

## Screening Hub Genes in Microbial Keratitis Based on Gibbs Sampling Analysis

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**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  $adj\_a_{pi}$  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 pow-

erful 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- $\beta$  (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.

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## 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 keratitis 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.

### KEGG 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  $\alpha_{pi}$ , 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  $\alpha_{pi}$  and  $P$  value, correction coefficient ( $R_{value}$ ) was calculated and then  $adj\_alpha_{pi}$  (multiplying  $\alpha_{pi}$  by  $R_{value}$ ) was calculated. Key differential pathways were selected judging by  $adj\_alpha_{pi}$  greater than 0.8. Related computational formulas were as follows.

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

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

$$adj\_alpha_{pi} = R_{value} \times \alpha_{pi}$$

### 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  $\alpha_{pi}$  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  $\alpha_{pi}$  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  $\alpha_{pi} > 0.8$ , including NF-kappa B signaling, TNF signaling, Cytokine-cytokine receptor interaction, Phagosome, Hemato-

poietic 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

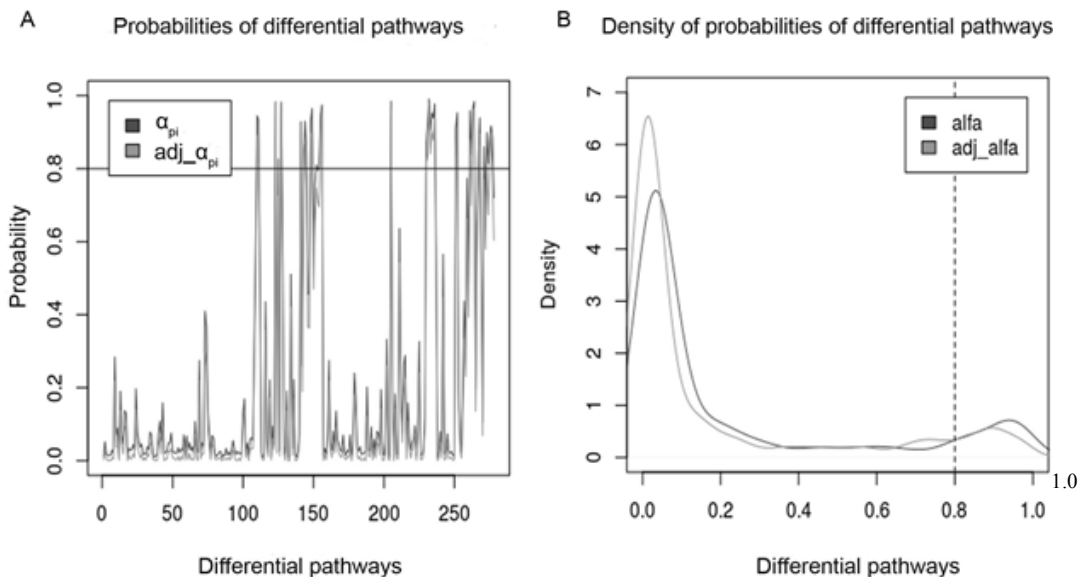


Fig. 1. The probabilities distribution of 278 pathways. The X axis denoted the pathways, and the Y axis represented the posterior value of the pathways. A. The adjusted posterior value distribution of 278 pathways. B. The density of 278 differential pathways posterior value distribution. Pathway was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

