq method. In conclusion,
117 we observed enriched expression of transcripts of defensive molecules against
118 microorganism, suggesting that reducing the microorganisms burden in AKC patients
119 might be a novel therapeutic target for refractory AKC. We also found abundant
120 immunoglobulin-encoded transcripts in refractory AKC samples, suggesting insufficient
121 anti-T cell/B cell therapy. The RNA-seq transcriptome study is useful to elucidate gene
122 pathways relevant to AKC, and provides us with clues to new therapeutic targets.

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140 conflicts of interest.

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142 **References**

- 143 1. Bielory B, Bielory L. Atopic dermatitis and keratoconjunctivitis. *Immunol*
144 *Allergy Clin North Am* 2010; 30:323-36.

- 145 2. Miyazaki D, Fukushima A, Ohashi Y, Ebihara N, Uchio E, Okamoto S, et al.
146 Steroid-Sparing Effect of 0.1% Tacrolimus Eye Drop for Treatment of Shield
147 Ulcer and Corneal Epitheliopathy in Refractory Allergic Ocular Diseases.
148 Ophthalmology 2017; 124:287-94.
- 149 3. Chen JJ, Applebaum DS, Sun GS, Pflugfelder SC. Atopic keratoconjunctivitis: A
150 review. J Am Acad Dermatol 2014; 70:569-75.
- 151 4. Matsuda A, Ebihara N, Yokoi N, Maruyama K, Hamuro J, Kinoshita S, et al.
152 Lymphoid neogenesis in the giant papillae of patients with chronic allergic
153 conjunctivitis. J Allergy Clin Immunol 2010; 126:1310-2 e1.
- 154 5. Henriquez AS, Allansmith MR. Russell bodies in contact-lens-associated giant
155 papillary conjunctivitis. Arch Ophthalmol 1979; 97:473-8.
- 156 6. Foster TJ. Immune evasion by staphylococci. Nat Rev Microbiol 2005;
157 3:948-58.
- 158 7. Fujishima H, Okada N, Matsumoto K, Fukagawa K, Igarashi A, Matsuda A, et al.
159 The usefulness of measuring tear periostin for the diagnosis and management of
160 ocular allergic diseases. J Allergy Clin Immunol 2016; 138:459-67 e2.
- 161 8. Suarez-Farinas M, Ungar B, Correa da Rosa J, Ewald DA, Rozenblit M,
162 Gonzalez J, et al. RNA sequencing atopic dermatitis transcriptome profiling

163 provides insights into novel disease mechanisms with potential therapeutic
164 implications. J Allergy Clin Immunol 2015; 135:1218-27.

165 9. Wang W, Gao Z, Wang H, Li T, He W, Lv W, et al. Transcriptome Analysis
166 Reveals Distinct Gene Expression Profiles in Eosinophilic and Noneosinophilic
167 Chronic Rhinosinusitis with Nasal Polyps. Sci Rep 2016; 6:26604.

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170 **Key words:** atopic keratoconjunctivitis; RNA sequencing; immunoglobulin genes

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1 **Table 1. List of highly expressed genes in AKC (log2 fold>3, FPKM of AK >100)**

Gene	FPKM (control)	FPKM (AKC)	log2 (fold_change)	P_Value
BPIFA1 ,SPLUNC-1 Antimicrobial Protein	0	143.10	inf	5.00E-05
CA2, Carbonic Anhydrase 2	0.15	134.83	9.86	0.0001
LTF, Lactotransferrin	1.14	277.83	7.93	5.00E-05
POSTN	8.99	2021.92	7.81	5.00E-05
IGHV3-7	1.97	407.73	7.69	5.00E-05
CLC, Eosinophil Lysophospholipase	1.25	141.87	6.83	5.00E-05
LTB, Lymphotoxin Beta	1.37	153.64	6.81	0.0001
KRT6A	11.34	1161.46	6.68	5.00E-05
LYZ, Lysozyme	2.08	163.27	6.29	0.0012
CCL13	2.19	169.69	6.28	5.00E-05
IGHG2	18.33	1415.96	6.27	5.00E-05
IGHA2	13.05	904.77	6.12	5.00E-05
IGHV3OR16-13	4.89	330.38	6.08	5.00E-05
IGLV3-21	1.61	105.49	6.03	5.00E-05
IGLV10-54	4.34	256.28	5.89	5.00E-05
KRT16	4.68	260.24	5.80	5.00E-05
IGHG4	5.96	325.74	5.77	5.00E-05
IGLV2-11	12.05	611.05	5.66	5.00E-05
IGKV2-30	5.26	240.37	5.51	0.0002
IGLC7	2.36	101.88	5.43	5.00E-05

