© Kamla-Raj 2018

Int J Hum Genet, 18(2): 137-151 (2018)
DOI: 10.31901/24566330.2018/18.2.697

Screening Hub Genes in Microbial Keratitis Based on Gibbs Sampling Analysis

Qiao-Ling Wang[1], Meng-Hui Zhang[2] and Xi Chen[3*]

[1]Department of Ophthalmology, The Second People's Hospital of Jinan, Jinan 250012, Shandong, P.R. China
[2]Department of General Surgery, The Fourth People's Hospital of Jinan, Jinan 250031, Shandong, P.R. China
[3]Department of Ophthalmology, The Ninth People's Hospital of Chongqing, Chongqing 400700, P.R. China

KEYWORDS Gibbs Sampling. Hub Gene. Key Differential Pathway. Markov Chain. Microbial Keratitis. Probability

ABSTRACT This study aimed to identify potential key pathways and hub genes. The enrichment pathways were chosen through merging the gene expression data from microarray data with KEGG pathways. Gibbs sampling was performed to screen out significant pathways and the pathway gene set was determined. Finally, hub genes were identified using Gibbs sampling and statistics. A total of 278 pathways were chosen according to gene overlap greater than 5. Markov chain (MC) was established based on the enrichment of the gene expression profile in each pathway using Gibbs sampling and then 26 significant pathways were determined judging by adj_$á_{pi}$ greater than 0.8. Additionally, 1167 pathway gene sets were found out. Finally, 26 pathways were chosen as key pathways and 5 hub genes were identified on basis of their importance, which will contribute to elucidating potential molecular mechanism of microbial keratitis. We identified potential key pathways and hub genes in microbial keratitis.

INTRODUCTION

Microbial keratitis is an infective ophthalmic disease, caused by spectrums of pathogenic microorganisms including bacteria, viruses, fungi, Acanthamoeba and other prokaryote pathogens. In spite of recent advances in antimicrobial measures, due to ensuing excessive inflammatory response and ocular tissue damage, large numbers of patients' conditions fail to achieve effective controls or continue to deteriorate, and eventually patients have to undergo corneal transplantation or even enucleation. Accordingly, it is imperative to have a bearing on relevant physiopathologic mechanisms, which inevitably will be a breakthrough for diagnosing, treating or monitoring microbial keratitis.

Microbial keratitis initiation and progression relate to intricate biological process that involves various genes alterations and diverse molecular mechanisms underlying the pathophysiology. With the aid of bioinformatics analyses, a powerful approach emerging of proteome analysis in tear protein profile has indicated that glutaredoxin-related protein, lipocalin, prolactin inducible protein, serum albumin precursor, apolipoprotein, lacritin precursor (Ananthi et al. 2008; Ananthi et al. 2013; Kandhavelu et al. 2017). Several molecular pathways were reported to involve in the development of microbial keratitis, namely, p38 mitogen-activated protein kinases (MAPK) pathway (Hua et al. 2017), Toll-like receptor-3 (TLR3)/ TIR domain-containing adaptor inducing IFN-β (TRIF) pathway (Park et al. 2015), spleen-tyrosine kinase (Syk) signaling (Liu et al. 2015). With the increasingly biological importance of highly connective hub genes that play a pivotal role in maintaining interaction network, hub genes or key pathways identified were particularly crucial for uncovering the pathogenesis of microbial keratitis. In the wake of rapidly diverse bioinformatics methodologies, the understanding of underlying physiopathologic molecular mechanisms of disorders has been greatly improved. Here, in this work, coupled with microarray data, Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment, Gibbs sampling, the researchers identified key pathways and hub genes associated with microbial keratitis.

Address for correspondence:
Xi Chen
Department of Ophthalmology,
The Ninth People's Hospital of Chongqing,
Chongqing 400700, P.R. China
E-mail: wqlcx123@163.com

Objective

In the present study, the researchers achieved 278 enrichment pathways based on genes intersection greater than 5 from microarray data performed on samples with 15 keratititides and 12 normal. Moreover, Gibbs sampling was implemented to identify 26 key differential pathways. Then, the researchers applied Gibbs sampling to acquire 5 hub genes through differential pathway gene sets. Overall, hub genes analysis improved comprehending of microbial keratitis through providing relevant gene changes that occur during microbial keratitis progression and discovered the key biomarkers with potential utility for clinical diagnosis, therapy, and surveillance of microbial keratitis progression.

METHODOLOGY

Microarray Data Acquisition and Preprocessing

The gene expression profile, with the accession number of E-GEOD-58291 (Chidambaram et al. 2017), was downloaded from ArrayExpress (http://www.ebi.ac.uk/arrayexpress/). In the microarray data of E-GEOD-58291, there were 15 keratitis samples and 12 normal samples. The probe annotation data were downloaded and then each probe was mapped to matched gene symbols. Amongst which the probe was discarded if it can't match any one gene, or the average expression value was computed if there were multiple probes match one gene. After data preprocessing, the researchers eventually acquired the gene expression matrix containing 14,832 genes.

EGG Pathway Enrichment

Pathway enrichment analysis of gene expression matrix was conducted based on the KEGG pathway database. The researchers merged the gene expression data with KEGG regulatory pathways and chose 278 pathways judging by gene overlap greater than 5.

Gibbs Sampling

In order to obtain the pivotal pathways and key genes, Gibbs sampling was introduced. Gibbs sampling is a Markov chain (MC) Monte Carlo (MCMC) algorithm extensively applied in statistical inference that generates samples from a sequence of different conditional probability distributions. To executing Gibbs sampling, the above 278 pathways were converted into MC.

According to the enrichment of the gene expression profile in each pathway, the mean value of gene expression in each pathway was computed in normal and keratitis states. The normal state served as the initial state and the keratitis state served as prior state, then the third state was inferred by the prior state, in turn the state n was gained according to this model extrapolated.

An empty Gibbs sampling set comprising a k-dimensional (k = 278) random vector was defined. Following, n samples MC data set containing the initial value and prior value were deposited into the empty Gibbs sampling set. Then, a k-dimensional vector was initialized, k-1 elements of this vector were fixed, the remaining element was extracted, like this cycled k times which was equal to updating the whole vectors and also generate a novel sample. The third state was obtained. Ultimately, through n cycles of Gibbs sampling, a MC was established.

Differential Pathways Analysis

On basis on the posterior value of pathways generated by the MC and utilizing the probability formula α_{pi}, the probability of each pathway was acquired. Then, by statistical analysis (student's t-test) of the pathways expression in keratitis and normal states, the *P* values were calculated and according to it, the pathways were ranked ($rank_i$). Combining α_{pi} and *P* value, correction coefficient (R_{value}) was calculated and then adj-α_{pi} (multiplying α_{pi} by R_{value}) was calculated. Key differential pathways were selected judging by adj_α_{pi} greater than 0.8. Related computational formulas were as follows.

$$\alpha_{pi} = \frac{\sum_{i-2000}^{1000} Pi}{1000 - 2000 + 1}$$

$$R_{value} = 1 - \frac{rank_i}{n}$$

$$\text{adj\_}\alpha_{pi} = R_{value} \times \alpha_{pi}$$

Hub Genes Identification

The genes in the differential pathways were analyzed to find the pathway gene set and their frequencies in differential pathways were count-

ed. Subsequently, pathway gene set were converted into MC and then Gibbs sampling was performed. Finally, differential pathway genes of which alfa-adj were greater than 0.8 were chosen as hub genes.

RESULTS

KEGG Pathway Enrichment Analysis

By data preprocessing, a total of 14832 genes were obtained and these genes were enriched into the KEGG pathway with 287 pathways containing 6,894 genes. The enrichment analysis suggested that 278 pathways were determined based on the gene overlap greater than 5.

Differential Pathways Identification

To further determine key pathways, the researchers used the Gibbs sampling via computing adj_α_{pi} to gain the probabilities distribution of enriched pathways shown in Figure 1, in the image, a total of 26 key pathways were identified according to the adj-$\alpha_{pi} > 0.8$, including NF-kappa B signaling, TNF signaling, Cytokine-cytokine receptor interaction, Phagosome, Hematopoietic cell lineage, Toll-like receptor signaling et al. As shown in Table 1, the expression values of these pathways in normal and keratitis states suggested that these pathways were upregulated significantly in keratitis groups compared to normal group ($P < 0.05$), correspondingly, the heat map of pathways expression levels were presented in Figure 2. Compared with normal group, these 26 significant pathways in keratitis group were higher expressed.