Source: Author

Table 1: Comparison of expression values with  $\text{adj\_}a_{\text{pr}} > 0.8$  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;A TP6V1E1;ATP6V1F; ATP6V1G1; ATP6V1G2; ATP6V0E1;ATP6V0E2;TCIRG 1; ATP6V0A2; ATP6V0A4; ATP6V0A1; ATP6V0D1;ATP6V1H;ATP6API;AT P6V0C; ATP6V0B; CTSK;ACF5;MMP1;MMP3; CTSL; CSF2;CCL5; CCL2; CCL3;CCL3L1 ;CCL3L2; CCL20; CXCL5;CXCL6; CXCL8;CXCL12; VEGFA;ANGPT1; TEK;IFNG;CXCL1 ;CXCL1;CXCL2;CXCL5;CXCL6;PPBP;CXCL8;CXCL9;C XCL10;CXCL11; CXCL12; CXCL13;CXCL16;PP4V1;CXCL14;CXCL1;CXCL17;CXCL18;CXCL22;CXCL24;CXCL26;CXCL29;CXCL19C CL21;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;T NFSF8;TNFSF9;TNFSF4;TNFSF13;EDAA;TGFB2;TGFB3;INHBB;INHBE;AMH;BM P2;BMP4;BMP7;GDF5;IL25;IL 1A;IL1B;IL18; CXCR2; CXCR1; CXCR3; CXCR4; CXCR5;CXCR6;ACKR3;CX3CR1;CC R6;CCR7;CCR2;CCR5;CCR1;CCR3; CCR10;IL 6R;IL6ST;IL1RA;LIFR;OSMR;CNTFR;CSF3R;LEPR;IL4R;IL13RA1;IL12RB1;CSF2RA;CSF2RB;IL3R A;IL5RA;IL2RA;IL2RB;IL2RG;IL7R;IL15RA;IL21R;EPOR;GHR;PRLR;MPL;PDGFRA;PDGFRB;KD R;MET;EGFR; CSF1R;KIT;FLT3;IFNAR1;IFNAR2;IFNGR1;IFNGR2;IL1 0RA;IL10RB;IL20RA;IL20RB;IL22RA1;IFNLR1;TNFRSF10A;TNFRSF10B;TNFRSF10C;TNF RSF10D;TNFRSF11B;TNFRSF11A;TNFRSF25;TNFR SF12A; TNFRSF21; TNFRSF1B; TNFRSF 1A;LTBR;TNFRSF14;TNFRSF6B; FAS;CD40;CD27; TNFRSF8; TNFRSF9; TNFRSF4;TNFRSF7;TNFRSF13B;EDAR;EDA2R;TNFRSF19;RELT ;TGFB2;TGFBRI;A CVR2A; ACVR1; ACVR1B;AMHR2; BMPR2;BMPRI1A; BMPR1B; IL17RA; IL17RB;IL17RI;IL17RA;IL17R1;IL18R1;IL18RAP;PLEKHO2 TNF;TNFRSF1A;TRADD;FADD;CASP8;CASP10;CASP3;BID;BAX;CYCS;CA SP9;APAF1;A KT1;AKT3;BAD;BCL2;CAMK2D;CAMK2B;C AMK2G;IFNG;IFNGR1;I FNGR2;JAK1; JAK2;STAT1;CHITA;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;CAR D9;MALT1;BCL10;NOD2;RIPK2;HSPA9;HSPD1;LBP;TLR2;TLR1;TL R6;TLR4;CD14;TIRAP;MYD88;IRAK4;IRAK1;IRAK2;TRAF6;NFKB1;RELA;M APK11;MAPK12;MAPK13;MAPK14;MAPK1;MAPK3;MAP K8;MAPK10; MAPK9; NOS2;IL6;IL18;IL23A;IL1A;IL1B;CEBPB;CEBPB;CD209;ARHGEF12;R HOA; LSP1; PLK3; KSR1;RAF1;TGFB2;TGFB3;CYP27B1;VDR;CAMP;C3;CRI;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
Cytokine-cytokine receptor interaction	195	
Tuberculosis	150	