IGLV2-14	8.72	376.73	5.43	5.00E-05
IGKV4-1	26.12	960.69	5.20	5.00E-05
IGHV5-51	3.57	125.90	5.14	0.0002
CCL18	12.62	433.88	5.10	5.00E-05
CPA3	4.80	158.76	5.05	5.00E-05
TRAC, T-Cell Receptor Alpha Constant	10.38	316.92	4.93	5.00E-05
IGKV3-20	79.88	2337.29	4.87	5.00E-05
IGKV2-24	9.87	282.17	4.84	5.00E-05
TPSB2, Tryptase Beta 2	4.75	129.68	4.77	5.00E-05
MMP10	10.45	284.64	4.77	5.00E-05
IGKV1-5	19.88	513.26	4.69	5.00E-05
CORO1A, Coronin 1A	15.82	355.27	4.49	5.00E-05
LCP1, Lymphocyte Cytosolic Protein 1	4.72	102.90	4.45	5.00E-05
IGLV2-23	21.62	439.14	4.34	5.00E-05
IGLC1, Immunoglobulin Lambda Constant 1	315.35	6238.51	4.31	0.0001
TRBC2, Killer Cell Lectin Like Receptor B1	9.50	184.49	4.28	5.00E-05
MMP7	16.37	309.92	4.24	5.00E-05
IGHG3	22.15	412.07	4.22	5.00E-05
CD79A, B Lymphocyte Antigen Receptor	6.23	113.23	4.18	5.00E-05
IGHM	80.53	1451.70	4.17	5.00E-05
RAC2, Small GTP Binding Protein Rac2	6.77	116.43	4.10	5.00E-05
IL32	12.38	207.17	4.06	5.00E-05
IGHG1	67.20	1124.28	4.06	5.00E-05

CTSG, Cathepsin G	6.63	109.13	4.04	5.00E-05
IGLV1-51	25.58	366.34	3.84	5.00E-05
HLA-DQA1	20.62	277.45	3.75	5.00E-05
VMO1, Vitelline Membrane Outer Layer 1 Homolog	13.38	177.56	3.73	5.00E-05
UBD, Ubiquitin D	12.51	164.71	3.72	5.00E-05
PIP, Prolactin-inducible Protein	27.04	344.11	3.67	5.00E-05
CXCL1	30.78	356.71	3.53	5.00E-05
SRGN, Serglycin	25.21	270.72	3.42	5.00E-05
LAMC2, Laminin Subunit Gamma 2	10.95	114.08	3.38	5.00E-05
SPRR1B, Small Proline Rich Protein 1B, Cornifin	58.32	603.70	3.37	5.00E-05
CD3D, CD3delta	18.58	190.12	3.35	0.00045
LST1, Leukocyte Specific Transcript 1	25.47	255.64	3.33	5.00E-05
CAPN13, Calpain 13	17.47	174.69	3.32	5.00E-05
LAPTM5, Lysosomal Protein Transmembrane 5	17.01	169.80	3.32	5.00E-05
KRT17	90.54	882.73	3.29	5.00E-05
COTL1, Coactosin Like F-Actin Binding Protein 1	20.50	199.26	3.28	5.00E-05
SERPINB10,SERPINB2	16.91	164.28	3.28	5.00E-05
DEPDC5, DEP Domain Containing 5	28.22	268.48	3.25	5.00E-05
IGLC3	84.93	780.26	3.20	5.00E-05
LUM, Lumican	37.87	346.83	3.20	5.00E-05
C2,CFB	243.91	2183.46	3.16	5.00E-05
IGKV3-11	13.90	120.16	3.11	5.00E-05
SAA1	32.58	275.99	3.08	5.00E-05

HLA-DPB1	85.23	694.07	3.03	5.00E-05
COL1A1	14.62	118.45	3.02	5.00E-05
CD53, Tetraspanin-25	16.53	133.74	3.02	5.00E-05