Pathway Gene Set Selection

In an effort to find out the hub genes are of importance in gene expression network, 1167 genes in significant pathways were needed to be analyzed. Gene set in 26 significant pathways comprised 82 genes in Rheumatoid arthritis signaling pathway, 195 genes in Cytokine-cytokine receptor interaction signaling pathway, 150 genes in Tuberculosis signaling, 158 genes in Chemokine signaling pathway, 115 genes in Osteoclast differentiation signaling pathway, 49 genes in Staphylococcus aureus infection pathway et al. Several genes may appear in multipathways and a total of 1167 genes were contained in 26 key differential pathways and the

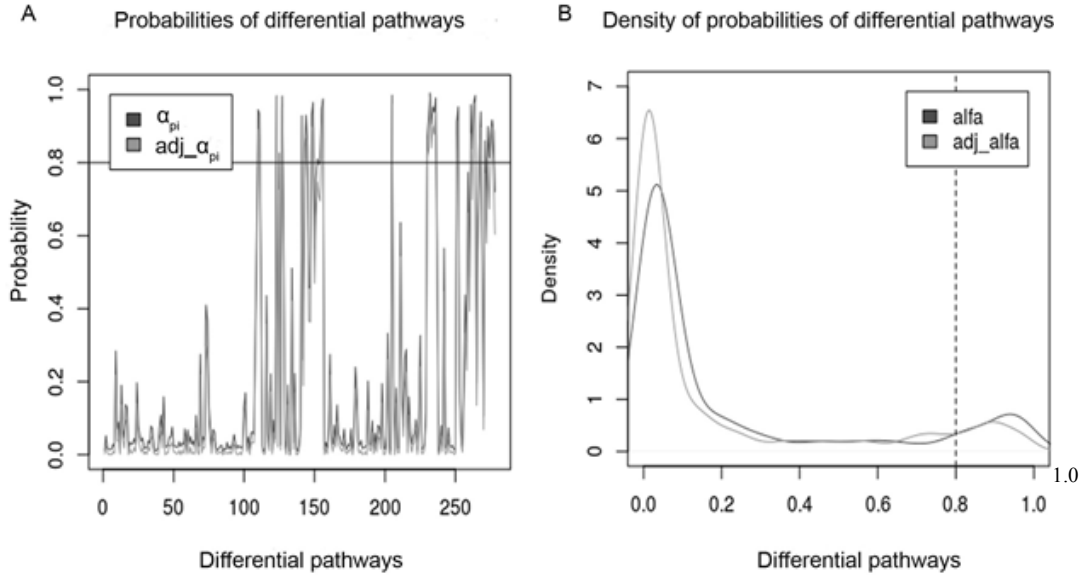

Fig. 1. The probabilities distribution of 278 pathways. The X axis denoted the pathways, and the Y axis represented the posterior value of the pathways. A. The adjusted posterior value distribution of 278 pathways. B. The density of 278 differential pathways posterior value distribution. Pathway was considered as the key differential pathway judging by adj_$\alpha_{pi} > 0.8$.

Source: Author

Table 1: Comparison of expression values in 26 differential pathways with adj_á$_{pi}$ > 0.8 between two groups

Pathway	*Number*	*Member*
Rheumatoid arthritis	82	CD80;CD86;CD28;CTLA4;HLA-DMA;HLA-DMB;HLA-DOA;HLA-DOB;HLA DPA1;HLA-DPB1;HLA-DQA1;HLA-DQA2;HLA-DQB1;HLA-DRA;HLA-DRB1;HLA-DRB3;HLA-DRB4;HLA-DRB5; ITGAL;ITGB2;ICAM1;IL15;TNFSF13;TNFSF13B;LTB ;TNF; IL1A;IL1B; IL6;IL11;IL18;TLR2;TLR4 ;JUN;FOS;TGFB2;TGFB3; IL23A; CSF1; TNFRSF11A; ATP6V1A;ATP6V1B1;ATP6 V1B2; ATP6V1C1; ATP6V1D; ATP6V1E2;ATP6V1E1;ATP6V1F; ATP6V1G1; ATP6V1G2; ATP6V0E1;ATP6V0E2;TCIRG 1; ATP6V0A2; ATP6V0A4; ATP6V0A1; ATP6V0D1;ATP6V1H;ATP6AP1;AT P6V0C; ATP6V0B; CTSK;ACP5;MMP1;MMP3; CTSL; CSF2;CCL5; CCL2; CCL3;CCL3L1 ;CCL3L3;CCL20; CXCL5;CXCL6; CXCL8;CXCL12; VEGFA;ANGPT1; TEK;IFNG;CXCL1
Cytokine-cytokine receptor interaction	195	CXCL1;CXCL2;CXCL5;CXCL6;PPBP;CXCL8;CXCL9;C XCL10;CXCL11; CXCL12; CXCL13;CXCL16;PF4V1;CXCL14;CX3CL1;CCL1;CCL16;CCL17;CCL18;CCL22;CCL24;CCL26;CCL20;CCL19;CCL21;CCL2;CCL4;CCL3;CCL3L1;CCL3L3;CCL13;CCL7;CCL5;CCL2 3;CCL8;CCL11; CCL28;IL6;IL11;OSM;LIF;CLCF1;CTF1;CSF3;LEP;IL23A;CSF2;IL7;IL15;PDGFC;PDGFD; PDGFA; PDGFB;VEGFA;VEGFB;VEG FC;HGF;EGF;CSF1;KITLG;FLT3L G;IFNW1; IFNG; IL10;IL19;IL24;TNFSF15;TNFSF10;TNFSF 12;TNF;LTA;LTB;TNFSF14;CD40LG;CD70;TNFSF8;TNFSF9;TNFSF4;TNFSF13;TNFSF13B;EDA;TGFB2;TGFB3;INHBB;INHBE;AMH;BMP2;BMP4;BMP7;GDF5;IL25;IL 1A;IL1B;IL18; CXCR2; CXCR1; CXCR3; CXCR4; CXCR5;CXCR6;ACKR3;CX3CR1;CC R6;CCR7;CCR2;CCR5;CCR1;CCR3; CCR10;IL6R;IL6ST;IL11RA;LIFR;OSMR;CNTFR;CSF3R;LEPR;IL4R;IL13RA1;IL12RB1;CSF2RA;CSF2RB;IL3RA;IL5RA;IL2RA;IL2RB;IL2RG;IL7R;IL15RA;IL21R;EPOR;GHR;PRLR;MPL;PDGFRA;PDGFRB;KDR;MET;EGFR;CSF1R;KIT;FLT3;IFNAR1;IFNAR2;IFNGR1;IFNGR2;IL10RA;IL10RB;IL20RA;IL20RB;IL22RA1;IFNLR1;TNFRSF10A;TNFRSF10B;TNFRSF10C;TNFRSF10D;TNFRSF11B;TNFRSF11A;TNFRSF25;TNFR SF12A; TNFRSF21; TNFRSF1B; TNFRSF 1A;LTBR;TNFRSF14;TNFRSF6B; FAS;CD40;CD27; TNFRSF8; TNFRSF9; TNFRSF4;TNFRSF17;TNFRSF13B;EDAR;EDA2R;TNFRSF19;RELT ;TGFBR2;TGFBR1;ACVR2A; ACVR1; ACVR1B;AMHR2; BMPR2;BMPR1A; BMPR1B; IL17RA; IL17RB;IL1R1;IL1RAP;IL1R2;IL18R1;IL18RAP;PLEKHO2
Tuberculosis	150	TNF;TNFRSF1A;TRADD;FADD;CASP8;CASP10;CASP3;BID;BAX;CYCS;CA SP9;APAF1;AKT1;AKT3;BAD;BCL2;CAMK2D;CAMK2B;C AMK2G;IFNG;IFNGR1;I FNGR2;JAK1; JAK2;STAT1;CIITA;RFX5;RFXANK;RFXAP;NFYA;NFYB;NFYC;CREB1;HLA-DMA; HLA-DMB;HLA-DOA;HLA-DOB;HLA-DPA1;HLA-DPB1;HLA-DQA1;HLA-DQA2;HLA-DQB1;HLA-DRA;HLA-DRB1;HLA-DRB3;HLA-DRB4;HLA-DRB5;CD74; CREBBP;EP300;IL10;IL10RA;IL10RB;CTSS;CLEC4E;FCER1G;CLEC7A;SRC;SYK;CARD9;MALT1;BCL10;NOD2;RIPK2;HSPA9;HSPD1;LBP;TLR2;TLR1;TLR6;TLR4;CD14;TIRAP;MYD88;IRAK4;IRAK1;IRAK2;TRAF6;NFKB1;RELA;MAPK11;MAPK12;MAPK13;MAPK14;MAPK1;MAPK3;MAP K8;MAPK10; MAPK9; NOS2;IL6;IL18;IL23A;IL1A;IL1B;CEBPB;CEBPG;CD209;ARHGEF12;R HOA; LSP1; PLK3; KSR1;RAF1;TGFB2;TGFB3;CYP27B1;VDR;CAMP;C3;CR1;ITG AX;ITGB2; ITGAM;PLA2R1;MRC1;SPHK1;SPHK2;CALM L3;CALM2; CALM3; CALM1;CALML5; PIK3C3;RAB 5A; RAB5B;RAB5C; EEA1;RAB7A; CTSD;T CIRG1; ATP6V0A2; ATP6V0A4; A TP6V0A1; ATP6V0D1;A TP6V1H; ATP6A P1; ATP6V0C;ATP6V0B; LAMP1;LAMP2; PPP3CA; PPP3CB; PPP3CC; PPP3R1; CORO1A; FCGR1A;FCGR2A;FCGR2B;FCGR3B

Table 1: Contd...