**Table 1: Contd...**

Pathway	Number	Member
Chemokine signaling pathway	158	CXCL1;CXCL2;CXCL5;CXCL6;PPBP;CXC L8;CXCL9;CXCL10;CXCL11;CXCL12;CXCL13;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;CX3GR1;CCR6;CCR7;CCR2;CCR5;CCR1;CCR 3;CCR10; JAK2 ;STAT1;STAT2; STAT3; STAT5B;GNAIL;GNAI3;G NAI2; ADCY2;ADCY3;ADCY6;ADCY7;A DCY9; PRKACB; PRKX;LYN;HCK;FGR;SRC;SHC1;SHC2;SH C3;SHC4; GRB2;SOS1;SOS2; HRAS;KRAS;NRAS; RAF1;BRA;F;MA P2K1; MAPK1;MAPK3;PIK3R1;PIK3R5;PIK3R2;PIK3R3;PIK3CA;PIK3CD;PIK3CB;PIK 3CG; PRKCK;AKT1;AKT3;FOX O3;CHUK;IKKBK;IKBK;N FKBI;R ELA; GSK3B;ITK; VAV3;VAV1; VAV2;RAC1 ;RAC2;PAK1;CDC42;WAS; WASL;RHOA; ROCK1; ROCK2; GNBI;GNB2; GNB4;GNB5;GNG2;GNG4;GNG5;GNG7;GNG8 ;GNG10;GNG11; GNG12;GNGT1 ;GNGT2;PREX1;EL MO1;DOCK2;PTK2;PXN;BCA RI;CRK; CRKL;PTK2B; PLCB1;PLCB2;PLCB4;R ASGRP2; RAPIA;RAPIB; PARD3; TIAMI1; 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; IL1RI; TNF;TNFRSF1A;TGFB2 ;TGFBRI;TGFB2; TNFRSF11A;TNFRSF11B; TRAF2;TRAF6;LCK;FYN;MAP3K 14;CHUK;RELB;NFKB2; MAP3K7;TAB1;TAB2; IKBK;IKKBK;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 GRIA;FCGR2A;FCGR3B;FCGR3B;TREM2;SIRPA ;SIRPB1;TYROBP; SYK;LCP2;PLCG2;PPP 3CA;PPP3CB;PPP3CC;PPP3RI ;CREB1;SPI1;MITF;CTSK; ACP5; ITGB3; PPARG;IFNARI; IFNAR2;JAK1;TYK2; STAT2;IRF9;SOCS1; SOCS3;GAB2;FHL2; CYLD;SQSTM1
Staphylococcus aureus infection	49	FGG;C3;CFB;CFD; CFH;MASP1;CIQA;CIQB;CIQC;CIR;CIS;C2;C4A;C5;C3A RI;C5ARI; FCGR1A;FCGR2A; FCGR2B;FCGR3B;FCAR; FPR3;FPR2;FPR1; CFI;SELP;G;SELP; ICAMI;ITGAL;ITGAM;ITGB2;DSG1;HLA-DMA;HLA-DMB;HLA-DOA;HLA-DOB;HLA-DPA1;HLA-DPB1;HLA-DQA1;HLA-DQA2;HLA-DQB1;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;ATP6V IE2; ATP6V1E1;ATP6V1F; ATP6V1G1; ATP6V1G2;ATP6V0E1;ATP6V0E2;TCIRG1;AT P6V0A2;ATP6V0A4;A TP6V0A1; ATP6V0D1;ATP6V1H;ATP6V0C;ATP6V0B;ATP6A1;RAB7A;R AB7B;DYNC1H1; DYNC2H1;DYNC111;DYNC112;DYNC1L12;TUBA1B;TUBA4A;TUBA1 A;TUBA1C; TUBA3D;TUB AL3;TUB B6;TUB B;TUB B2A;TUB B2;TUB B8;TUB B2B;TUB B2C;TUB B2D;TUB B2E;TUB B2F;TUB B2G;TUB B2H;TUB B2I;TUB B2J;TUB B2K;TUB B2L;TUB B2M;TUB B2N;TUB B2O;TUB B2P;TUB B2Q;TUB B2R;TUB B2S;TUB B2T;TUB B2U;TUB B2V;TUB B2W;TUB B2X;TUB B2Y;TUB B2Z;TUB B3;TUB B4;TUB B5;TUB B6;TUB B7;TUB B8;TUB B9;TUB B10;TUB B11;TUB B12;TUB B13;TUB B14;TUB B15;TUB B16;TUB B17;TUB B18;TUB B19;TUB B20;TUB B21;TUB B22;TUB B23;TUB B24;TUB B25;TUB B26;TUB B27;TUB B28;TUB B29;TUB B30;TUB B31;TUB B32;TUB B33;TUB B34;TUB B35;TUB B36;TUB B37;TUB B38;TUB B39;TUB B40;TUB B41;TUB B42;TUB B43;TUB B44;TUB B45;TUB B46;TUB B47;TUB B48;TUB B49;TUB B50;TUB B51;TUB B52;TUB B53;TUB B54;TUB B55;TUB B56;TUB B57;TUB B58;TUB B59;TUB B60;TUB B61;TUB B62;TUB B63;TUB B64;TUB B65;TUB B66;TUB B67;TUB B68;TUB B69;TUB B70;TUB B71;TUB B72;TUB B73;TUB B74;TUB B75;TUB B76;TUB B77;TUB B78;TUB B79;TUB B80;TUB B81;TUB B82;TUB B83;TUB B84;TUB B85;TUB B86;TUB B87;TUB B88;TUB B89;TUB B90;TUB B91;TUB B92;TUB B93;TUB B94;TUB B95;TUB B96;TUB B97;TUB B98;TUB B99;TUB B100;TUB B101;TUB B102;TUB B103;TUB