1 **Table 2. List of highly expressed genes with possible relevance to AKC**

2 **pathophysiology**

Cytokines and receptors

Gene	FPKM (control)	FPKM (AKC)	log2 (fold_change)	p_value
IL4	0	10.56	inf	5.00E-05
IL13	0.08	35.66	8.74	0.0008
CCL13	2.19	169.69	6.28	5.00E-05
CCL18	12.62	433.88	5.10	5.00E-05
CCR3	0.56	12.97	4.52	0.0006
IL7R	0.80	14.69	4.20	5.00E-05
IL32	12.38	207.17	4.06	5.00E-05
IL2RG	7.22	93.06	3.69	5.00E-05
IL27RA	1.29	15.92	3.63	5.00E-05
CXCL1	30.78	356.71	3.53	5.00E-05
IL17RB	1.91	13.94	2.87	0.00045
CCL2	15.21	95.53	2.65	5.00E-05
CCL5 (RANTES)	9.28	52.10	2.49	5.00E-05
TNFRSF12A (TWEAKR)	18.48	96.51	2.38	5.00E-05
CCL4	5.73	25.87	2.18	0.0009
CCL19	24.38	105.45	2.11	5.00E-05
IL1R1	15.33	63.22	2.04	5.00E-05
IFNGR2	31.49	124.48	1.98	5.00E-05
IL4R	13.65	53.31	1.97	5.00E-05
IL33	6.41	19.86	1.63	5.00E-05

Inflammatory cells

Gene	FPKM (control)	FPKM (AKC)	log2 (fold_change)	p_value
KRT6A	11.34	1161.46	6.68	5.00E-05
KRT16	4.68	260.24	5.80	5.00E-05
TPSAB1 (Tryptase)	1.36	54.36	5.32	0.0002
CPA3 (Carboxypeptidase A3)	4.80	158.76	5.05	5.00E-05

ITGAX (CD11c)	0.61	11.14	4.18	5.00E-05
CD207 (Langerin)	0.80	10.39	3.70	5.00E-05
CD4	8.61	42.38	2.30	5.00E-05
C1QC	83.43	22.03	1.92	5.00E-05
FCER1A	7.26	25.77	1.83	5.00E-05
PRG2 (Major Basic Protein)	13.08	35.04	1.42	0.00015
CFI	70.16	28.95	1.28	5.00E-05

Remodeling associated

Gene	FPKM (control)	FPKM (AKC)	log2 (fold_change)	p_value
POSTN	8.99	2021.92	7.81	5.00E-05
TNC	1.70	63.31	5.21	5.00E-05
MMP1	3.49	97.16	4.80	5.00E-05
MMP10	10.45	284.64	4.77	5.00E-05
MMP7	16.37	309.92	4.24	5.00E-05
COL4A1	1.45	16.26	3.49	5.00E-05
COL4A2	10.65	97.89	3.20	5.00E-05
COL1A1	14.62	118.45	3.02	5.00E-05
COL6A2	29.27	157.56	2.43	5.00E-05
TIMP1	267.11	1381.38	2.37	5.00E-05
FN1	17.53	76.32	2.12	5.00E-05
MMP14	24.73	104.86	2.08	5.00E-05
COL6A1	41.03	170.50	2.05	5.00E-05
COL3A1	40.93	167.01	2.03	5.00E-05
SPARC	66.42	238.54	1.84	5.00E-05
COL5A2	3.49	11.19	1.68	5.00E-05
COL1A2	78.53	193.57	1.30	5.00E-05

Adhesion molecules

Gene	FPKM (control)	FPKM (AKC)	log2 (fold_change)	p_value
MADCAM1	0.32	15.43	5.60	5.00E-05
ITGA2B	1.15	32.48	4.82	0.0002
ITGAX	0.61	11.14	4.18	5.00E-05
ITGAL	1.45	24.90	4.10	5.00E-05