Pathway	*Number*	*Member*
Chemokine signaling pathway	158	CXCL1;CXCL2;CXCL5;CXCL6;PPBP;CXC L8;CXCL9;CXCL10;CXCL11;CXCL12;CX CL13;CXCL16;PF4V1;CXCL14;CX3CL1;CCL2;CCL3;CC L3L1; CCL3L3;CCL4; CCL5;CCL7;CCL8;CCL11;CCL13;C CL23; CCL19 ;CCL20; CCL21;CCL28; CCL1;CCL16; CCL17;CCL18;C CL22; CCL24;CCL26;CXCR2;CXCR1;CXCR3;CXCR4;C XCR5 ;CXCR6; CX3CR1;CCR6;CCR7;CCR2;CCR5;CCR1;CCR 3;CCR10; JAK2 ;STAT1;STAT2; STAT3; STAT5B;GNAI1;GNAI3;G NAI2; ADCY2;ADCY3;ADCY4;ADCY6;ADCY7;A DCY9; PRKACB; PRKX;LYN;HCK;FGR;SRC;SHC1;SHC2;SH C3;SHC4; GRB2;SOS1;SOS2; HRAS;KRAS;NRAS; RAF1;BRAF;MA P2K1; MAPK1;MAPK3;PIK3R1;PIK3R5;PIK 3R2;PIK3R3;PIK3CA;PIK3CD;PIK3CB;PIK 3CG; PRKCZ;AKT1;AKT3;FOX O3;CHUK;IKBKB;IKBKG;N FKBIA;NFKBIB;NFKB1;R ELA; GSK3B;ITK; VAV3;VAV1; VAV2;RAC1 ;RAC2;PAK1;CDC42;WAS; WASL;RHOA; ROCK1; ROCK2; GNB1;GNB2; GNB4;GNB5;GNG2;GNG4;GNG5;GNG7;GNG8 ;GNG10;GNG11; GNG12;GNGT1 ;GNGT2;PREX1;EL MO1;DOCK2;PTK2;PXN;BCA R1;CRK; CRKL;PTK2B; PLCB1;PLCB2;PLCB4;R ASGRP2; RAP1A;RAP1B; PARD3; TIAM1; NCF1; GRK4; GRK5;GRK6;ARRB1;ARRB2;PRKCB;PRKCD
Osteoclast differentiation	115	CSF1;CSF1R;GRB2; MAPK1; MAPK3; PIK3CA; PIK3CD; PIK3CB; PIK3CG; PIK3R1; PIK3R5;PIK3R2;PIK3R3 ;AKT1;AKT3;IF NG;IFNGR1; IFNGR2;STAT1; IL1A;IL1B; IL1R1; TNF;TNFRSF1A;TGFB2 ;TGFBR1;TGFBR2; TNFRSF11A;TNFRSF11B; TRAF2;TRAF6;LCK;FYN;MAP3K 14;CHUK;RELB;NFKB2; MAP3K7;TAB1;TAB2; IKBKG;IKBKB;NFKBIA;RELA;NF KB1;NFATC1; MAP2K1;MAPK11;MAPK12; MAPK13;MAPK14;M AP2K7;MAPK8;MAPK10;MAPK9; FOS;FOSB;FOSL2; FOSL1;JUN;JUND;JUN B;RAC1;CYBB;CYBA;NCF2;NCF1; NCF4;BTK;TEC;OSCAR; LILRB2; LILRB1;L ILRB5;LILRB4;LILRB3;LILRA3; LILRA2; LILRA4;LILRA6; LILRA5;FC GR1A;FCGR2A;FCGR2B;FCGR3B;TREM2;SIRPA ;SIRPB1;TYROBP; SYK;LCP2;PLCG2;PPP 3CA;PPP3CB;PPP3CC;PPP3R1 ;CREB1;SPI1;MITF;CTSK; ACP5; ITGB3; PPARG;IFNAR1; IFNAR2;JAK1;TYK2; STAT2;IRF9;SOCS1; SOCS3;GAB2;FHL2; CYLD;SQSTM1
Staphylococcus aureus infection	49	FGG;C3;CFB;CFD; CFH;MASP1;C1QA;C1QB;C1QC;C1R;C1S;C2;C4A;C5;C3A R1;C5AR1; FCGR1A;FCGR2A; FCGR2B;FCGR3B;FCAR; FPR3;FPR2;FPR1; CFI;SELPLG;SELP; ICAM1;ITGAL;ITGAM;ITGB2;DSG1;HLA-DMA;HLA-DMB;HLA-DOA;HLA-DOB;HLA-DPA1;HLA-DPB1;HLA-DQA1;HLA-DQA2;HLA-DQB1;HLA-DRA;HLA-DRB1;HLA-DRB3;HLA-DRB4;HLA-DRB5;PTAFR;IL10;KRT10 Phagosome 132 VAMP3; STX12; STX7; ACTB;ACTG1;CORO1A;STX18;SEC22B;HLA-A;HLA-B;HLA-C;HLA-F; HLA-G;HLA-E; HLA-DMA;HLA-DMB;HLA-DOA;HLA-DOB;HLA-DPA1;HLA-DPB1; HLA-DQA1; HLA-DQA2;HLA-DQB1;HLA-DRA;HLA-DRB1;HLA-DRB3;HLA-DRB4; HLA-DRB5; RAB5A;RAB5B;RAB5C; EEA1;PIK3C3;TFRC; HGS;ATP6V1A; ATP6V1B1; ATP6V1B2;ATP6 V1C1;ATP6V1D;ATP6V1E2; ATP6V1E1;ATP6V1F; ATP6V1G1; ATP6V1G2;ATP6V0E1;ATP6V0E2;TCIRG1;AT P6V0A2;ATP6V0A4;A TP6V0A1; ATP6V0D1;ATP6V1H;ATP6V0C;ATP6V0B;ATP6AP1;RAB7A;R AB7B;DYNC1H1; DYNC2H1;DYNC1I1;DYNC1I2;DYNC1LI2;TUBA1B;TUBA4A;TUBA1 A;TUBA1C; TUBA3D;TUBAL3;TUBB6;TUBB;TUBB2A;TUBB3;TUBB8;TUBB2B;TUBB4B;LAMP1;LAMP2;M6PR;MPO;CTSL;CTSS;SEC61A1;SEC61A2;SEC61B;SEC61G;TAP1;TA

Table 1: Contd...