B104;TUB B105;TUB B106;TUB B107;TUB 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B330;TUB B331;TUB B332;TUB B333;TUB B334;TUB B335;TUB B336;TUB B337;TUB B338;TUB B339;TUB B340;TUB B341;TUB B342;TUB B343;TUB B344;TUB B345;TUB B346;TUB B347;TUB B348;TUB B349;TUB B350;TUB B351;TUB B352;TUB B353;TUB B354;TUB B355;TUB B356;TUB B357;TUB B358;TUB B359;TUB B360;TUB B361;TUB B362;TUB B363;TUB B364;TUB B365;TUB B366;TUB B367;TUB B368;TUB B369;TUB B370;TUB B371;TUB B372;TUB B373;TUB B374;TUB B375;TUB B376;TUB B377;TUB B378;TUB B379;TUB B380;TUB B381;TUB B382;TUB B383;TUB B384;TUB B385;TUB B386;TUB B387;TUB B388;TUB B389;TUB B390;TUB B391;TUB B392;TUB B393;TUB B394;TUB B395;TUB B396;TUB B397;TUB B398;TUB B399;TUB B400;TUB B401;TUB B402;TUB B403;TUB B404;TUB B405;TUB B406;TUB B407;TUB B408;TUB B409;TUB B410;TUB B411;TUB B412;TUB B413;TUB B414;TUB B415;TUB B416;TUB B417;TUB B418;TUB B419;TUB B420;TUB B421;TUB B422;TUB B423;TUB B424;TUB B425;TUB B426;TUB B427;TUB B428;TUB B429;TUB B430;TUB B431;TUB B432;TUB B433;TUB B434;TUB B435;TUB B436;TUB B437;TUB B438;TUB B439;TUB B440;TUB B441;TUB B442;TUB B443;TUB B444;TUB B445;TUB B446;TUB B447;TUB B448;TUB B449;TUB B450;TUB B451;TUB B452;TUB B453;TUB B454;TUB B455;TUB B456;TUB B457;TUB B458;TUB B459;TUB B460;TUB B461;TUB B462;TUB B463;TUB B464;TUB B465;TUB B466;TUB B467;TUB B468;TUB B469;TUB B470;TUB B471;TUB B472;TUB B473;TUB B474;TUB B475;TUB B476;TUB B477;TUB B478;TUB B479;TUB B480;TUB B481;TUB B482;TUB B483;TUB B484;TUB B485;TUB B486;TUB B487;TUB B488;TUB B489;TUB B490;TUB B491;TUB B492;TUB B493;TUB B494;TUB B495;TUB B496;TUB B497;TUB B498;TUB B499;TUB B500;TUB B501;TUB B502;TUB B503;TUB B504;TUB B505;TUB B506;TUB B507;TUB B508;TUB B509;TUB B510;TUB B511;TUB B512;TUB B513;TUB B514;TUB B515;TUB B516;TUB B517;TUB B518;TUB B519;TUB B520;TUB B521;TUB B522;TUB B523;TUB B524;TUB B525;TUB B526;TUB B527;TUB B528;TUB B529;TUB B530;TUB B531;TUB B532;TUB B533;TUB B534;TUB B535;TUB B536;TUB B537;TUB B538;TUB B539;TUB B540;TUB B541;TUB B542;TUB B543;TUB B544;TUB B545;TUB B546;TUB B547;TUB B548;TUB B549;TUB B550;TUB B551;TUB 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B885;TUB B886;TUB B887;TUB B888;TUB B889;TUB B890;TUB B891;TUB B892;TUB B893;TUB B894;TUB B895;TUB B896;TUB B897;TUB B898;TUB B899;TUB B900;TUB B901;TUB B902;TUB B903;TUB B904;TUB B905;TUB B906;TUB B907;TUB B908;TUB B909;TUB B910;TUB B911;TUB B912;TUB B913;TUB B914;TUB B915;TUB B916;TUB B917;TUB B918;TUB B919;TUB B920;TUB B921;TUB B922;TUB B923;TUB B924;TUB B925;TUB B926;TUB B927;TUB B928;TUB B929;TUB B930;TUB B931;TUB B932;TUB B933;TUB B934;TUB B935;TUB B936;TUB B937;TUB B938;TUB B939;TUB B940;TUB B941;TUB B942;TUB B943;TUB B944;TUB B945;TUB B946;TUB B947;TUB B948;TUB B949;TUB B950;TUB B951;TUB B952;TUB B953;TUB B954;TUB B955;TUB B956;TUB B957;TUB B958;TUB B959;TUB B960;TUB B961;TUB B962;TUB B963;TUB B964;TUB B965;TUB B966;TUB B967;TUB B968;TUB