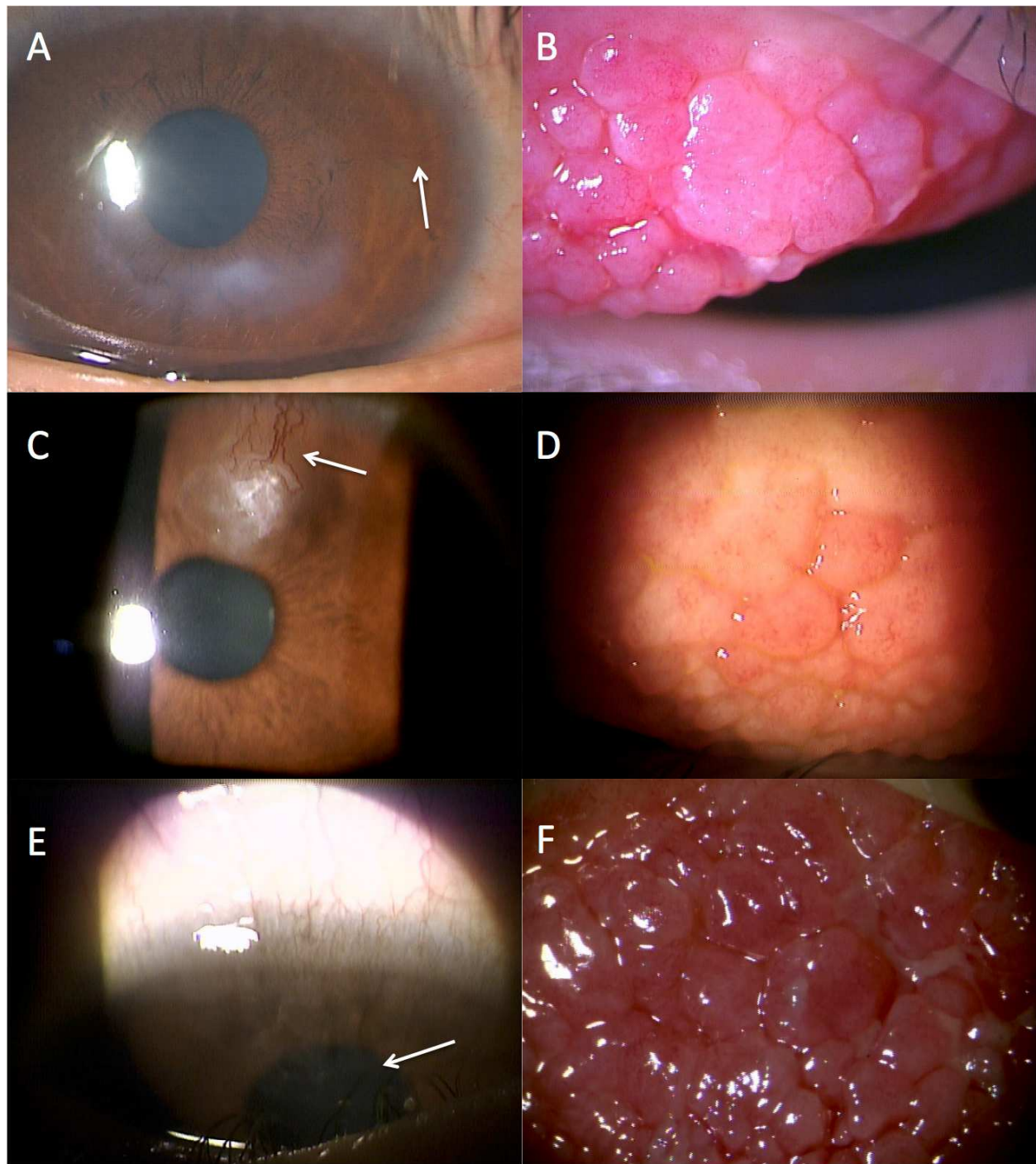
VCAM1	11.66	0.93	3.65	5.00E-05
ITGB2	9.53	87.57	3.20	5.00E-05
ICAM1	4.47	40.19	3.17	0.0001
ITGA5	3.26	19.17	2.56	0.0015
ITGB6	6.92	29.74	2.10	0.00015
PECAM1	7.38	31.64	2.10	5.00E-05
ICAM3	19.76	79.84	2.01	0.004
ICAM2	7.33	22.78	1.64	0.0001
ITGA3	71.00	181.41	1.35	0.00065
ITGAV	7.27	17.08	1.23	5.00E-05