Pathway	*Number*	*Member*
		P2;CALR;CANX;FCAR;FCGR1A;FCGR2A;FCGR2B;FCGR3B;C1R;ITGAM;ITGB2 ; C3;COLEC11;COLEC12;SFTPD;ITGAV;ITGA2;ITGA5;ITGB1;ITGB 3;ITGB5;THBS1;COMP;THBS2;THBS3;THBS4 ;TLR2;TLR6;TLR4;CD14;PLA2R1; MRC1;CD209;CLEC7A;MSR1;MARCO;OLR1;SCARB1;CD36;CYBA;CYBB;RAC1;NCF1;NCF2;NCF4
Graft-versus-host disease	35	IL6;IL1A;IL1B;TNF;HLA-DMA;HLA-DMB;HLA-DOA;HLA-DOB;HLA-DPA1;HLA-DPB1;HLA-DQA1;HLA-DQA2;HLA-DQB1;HLA-DRA;HLA-DRB1;HLA-DRB3;HLA-DRB4;HLA-DRB5;CD80;CD86;CD28;HLA-A;HLA-B;HLA-C;HLA-F;HLA-G;HLA-E;FAS;PRF1;GZMB;IFNG;KLRD1;KIR2DL3;KIR3DL1;KLRC1
Type I diabetes mellitus	37	GAD1;PTPRN;PTPRN2;CPE;HSPD1;HLA-DMA;HLA-DMB;HLA-DOA;HLA-DOB;HLA-DPA1;HLA-DPB1;HLA-DQA1;HLA-DQA2;HLA-DQB1;HLA-DRA;HLA-DRB1;HLA-DRB3;HLA-DRB4;HLA-DRB5;CD80;CD86;CD28;IFNG;HLA-A;HLA-B;HLA-C;HLA-F;HLA-G;HLA-E;FAS;PRF1;GZMB;LTA;TNF;IL1A;IL1B;ICA1
Hematopoietic cell lineage	74	KITLG;IL7;CSF2;FLT3LG;CSF3;IL6;IL11;IL1A;IL1B;TNF ;CSF1;CD34;FLT3; CD44;KIT;IL2RA;IL7R;TFRC;CD7;CD2;CD5;CD1A;CD1B;CD1C;CD1D;CD1E;CD4;CD8A;CD3D;CD3E;CD3G;MME; CD9;CD19; CD22; CD24;MS4A1;CR2; CD37;FCER2; CR1;CSF2RA;IL3RA;CD3 3;IL4R; IL6R;FCGR1A; CSF1R;ANPEP; ITGAM;CD14; IL1R1;IL1R2;CSF3R;IL5 RA;EPOR; CD36;CD55;CD59;IL11RA;ITGB3; GP1BA;ITGA1; ITGA2; ITGA3; ITGA4;ITGA5;ITGA6;HLA-DRA;HLA-DRB1;HLA-DRB3;HLA-DRB4;HLA-DRB5;CD38
Inflammatiory bowel disease (IBD)	51	TLR2;TLR4;TLR5;NFKB1;RELA;NOD2;HLA-DMA;HLA-DMB;HLA-DOA;HLA-DOB;HLA-DPA1;HLA-DPB1;HLA-DQA1;HLA-DQA2;HLA-DQB1;HLA-DRA;HLA-DRB1;HLA-DRB3;HLA-DRB4;HLA-DRB5;IFNG;IFNGR1;IFNGR2;STAT 1;TBX21;IL12RB1;STAT4;IL18;IL 18R1;IL18RAP;JUN;TNF;IL6 ;IL1A; IL1B;TGFB2;TGFB3;S MAD2;SMAD3;STAT3 ;IL21R;IL23A; RORC;RORA; IL17F;IL4R;IL2RG;STAT6;IL10;MAF;NFATC1
Intestinal immune network for IgA production	38	CD80;CD86; HLA-DMA;HLA-DMB;HLA-DOA;HLA-DOB; HLA-DPA1;HLA-DPB1;HLA-DQA1;HLA-DQA2;HLA-DQB1;HLA-DRA; HLA-DRB1; HLA-DRB3;HLA-DRB4;HLA-DRB5;CD28;IL6;IL10;TNFSF13; TNFSF13B;TNFRSF13B;TNFRSF17;CD40;C D40LG;ICOS;ICOSLG;ITGA4;ITGB7; CXCL12; CXCR4; CCL28; CCR10; LTBR; MAP3K14; IL15; IL15RA;PIGR
Leishmaniasis	66	TLR2;TLR4;MYD88;IRAK1;IRAK4;TRAF6;MAP3K7; TAB1;TAB2;NFKBIB; NFKBIA;NFKB1;RELA;IL1A;IL1B;TNF;NOS2;IL10;TGFB2;TGFB3;C3;CR1 ;ITGAM;ITGB2; FCGR1A;FCGR2A;FCGR3B;ITGA4;ITG B1;PTGS2;NCF1; NCF2;NCF4; CYBA; MAPK1;MAPK3;ELK1;FOS;JUN;MAPK11; MAPK12;MAPK13; MAPK14; IFNG;IFNGR1; IFNGR2;JAK1;JAK2;STAT1;HLA-DMA;HLA-DMB;HLA-DOA;HLA-DOB; HLA-DPA1;HLA-DPB1;HLA-DQA1;HLA-DQA2;HLA-DQB1;HLA-DRA;HLA-DRB1; HLA-DRB3;HLA-DRB4;HLA-DRB5;MARCKSL1;PTPN6;PRKCB
Natural killer cell mediated cytotoxicity	98	HLA-A;HLA-B;HLA-C;HLA-F;HLA-G;HLA-E;KIR3DL1; KIR2DL3 ;KIR2DL4; KLRC1; KLRD1;PTPN6;PTPN11;ICAM1;ICA M2;ITGAL; ITGB2;PTK2B; VAV3; VAV1; VAV2;RAC1;RAC2;RAC3;PAK1;MAP2K1;MAP 2K2; MAPK1; MAPK3;TNF;CSF2; IFNG;KIR2DS3;TYROBP;LCK;FCGR3B;FCER1G;CD247;ZAP

Table 1: Contd...

Pathway	*Number*	*Member*
		70;SYK;LCP2;LAT;PLCG1; PLCG2;PIK3CA; PIK3CD;PIK3CB ;PIK3CG;PIK3R1; PIK3R5; PIK3R2;PIK3R3;FY N;SHC1;SHC2;SHC3; SH C4;GRB2;SOS1; SOS2; HRAS; KRAS;NRAS; ARAF;BRAF;RAF1; MICB; MIC A; ULBP1; ULBP2; RAET1G; RAET1L; RAET1E; HCST;CD48;PP P3CA; PPP3CB; PPP3CC; PPP3R1;NFATC1; PRK CA; PRKCB;SH2D1B;SH2D1A;IFNGR1;IFN GR2; IFNAR1; IFNAR2;TNFSF10; TNFRSF10A; TNFRSF10B; TNFRSF10C; TNFRSF10D; FAS; GZMB;PRF1;CASP3;BID
Influenza A	146	PRSS3;PRSS2; TMPRSS2; TMPRSS4;OAS1;OAS2; OAS3;RNASEL; DNAJB1;DNAJC3; HSPA8; HSPA 1A;HSPA2;HSPA1L;H SPA1B;EIF2AK1; EIF2AK2;EIF2AK3; EIF2AK4;EIF2S1; MAP2K3; MAPK11;MAPK12;MAPK13;MAPK14;M AP2K4; MAP2K7; MAPK8 ;MAPK10;MAPK9;JUN; ATF2;DDX58;IFIH1; MAVS;NLRX1;IKBKB; NFKBIB; NFKBIA; NFKB 1;RELA;TRIM25;TLR3;TICAM1; TBK1;IKBKE;IRF3; CREBBP; EP300; PIK3R1;PIK3R5;PIK3R2;PIK3R3;PIK3CA;PIK3CD ;PIK3CB; PIK3CG; AKT1;AKT3; TLR4;TLR7;MYD88; IRAK4;IRF7;IL1A;IL1B; IL6;TN F;CXCL8;CCL2; CCL5; CXCL10;ICAM1;IFNA R1;IFNAR2;JAK1; TYK2;STA T1;STAT2;IRF9; SOCS3; MX1; ADAR; PML;IFNG;IFNGR1; IFNGR2;JAK2; CIITA;HLA-DMA;HLA-DMB;HLA-DOA; HLA-DOB;HLA-DPA1;HLA-DPB1;HLA-DQA1;HLA-DQA2;HLA-DQB1; HLA-DRA; HLA-DRB1;HLA-DRB3;HLA-DRB4;HLA-DRB5; NLRP3;PYCARD; CASP1; IL18; IL33;TNFSF 10;TNFRSF10A;TNFRSF10B;TNFRSF10C;T NFRSF10D; FAS; TNFRSF1A; VDAC1;CYCS;C ASP9;GSK3B;KPNA1;KPNA2;XPO1;A GFG1; RAF1; MAP2K1; M AP2K2; MAPK1;M APK3;RSAD2;FDPS;ACTB;ACTG1;IV NS1ABP; CPSF4; NXF1; NXF3; NXT1; NXT2;RAE1;HNRNPUL1; NUP98;FURIN;TMPRSS13; SLC25A6; PRKCA; PRKCB
Antigen processing and presentation	62	IFNG;TNF;PSME1;PS ME2;PSME3;HSPA8; HSPA1A;HSPA2;HSPA1L; HSPA1B; HSPA4; HSP90AA1;HSP90AB1;HLA-A;HLA-B;HLA-C;HLA-F;HLA-G;HLA-E; CANX;B2M;CALR;TAPBP;TAP1;T AP2;CD8A; KIR3DL1; KIR2DL3; KIR2DL4; KLRC1; KLRD1;KIR2DS3;IFI30;LGMN;CTSB;HLA-DMA; HLA-DMB;HLA-DOA;HLA-DOB;HLA-DPA1;HLA-DPB1;HLA-DQA1;HLA-DQA2;HLA-DQB1;HLA-DRA;HLA-DRB1;HLA-DRB3;HLA-DRB4;HLA-DRB5; CD74; CTSL;CTSS; CD4;CIITA; RFX5; RFXANK; RFXAP;CREB1;NFYA;NFYB;NFYC;HSPA5
NF-kappa B signaling pathway	84	LCK;ZAP70;LAT;PLCG1; PRKCQ;SYK;LYN; BTK;PLCG2;CARD11; BCL10; MALT1;IL1B;IL1R1;MYD88 ;IRAK1; IRAK4;TRAF6;TNF; TNFRSF1A;RIPK1; TRADD; TRAF2; TRAF5; BIRC2;BIRC3; DDX58;TRIM25;LB P;CD14; TLR4;LY96;TIRAP; TICAM1; CD40LG; CD40; TRAF3; TNFRSF11A; LTA; LTB;TNFSF14;LTBR;M AP3K14; MAP3K7; TAB1; TAB2; TNFSF13B; IKBKG;CHUK;IKBKB;PIAS4;UBE2I;ATM ;PIDD1; ERC1; NFKBIA; NFKB1; RELA;CFLAR;XIAP;BCL 2L1;BCL2; TRAF1; BCL2A1; NFKB2;CXCL8; TNFAIP3;PTGS2;CCL4;VCAM1 ;PLAU;CSNK2A1; CSNK2A2; CSNK2B; RELB;CCL13;CCL19; CCL21;CXCL12;ICAM1; PARP1; CXCL2; GADD45B; PRKCB
Pertussis	67	ITGA5;ITGB1;ITGAM;ITGB2;CALML3 ;CALM2;CALM3; CALM1;CALML5; RHOA;CFL1;CFL2;CASP3; CASP7;CASP1;PYCA RD;NLRP3;IL1B;C1QA; C1QB;C1QC; C1R;C1S;C2;C 4A;C3;C5;SERPING1;C4BPA ;LY96;TLR4; CD14;TIRAP; MYD88;I RAK4; IR AK1;TRAF6;NFKB1;RELA;TICAM1;IRF3;M APK11; MAPK12; MAPK13; MAPK14; MAPK8;MAPK10; MAPK9;MAPK1; MAPK3; FOS;JUN; IL6;IL23A;TNF;IL10; IRF1;IRF8;GNAI1;GNAI3;GNAI2 ;CXCL8;CXCL5; CXCL6;NOD1;IL1A;NOS2

Table 1: Contd...

Pathway	*Number*	*Member*
Allograft rejection	31	HLA-A; HLA-B;HLA-C;HLA-F;HLA-G;HLA-E;CD80;CD86; CD28; FAS; PRF1; GZMB; HLA-DMA;HLA-DMB;HLA-DOA;HLA-DOB;HLA-DPA1;HLA-DPB1; HLA-DQA1;HLA-DQA2;HLA-DQB1;HLA-DRA;HLA-DRB1;HLA-DRB3;HLA-DRB4;HLA-DRB5;CD40LG;CD40;IFNG;TNF;IL10
Toll-like receptor signaling pathway	84	TLR1;TLR2;TLR6;LBP;CD14;LY96; TLR3;TLR4;TLR5;TLR7; TLR8;CTSK;RAC1; PIK3CA;PIK3CD;PIK3CB;PIK3CG;PIK3R1;PIK3R5;PIK3R 2;PIK3R3;AKT1; AKT3;TOLLIP;MYD88;TIRAP;FADD;CASP8;IRAK4;IRAK1;TRAF6;TAB1;TAB2;MAP3K7;IKBKG;CHUK;IKBKB;NFKBIA;NFKB1;RELA;MAP3K8;MAP2K1;MAP2K2;MAPK1;MAPK3;MAP2K3;MAP2K4;MAP2K7;MAPK11;MAPK12;MAPK13;MAPK14;MAPK8;MAPK10;MAPK9;JUN;FOS;TNF;IL1B;IL6;CXCL8;CCL5;CCL3;CCL3L1;CCL3L3;CCL4;TICAM1;R IPK1;IRF5;IRF7;SPP1;IKBKE;TBK1; TRAF3;IRF3;CD40;CD80;CD86;IFNAR1;IFNAR2;STAT1;CXCL10;CXCL9;CXCL11
TNF signaling pathway	104	TNF; TNFRSF1A;BAG4;TRADD;T RAF2;TRAF5;RIPK1;B IRC2; BIRC3; MAP3K7;TAB1; TAB2;MAP2K4;MAP2K7;MAPK 8;MAPK10; MAPK9;JUN; ITCH; CFLAR;MAP2K3; MAPK11;MAPK12; MAPK13; MAPK14;CEBP B; MAP3K5; MAP3K14; IKBKG;IKBKB; CHUK; NFKBIA; RELA;NFKB1;MAP3K8;MAP2K 1;MAPK1;MAPK3; RPS6KA5; RPS6KA4; CREB1; CREB3;CREB3L2;CREB3L4;ATF2;ATF4;CREB5;ATF 6B;RIPK3; MLKL; PGAM5; DNM1L; FADD;CASP8;CASP10; CASP7;CASP3; CCL2;CCL5; CCL20; CXCL 1;CXCL2; CXCL10; CX3CL1;CSF1; CSF2;FAS;IL18R1;I L1B;IL6; IL15;LIF; LTA;BCL3;SOCS3;TNFA IP3; TRAF1; FOS;JUNB;MMP3;MMP9; MMP14;E DN1; NOD2;ICAM1;SELE;VCAM1;PTG S2;TNFRSF1B;TRAF3;PIK3CA ;PIK3CD;PIK3CB;PIK3CG; PIK3R1;PIK3R5; PIK3R2;PIK3R3; AKT1;AKT3 MAGI2;JAG1; CXCL5;VEGFC
Autoimmune thyroid disease	31	CTLA4;TPO;HLA-DMA;HLA-DMB;HLA-DOA;HLA-DOB;HLA-DPA1;HLA-DPB1;HLA-DQA1;HLA-DQA2;HLA-DQB1;HLA-DRA;HLA-DRB1;HLA-DRB3;HLA-DRB4;HLA-DRB5;CD80;CD86;CD28;HLA-A;HLA-B;HLA-C;HLA-F;HLA-G;HLA-E;FAS;PRF1;GZMB;CD40LG;CD40;IL10
Systemic lupus erythematosus	78	C1QA;C1QB;C1QC;C2;C4A; H2AFX;HIST2H2AC; HIST3H2A;HIST1H2AE; H2AFY2;H2AFY;H2AFJ; HIST2H2AB;H2A FV;H2AFZ; HIST1H2AC; HIST1H2BH; HIST2H2BF; HIST1H2BG;HIST1H2BC;HIST1H 2BD; HIST1H2BE; HIST2H2BE; HIST1H2BK; H2BFM;H3F3 C;H3F3B; HIST1H3D; HIST2H3D;HIST1H3E;HI ST1H3G; HIST1H3F;HIST1H4C;HIS T1H4H;HIST1H4E; HIST1H4A; S NRPB; SNRPD1;SNRPD3; GRIN2A; TRIM21;TROVE2;SSB; ACTN1;ACTN4; HLA-DMA;HLA-DMB;HLA-DOA; HLA-DOB;HLA-DPA1;HLA-DPB1;HLA-DQA1;HLA-DQA2; HLA-DQB1;HLA-DRA;HLA-DRB1; HLA-DRB3;HLA-DRB4;HLA-DRB5;CD80; CD86;CD28; CD40LG;CD40;TNF; IFNG;IL10;C1R;C1S;C3;C5; C7;C8A;CTSG;ELANE ;FCGR1A;FCGR2A; FCGR3B
Viral myocarditis	57	CXADR;CD55;FYN;CAV1;ABL1;ABL2;RAC1;RAC2;RAC3;S GCD;SGCA;SGCB;DAG1;DMD;ACTB; ACTG1;EIF4G3;EIF4G1; EIF4G2;CCND1; CASP8;BID;CYCS; CASP9; CASP3;MYH7;MYH6;CD40LG;CD40;HLA-DMA;HLA-DMB; HLA-DOA; HLA-DOB;HLA-DPA1;HLA-DPB1;HLA-DQA1;HLA-DQA2;HLA-DQB1;HLA-DRA;HLA-DRB1;HLA-DRB3;HLA-DRB4;HLA-DRB5;HLA-A;HLA-B;HLA-C;HLA-F;HLA-G;HLA-E;CD80;CD86;CD28;PRF1;ITGAL;ITGB2;ICAM1;LAMA2

Table 1: Contd...

Pathway	*Number*	*Member*
Asthma	23	HLA-DMA;HLA-DMB;HLA-DOA;HLA-DOB;HLA-DPA1;HLA-DPB1;HLA-DQA1;HLA-DQA2;HLA-DQB1;HLA-DRA;HLA-DRB1;HLA-DRB3;HLA-DRB4;HLA-DRB5;CD40LG;CD40;FCER1A;MS4A2;FCER1G;IL10;CCL11;TNF;PRG2
Amoebiasis	92	IL1B;IL1R1;IL1R2;NFKB1;RELA;HSPB1; COL1A1;COL1A2;COL3A1;COL5A1;COL5A2; COL11A 1;COL11A2; COL5A3;COL24A1; COL4A2;COL4A4; COL4A6;COL4A1; COL4A5; COL4A3; FN1;LAM A1;LAMA2; LAMA3;LAMA 5; LAMA4 ;LAMB1 ;LAMB2 ;LAMB3; LAMB4;LA MC1;LAMC2; LAMC3;CASP3; GNAQ;GNA11; GNA14; GNA15;PLCB1; PLCB2;PLCB4;PR KCA; PRKCB;GNAS; GNAL;PRKACB; PRKX;RAB5A; RAB5B;RAB5C; RAB7A;R AB7B; TLR2; TLR4; CD14; IL6;CSF2; CXCL8;TNF;IFNG;PTK2;VCL;ACTN 1; ACTN4; ARG2;ARG1; NOS2;ITGB2;ITGAM;PIK3R1;P IK3R5; PIK3R2;PIK3R3 ;PIK3CA;PIK3CD;P IK3CB; PIK3CG;SERPINB1; SER PINB2; SERPINB3; SERPINB4;SERPINB6; SERPINB9; SERPINB13; CTSG;IL10;TGFB2; TGFB3;C8A;CXCL1; CD1D

concrete results are presented in Supplement Table 1.