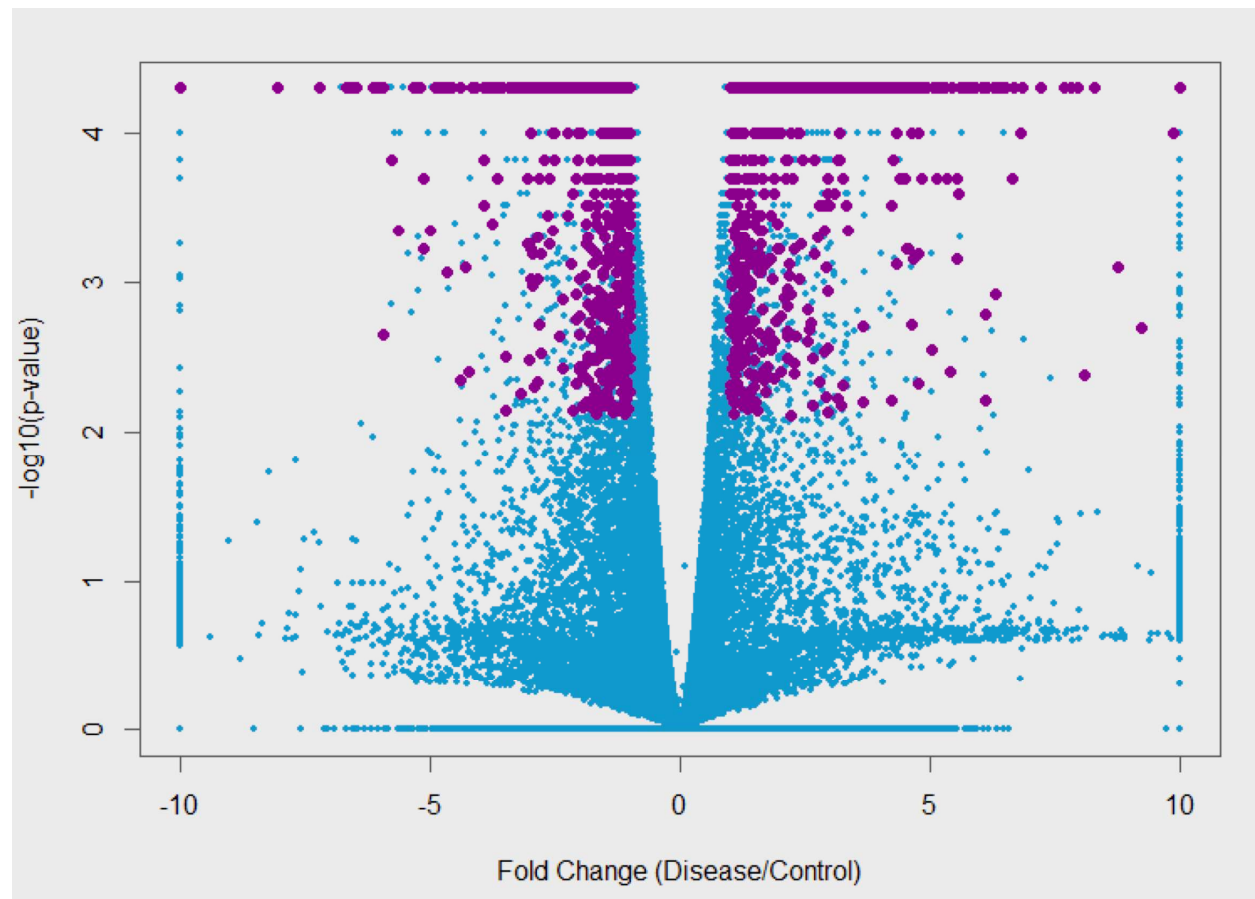
Figure Legend for supplementary Figure 1

Slitlamp photographs of AKC patients No.1 (A, B), No.2 (C, D) and No.3 (E, F). Pannus (corneal neovascular) formation was observed in all cases (arrows). Formation of giant papillae was also observed in all three cases (B, D, F).

Figure Legend for supplementary Figure 2

Volcano plots of differentially expressed genes ($FDR < 0.05$, fold change > 2 , purple color) are shown. The fold change (diseases/control) of each transcript is plotted horizontally and FDR in the form of $-\log_{10}$ (p-value) of each transcript is plotted vertically.