Hub Genes Identification

Finally, to acquire the potential hub genes involved in keratitis, Gibbs sampling was reutilized to calculate probabilities of pathway gene set. As presented in Figure 3, there were 5 hub genes identified based on adj_α_{pi} > 0.8, including *CXCL5*, *MARCO*, *FCER1G*, *RAC2*, *HCK*. Their expression values in normal and keratitis states suggested that they were enhanced markedly in keratitis group when compared with normal group (Fig. 4, $P < 0.05$), accordingly, the heat map of hub genes expression levels in two groups were exhibited in Figure 5. These hub genes were shown to be higher expressed in keratitis group on comparing normal group.

DISCUSSION

In the current study, the gene expression matrix with 14832 genes was gained through data preprocessing and then 278 pathways were obtained via KEGG enrichment analysis. Moreover, 26 significant pathways were chose by Gibbs sampling. Furthermore, 5 hub genes were screened by analyzing pathway gene set using Gibbs sampling. These results demonstrated that identified key pathways and hub genes would provide valuable information on the progress of microbial keratitis and offer new insights for investigating molecule mechanisms potentially involved in the microbial keratitis.

Microbial keratitis is recognized as the leading eye-threatening disease of keratitis, characterised by the presence of white/yellowish infiltrates in the corneal stroma, with/without an overlaying corneal epithelial defect, and related with strong signs of inflammation (Upadhyay et al. 2015), which caused considerable inconvenience to patients. In an effort to uncover the underlying molecular mechanisms that drive the microbial keratitis development, more and more researchers have spared no efforts to carry out experiments. It was suggested that activity of Toll-like receptors (TLRs) signaling resulting in the activation of nuclear factor-kB (NF-kB) and production of proinflammatory cytokines like TNF-α, enable to trigger the earliest immune responses that lead to inflammation in ocular immunology (Pandey et al. 2013). Beyond that,

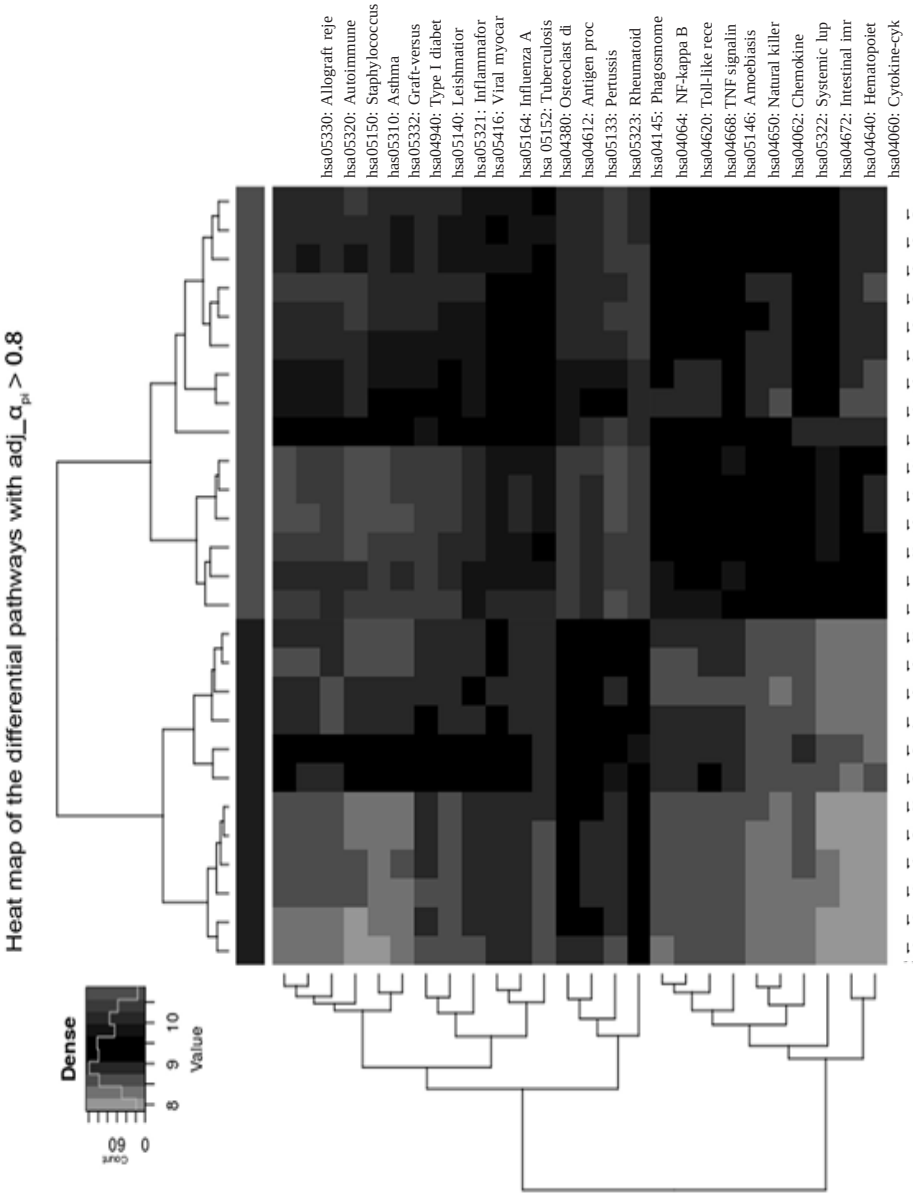

Fig. 2. Heat map of the top 26 significant pathways. Red: high expression level; Greeen: lower expression level.
Source: Author

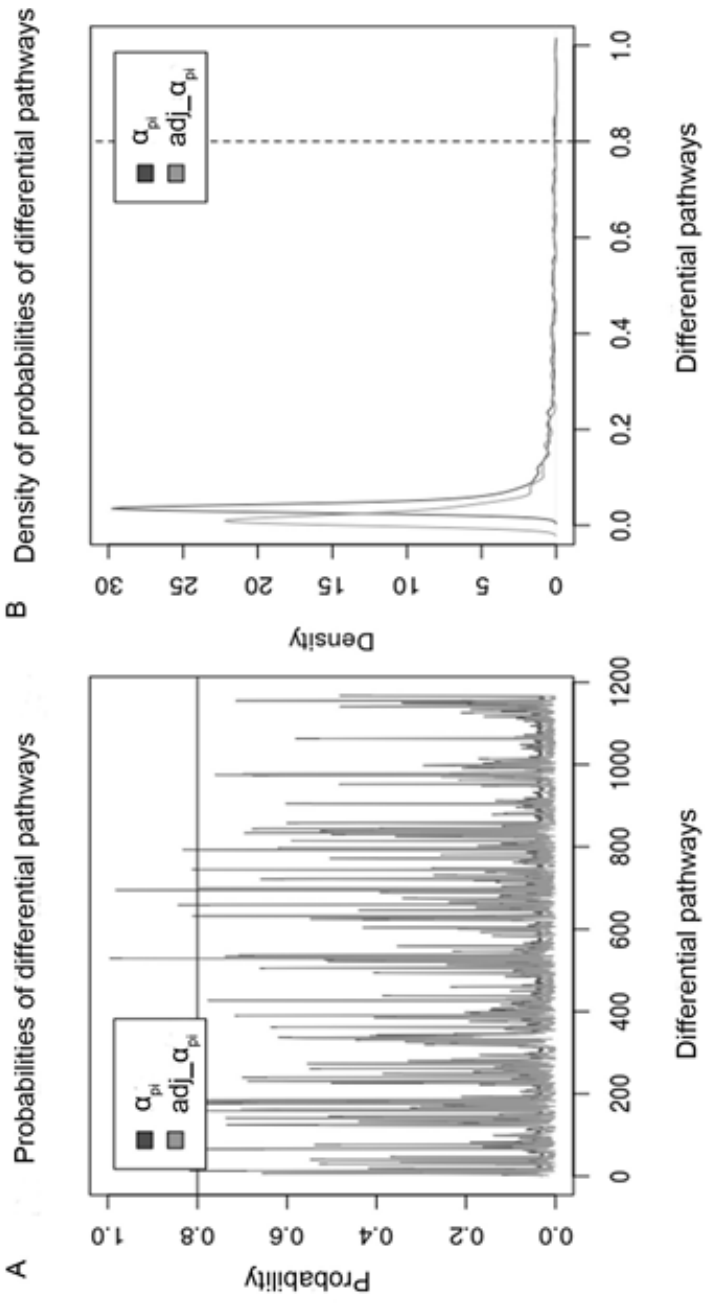

Fig. 3. The probabilities distribution of 1167 differential pathway gene set. The X axis denoted the differential pathway gene set, and the Y axis dented the posterior value of the differential pathway genes. A. The adjusted posterior value distribution of differential pathway gene set. B. The density of differential pathway genes posterior value distribution. Differential pathway genes were considered as hub gene judging by adj_α_{pi} > 0.8.
Source: Author