Supplementary Table 1. Clinical information of AKC and control (conjunctivochalasis) patients analyzed in this study**Supplementary Table****1**

	Age	Sex	Diagnosis	Corneal involvement	Treatment before resection	Antigen specific IgE	Bacterial c
AKC-1	12	M	AKC	Corneal opacity and neovascularization	Dex q.i.d (4weeks), Tac (8weeks)	house dust, mite, cedar pollen	negativ
AKC-2	9	F	AKC	Corneal opacity and neovascularization	Dex q.i.d (2 years), Tac (2 years)	house dust, mite, cedar pollen	Positive for <i>Stap</i>
AKC-3	14	M	AKC	Corneal opacity and neovascularization	Dex q.i.d (12 weeks), Tac (1 year)		negativ
Control-1	59	M	Conjunctivochalasis	none	none	N/A	N/A
Control-2	69	F	Conjunctivochalasis	none	none	N/A	N/A
Control-3	73	F	Conjunctivochalasis	none	none	N/A	N/A

Dex: 0.1% dexamethasone eyedrop; Tac: 0.1% Tacrolimus eye drop.

Supplementary Table 2. Enriched pathways in genes significantly increased in AKC samples detected by KEGG/GO analysis**GO analysis****KEGG
analysis**

Rank	Term	P-Value
1	Immune response	1.80E-37
2	Regulation of immune response	7.76E-23
3	Extracellular matrix organization	8.08E-21
4	Leukocyte migration	2.15E-20
5	Cell adhesion	1.65E-17
6	T cell receptor signaling pathway	3.18E-13
7	Complement activation	1.26E-12
8	T cell costimulation	3.20E-12
9	Proteolysis	5.39E-12
10	Receptor-mediated endocytosis	6.95E-12
11	Adaptive immune response	3.33E-11
12	Complement activation, classical pathway	3.62E-11
13	Fc-gamma receptor signaling pathway involved in phagocytosis	4.63E-11
14	Regulation of cell shape	6.94E-11
15	Inflammatory response	1.90E-10

Rank	Term
1	Staphylococcus aureus infection
2	Hematopoietic cell lineage
3	ECM-receptor interaction
4	Cell adhesion molecules
5	Primary immunodeficiency
6	NF-kappa B signaling pathway
7	Viral myocarditis
8	Asthma
9	HTLV-I infection
10	Focal adhesion
11	Chemokine signaling pathway
12	Allograft rejection
13	Amoebiasis
14	Rheumatoid arthritis
15	Leishmaniasis

Supplementary Table 3. Genes abundantly expressed in AKC samples (log2 fold>1, in order of FKPM-AKC)

Gene	FPKM (control)	FPKM (AKC)	log2 (fold_change)	p_value
S100A8	4532.45	10313.7	1.19	0.00525
IGLC1, Immunoglobulin Lambda Constant 1	315.353	6238.51	4.31	0.0001
S100A2	960.751	5785.03	2.59	5.00E-05
TMSB10, Thymosin Beta 10	2251.64	4545.9	1.01	0.00175
JCHAIN, Joining Chain of Multimeric IgA and IgM	574.961	3971.38	2.79	5.00E-05
CD74	1073.36	3704.58	1.79	0.0007

KRT14	927.303	3276.19	1.82	0.0028
IGLC2	403.872	2585.58	2.68	5.00E-05
IGKV3-20	79.8815	2337.29	4.87	5.00E-05
C2,CFB, Complement C2, Complement Factor B	243.909	2183.46	3.16	5.00E-05
POSTN	8.98861	2021.92	7.81	5.00E-05
HLA-DRA	271.691	1791.15	2.72	5.00E-05
S100P	556.965	1737.01	1.64	5.00E-05
RPS2, Ribosomal Protein S2	738.116	1598.66	1.11	0.00295
IGHM	80.5295	1451.7	4.17	5.00E-05
IGHG2	18.3306	1415.96	6.27	5.00E-05
TIMP1	267.112	1381.38	2.37	5.00E-05
AGR2, Protein Disulphide Isomerase	438.374	1186.22	1.44	5.00E-05
KRT6A	11.3436	1161.46	6.68	5.00E-05
IGHG1	67.2018	1124.28	4.06	5.00E-05
ANXA1	505.081	1052.88	1.06	0.001
HLA-DQB1	200.605	1019.57	2.35	5.00E-05
CTSC	149.658	1011.18	2.76	5.00E-05
CFL1	407.47	962.022	1.24	0.00035
IGKV4-1	26.1163	960.691	5.20	5.00E-05
IFITM3	455.205	922.417	1.02	0.0001
IGHA2	13.0459	904.774	6.12	5.00E-05

KRT17	90.5363	882.729	3.29	5.00E-05
WFDC21P	356.43	791.683	1.15	5.00E-05
HLA-DRB1, HLA-DRB6	135.095	782.348	2.53	5.00E-05
IGLC3	84.9334	780.262	3.20	5.00E-05
SERPINF1	362.353	747.261	1.04	5.00E-05
HSP90B1	268.587	694.512	1.37	0.0013
HLA-DPB1	85.2258	694.071	3.03	5.00E-05
MSMB	216.182	658.787	1.61	5.00E-05
IGLV2-11	12.0454	611.046	5.66	5.00E-05
CD59	239.943	606.478	1.34	5.00E-05
SPRR1B	58.3152	603.704	3.37	5.00E-05
FLNA	289.095	594.623	1.04	0.00585
SUMO2	260.566	542.375	1.06	0.0031
P4HB, Collagen Prolyl 4-Hydroxylase	186.287	529.435	1.51	0.00035
IGKV1-5	19.8796	513.258	4.69	5.00E-05
GNAS	240.277	501.758	1.06	5.00E-05
PI3, Peptidase Inhibitor 3	61.2659	479.666	2.97	5.00E-05
VPS29	191.211	478.001	1.32	5.00E-05
HLA-DPA1	64.7422	476.809	2.88	5.00E-05
TNFAIP2	196.036	470.901	1.26	0.0001
APOC1, APOE	72.8517	467.292	2.68	5.00E-05

HLA-DRB5	65.1421	452.205	2.80	5.00E-05
SH3BGRL3	179.948	443.762	2.27	0.0004

Supplementary Table 4. Genes significantly decreased in AKC samples (log2 fold<-2.0, FKPM of control samples>100)

Gene	FPKM (control)	FPKM (AKC)	log2(fold_change)
KRT15	3637.58	379.11	-3.26
ADIRF, Adipogenesis Regulatory Factor	2196.84	435.06	-2.34
TNNI2, Troponin I	1920.83	7.18	-8.06
FABP5	1614.60	398.33	-2.02
IGF2, Insulin-Like Growth Factor 2	1363.89	20.43	-6.06
TNNT3, Troponin T3	1201.19	17.23	-6.12
ALDH3A1	1076.85	107.67	-3.32
MGP, Matrix Gla Protein	970.01	156.48	-2.63
SSH3, Slingshot Protein Phosphatase 3	889.03	171.57	-2.37
COX7A1, Cytochrome C Oxidase Subunit 7A1	855.74	148.99	-2.52
SYT8, Synaptotagmin 8	807.91	7.87	-6.68
KRT7	721.73	164.78	-2.13
CSRP2, Cysteine And Glycine Rich Protein 2	687.55	86.41	-2.99

PER1, Period Circadian Clock 1	665.19	56.48	-3.56
HOPX	621.95	144.30	-2.11
DUSP1	594.97	87.41	-2.77
TMEM203, TPRN	592.31	31.77	-4.22
CCDC57	418.65	99.00	-2.08
TJP3, Tight Junction Protein 3	404.01	90.88	-2.15
APOD, Apolipoprotein D	388.35	67.82	-2.52
HOOK2, RTBDN	346.24	44.98	-2.94
DAPL1	263.09	26.39	-3.32
ERICH5	259.50	17.12	-3.92
SCGB3A1, Secretoglobin Family 3A Member 1	250.97	11.83	-4.41
HES1	236.84	44.54	-2.41
CXCL14	235.13	54.10	-2.12
FAM3B	230.27	18.93	-3.60
RN7SK	226.74	23.36	-3.28
CAPNS2	223.97	38.44	-2.54
CALML3	219.62	39.80	-2.46
NFATC2IP, Nuclear Factor of Activated T-Cells 2 Interacting Protein	215.88	8.55	-4.66
MROH6	211.12	49.50	-2.09
ALDH1A1	183.22	23.92	-2.94
RMRP	178.76	22.23	-3.01

MFAP4	177.83	23.14	-2.94
G3BP2	158.08	23.27	-2.76
FAM3D	144.34	33.50	-2.11
ARL4D	138.88	25.97	-2.42
CFD, Complement Factor D	130.83	25.31	-2.37
SCGB2A1	120.63	8.07	-3.90
PLAC9	110.92	13.28	-3.06
SNAI3, Snail Family Transcriptional Repressor 3	109.37	1.98	-5.79
CPAMD8, Alpha-2-Macroglobulin Domain Containing 8	104.53	8.95	-3.55
WNK2	104.25	12.75	-3.03