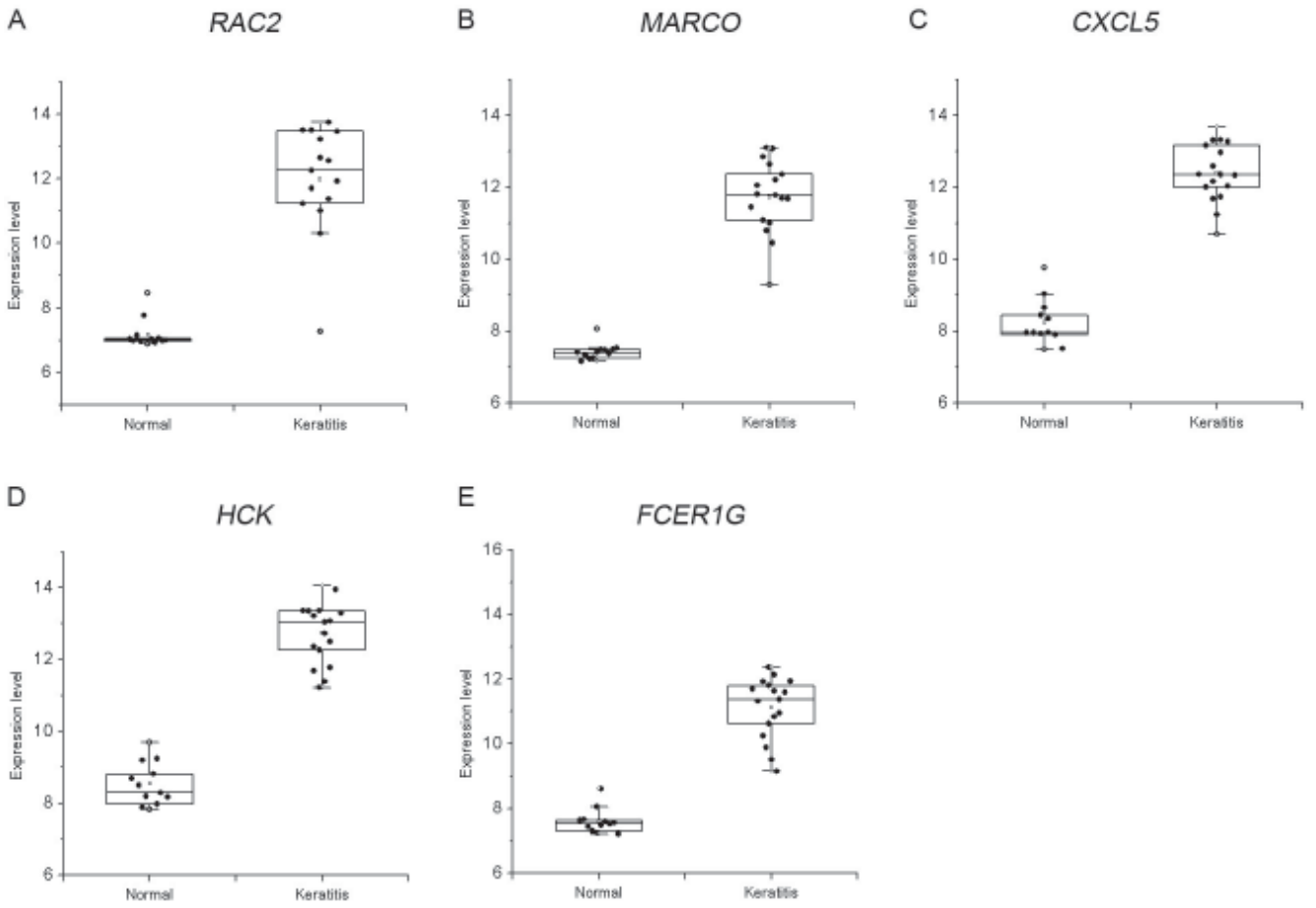

Fig. 4. The box scatter diagram of top 5 hub genes. A. Analysis of the *RAC2* expression level between two groups. B. Analysis of the *MARCO* expression level between two groups. C. Analysis of the *CXCL5* expression level between two groups. D. Analysis of the *HCK* expression level between two groups. E. Analysis of the *FCER1G* expression level between two groups. The X axis denoted groups, and the Y axis denoted the hub genes expression level
*Source:*Author

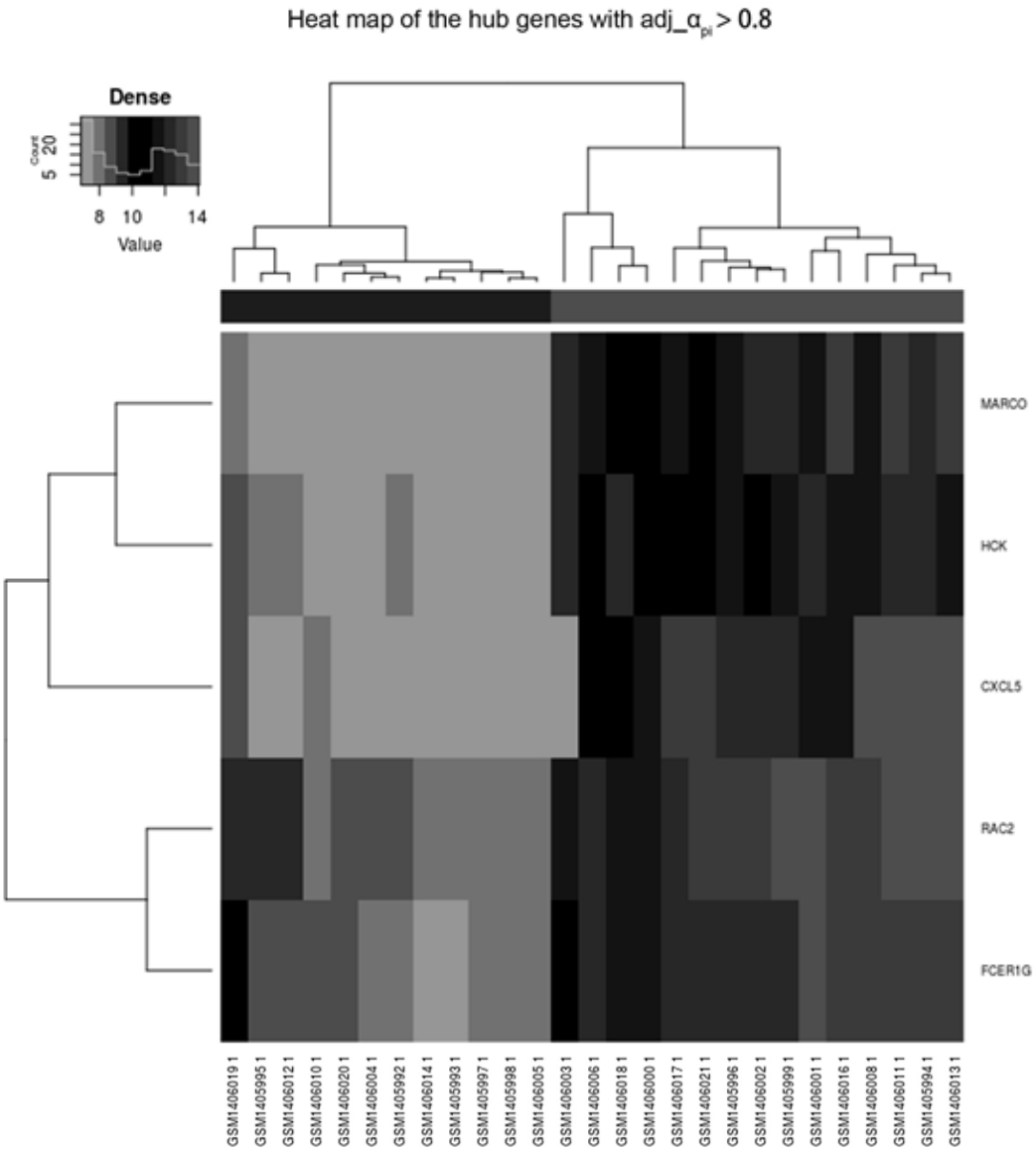

Fig. 5. Heat map of the top 5 hub genes. Red: high expression level; Greeen: lower expression level. *Source:* Author

activation of TLR2 and TLR4 through NF-kappaB could contribute to pathogenesis of keratomycosis (Jie et al. 2009). In addition, Toll-like receptor 4 signalling pathway was activated in a rat model of Acanthamoeba keratitis (Ren and Wu 2011). TNF-α and IL-6 was reported to facilitate corneal lymphangiogenesis during acute HSV-1 infection (Bryant-Hudson et al. 2014). Consistent with this. This study demonstrated that a series of inflammation signaling pathways, such as NF-kappa B signaling, TNF signaling pathway, Toll-like receptor signaling pathway were identified using bioinformatics measurement.

Previous research demonstrated that keratitis was associated with longstanding rheumatoid arthritis, besides, morbidity and mortality

of which were increasingly improved (Domngang Noche et al. 2016; Lee et al. 2016; Petrushkin et al. 2016). HLA-DR expression was reported to be considered as a biomarker of inflammation for multicenter clinical trials of ocular surface disease (Epstein et al. 2013). Accumulating evidence showed that peripheral ulcerative keratitis was associated with tuberculosis in a child (Al-Mendalawi 2016), mycobacterium tuberculosis may lead to formation of interstitial keratitis (Gupta et al. 2015). Cytokines and chemokines served as small proteins played an important proinflammatory or anti-inflammatory role in modulating the herpes simplex keratitis (Azher et al. 2017). It is reported that chemokine CXCL10 inhibition of hem- and lymph-angiogenesis emerged in inflamed corneas (Gao et al. 2017). It has also reported that IL-8, IL-6 and IL-1â expressions were increased in tears samples of patients with microbial keratitis compared with negative normal (Santacruz et al. 2015). The importance of long-lasting Ag presentation in inducing peripheral T cell tolerance was also determined in the model of herpes stromal keratitis autoimmune disease (Raimondi et al. 2006). Thus, in line with above evidence, results of this study determined that Rheumatoid arthritis pathway, Tuberculosis pathway, chemokine signaling pathway, Staphylococcus aureus infection signaling pathway were selected.

Based on Gibbs sampling, several hub genes like *RAC2*, *MARCO*, *CXCL5*, *HCK*, *FCER1G* were identified and these key genes may contribute to predict, monitor and even treat microbial keratitis. Lin et al demonstrated that CXCL5/lipopolysaccharide (LPS)-induced chemokine mediated neutrophil recruitment into the cornea during LPS keratitis (Lin et al. 2007). A previous investigation determined that CXCL5 as a proinflammatory chemokine was downregulated in mice corneal with alkali burns after treated with tofacitinib (Sakimoto and Ishimori 2016). Sweet et al demonstrated that the presence of FcR common γ-chain (Fcer1g) or/and MyD88 were responsible for proinflammatory responses to exogenous antigens while the absence of them reduced extrafollicular plasmablast response (Sweet and Nickerson 2017). It is suggested that Lck/Hck/Fgr triple knockout enhanced antiviral sensing and resistance substantially while ectopic expression of them dampened cells antiviral defense (Liu et al. 2017). Nonetheless, this study is only a bioinformatics screening for biomarkers in microbial keratitis preliminarily and further in-depth investigations in molecular pathogenesis and validations with several experiments are still demanded.

CONCLUSION

Collectively, this study found out several key pathways associated with microbial keratitis based on bioinformatics method, accordingly, monitoring these signaling pathways might aid prediction or treatment of microbial keratitis occurrence and development. Importantly, hub genes in microbial keratitis, namely, *RAC2*, *MARCO*, *CXCL5*, *HCK*, *FCER1G* were identified on the basis of pathway gene set using Gibbs sampling, which were likely to be prognostic and diagnostic implications of microbial keratitis.

RECOMMENDATIONS

Findings from the present study will provide the molecular basis for the understanding of microbial keratitis pathogenesis, implying a novel strategy for relieving the burden of microbial keratitis.

REFERENCES

Al-Mendalawi MD 2016. Peripheral ulcerative keratitis associated with chronic malabsorption syndrome and miliary tuberculosis in a child. *Oman J Ophthalmol*, 9: 121.

Ananthi S, Chitra T, Bini R, Prajna NV, Lalitha P, Dharmalingam K 2008. Comparative analysis of the tear protein profile in mycotic keratitis patients. *Mol Vis*, 14: 500-507.

Ananthi S, Venkatesh Prajna N, Lalitha P, Valarnila M, Dharmalingam K 2013. Pathogen induced changes in the protein profile of human tears from fusarium keratitis patients. *PLoS One*, 8: e53018.

Azher TN, Yin XT, Stuart PM 2017. Understanding the role of chemokines and cytokines in experimental models of herpes simplex keratitis. *J Immunol Res*, 2017: 7261980.

Bryant-Hudson KM, Gurung HR, Zheng M, Carr DJ 2014. Tumor necrosis factor alpha and interleukin-6 facilitate corneal lymphangiogenesis in response to herpes simplex virus 1 infection. *J Virol*, 88: 14451-14457.

Chidambaram JD, Kannambath S, Srikanthi P, Shah M, Lalitha P, Elakkiya S, Bauer J, Prajna NV, Holland MJ, Burton MJ 2017. Persistence of innate immune pathways in late stage human bacterial and fungal keratitis: Results from a comparative transcriptome analysis. *Front Cell Infect Microbiol*, 7: 193.

Domngang Noche C, Singwe-Ngandeu M, Bella AL 2016. Rheumatoid polyarthritis suspected in an HIV patient with scleritis, peripheral ulcerative keratitis, and anterior uveitis. *Int Med Case Rep J*, 9: 19-24.

Epstein SP, Gadaria-Rathod N, Wei Y, Maguire MG, Asbell PA 2013. HLA-DR expression as a biomarker of inflammation for multicenter clinical trials of ocular surface disease. *Exp Eye Res,* 111: 95-104.

Gao N, Liu X, Wu J, Li J, Dong C, Wu X, Xiao X, Yu FX 2017. CXCL10 suppression of hem- and lymph- angiogenesis in inflamed corneas through MMP13. *Angiogenesis,* 20: 505-518.

Gupta V, Shoughy SS, Mahajan S, Khairallah M, Rosenbaum JT, Curi A, Tabbara KF 2015. Clinics of ocular tuberculosis. *Ocul Immunol Inflamm,* 23: 14-24.

Hua X, Chi W, Su L, Li J, Zhang Z, Yuan X 2017. ROS-induced oxidative injury involved in pathogenesis of fungal keratitis via p38 MAPK activation. *Sci Rep,* 7: 10421.

Jie Z, Wu XY, Yu FS 2009. Activation of toll-like receptors 2 and 4 in aspergillus fumigatus keratitis. *Innate Immun,* 15: 155-168.

Kandhavelu J, Demonte NL, Namperumalsamy VP, Prajna L, Thangavel C, Jayapal JM, Kuppamuthu D 2017. Aspergillus flavus induced alterations in tear protein profile reveal pathogen-induced host response to fungal infection. *J Proteomics,* 152: 13-21.

Lee WS, Choi YJ, Yoo WH 2016. Rapid progressive peripheral ulcerative keratitis associated with long-standing rheumatoid arthritis. *J Rheumatol,* 43: 1909-1910.

Lin M, Carlson E, Diaconu E, Pearlman E 2007. CXCL1/KC and CXCL5/LIX are selectively produced by corneal fibroblasts and mediate neutrophil infiltration to the corneal stroma in LPS keratitis. *J Leukoc Biol,* 81: 786-792.

Liu S, Chen S, Li X, Wu S, Zhang Q, Jin Q, Hu L, Zhou R, Yu Z, Meng F et al. 2017. Lck/Hck/Fgr-mediated tyrosine phosphorylation negatively regulates TBK1 to restrain innate antiviral responses. *Cell Host Microbe,* 21: 754-768.

Liu Y, Zhao G, Lin J, Li C, Li Q, Che C, Wang Q, Hu L 2015. The role of Syk signaling in antifungal innate immunity of human corneal epithelial cells. *BMC Ophthalmol,* 15: 55.

Pandey RK, Yu FS, Kumar A 2013. Targeting toll-like receptor signaling as a novel approach to prevent ocular infectious diseases. *Indian J Med Res,* 138: 609-619.

Park GB, Hur DY, Kim YS, Lee HK, Yang JW, Kim D 2015. TLR3/TRIF signalling pathway regulates IL-32 and IFN-beta secretion through activation of RIP-1 and TRAF in the human cornea. *J Cell Mol Med,* 19: 1042-1054.

Petrushkin HJ, Stanford M, Fortune F, Jawad A 2016. Improving morbidity and mortality in peripheral ulcerative keratitis associated with rheumatoid arthritis. *Clin Exp Rheumatol,* 34: S18-S19.

Raimondi G, Zanoni I, Citterio S, Ricciardi-Castagnoli P, Granucci F 2006. Induction of peripheral T cell tolerance by antigen-presenting B cells. I. Relevance of antigen presentation persistence. *J Immunol,* 176: 4012-4020.

Ren MY, Wu XY 2011. Toll-like receptor 4 signalling pathway activation in a rat model of acanthamoeba keratitis. *Parasite Immunol,* 33: 25-33.

Sakimoto T, Ishimori A 2016. Anti-inflammatory effect of topical administration of tofacitinib on corneal inflammation. *Exp Eye Res,* 145: 110-117.

Santacruz C, Linares M, Garfias Y, Loustaunau LM, Pavon L, Perez-Tapia SM, Jimenez-Martinez MC 2015. Expression of IL-8, IL-6 and IL-1beta in tears as a main characteristic of the immune response in human microbial keratitis. *Int J Mol Sci,* 16: 4850-4864.

Sweet RA, Nickerson KM 2017. B cell-extrinsic Myd88 and Fcer1g negatively regulate autoreactive and normal B cell immune responses. *J Immunol,* 199: 885-893.

Upadhyay MP, Srinivasan M, Whitcher JP 2015. Diagnosing and managing microbial keratitis. *Community Eye Health,* 28: 3-6.

Paper received for publication on March 2018
Paper accepted for publication on May 2018