B969;TUB B970;TUB B971;TUB B972;TUB B973;TUB B974;TUB B975;TUB B976;TUB B977;TUB B978;TUB B979;TUB B980;TUB B981;TUB B982;TUB B983;TUB B984;TUB B985;TUB B986;TUB B987;TUB B988;TUB B989;TUB B990;TUB B991;TUB B992;TUB B993;TUB B994;TUB B995;TUB 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B1096;TUB B1097;TUB B1098;TUB B1099;TUB B1100;TUB B1101;TUB B1102;TUB B1103;TUB B1104;TUB B1105;TUB B1106;TUB B1107;TUB B1108;TUB B1109;TUB B1110;TUB B1111;TUB B1112;TUB B1113;TUB B1114;TUB B1115;TUB B1116;TUB B1117;TUB B1118;TUB B1119;TUB B1120;TUB B1121;TUB B1122;TUB B1123;TUB B1124;TUB B1125;TUB B1126;TUB B1127;TUB B1128;TUB B1129;TUB B1130;TUB B1131;TUB B1132;TUB B1133;TUB B1134;TUB B1135;TUB B1136;TUB B1137;TUB B1138;TUB B1139;TUB B1140;TUB B1141;TUB B1142;TUB B1143;TUB B1144;TUB B1145;TUB B1146;TUB B1147;TUB B1148;TUB B1149;TUB B1150;TUB B1151;TUB B1152;TUB B1153;TUB B1154;TUB B1155;TUB B1156;TUB B1157;TUB B1158;TUB B1159;TUB B1160;TUB B1161;TUB B1162;TUB B1163;TUB B1164;TUB B1165;TUB B1166;TUB B1167;TUB B1168;TUB B1169;TUB B1170;TUB B1171;TUB B1172;TUB B1173;TUB B1174;TUB B1175;TUB B1176;TUB B1177;TUB B1178;TUB B1179;TUB B1180;TUB B1181;TUB B1182;TUB B1183;TUB B1184;TUB B1185;TUB B1186;TUB B1187;TUB B1188;TUB B1189;TUB B1190;TUB B1191;TUB B1192;TUB B1193;TUB B1194;TUB B1195;TUB B1196;TUB B1197;TUB B1198;TUB B1199;TUB B1200;TUB B1201;TUB B1202;TUB B1203;TUB B1204;TUB B1205;TUB B1206;TUB B1207;TUB B1208;TUB B1209;TUB B1210;TUB B1211;TUB B1212;TUB B1213;TUB B1214;TUB B1215;TUB B1216;TUB B1217;TUB B1218;TUB B1219;TUB B1220;TUB B1221;TUB B1222;TUB B1223;TUB B1224;TUB B1225;TUB B1226;TUB B1227;TUB B1228;TUB B1229;TUB B1230;TUB B1231;TUB B1232;TUB B1233;TUB B1234;TUB B1235;TUB B1236;TUB B1237;TUB B1238;TUB B1239;TUB B1240;TUB B1241;TUB B1242;TUB B1243;TUB B1244;TUB B1245;TUB B1246;TUB B1247;TUB B1248;TUB B1249;TUB B1250;TUB B1251;TUB B1252;TUB B1253;TUB B1254;TUB B1255;TUB B1256;TUB B1257;TUB B1258;TUB B1259;TUB B1260;TUB B1261;TUB B1262;TUB B1263;TUB B1264;TUB B1265;TUB B1266;TUB B1267;TUB B1268;TUB B1269;TUB B1270;TUB B1271;TUB B1272;TUB B1273;TUB B1274;TUB B1275;TUB B1276;TUB B1277;TUB B1278;TUB B1279;TUB B1280;TUB B1281;TUB B1282;TUB B1283;TUB B1284;TUB B1285;TUB B1286;TUB B1287;TUB B1288;TUB B1289;TUB B1290;TUB B1291;TUB B1292;TUB B1293;TUB B1294;TUB B1295;TUB B1296;TUB B1297;TUB B1298;TUB B1299;TUB B1300;TUB B1301;TUB B1302;TUB B1303;TUB B1304;TUB B1305;TUB B1306;TUB B1307;TUB B1308;TUB B1309;TUB B1310;TUB B1311;TUB B1312;TUB B1313;TUB B1314;TUB B1315;TUB B1316;TUB B1317;TUB B1318;TUB B1319;TUB B1320;TUB B1321;TUB B1322;TUB B1323;TUB B1324;TUB B1325;TUB B1326;TUB B1327;TUB B1328;TUB B1329;TUB B1330;TUB B1331;TUB B1332;TUB B1333;TUB B1334;TUB B1335;TUB B1336;TUB B1337;TUB B1338;TUB B1339;TUB B1340;TUB B1341;TUB B1342;TUB B1343;TUB B1344;TUB B1345;TUB B1346;TUB B1347;TUB B1348;TUB B1349;TUB B1350;TUB B1351;TUB B1352;TUB B1353;TUB B1354;TUB B1355;TUB B1356;TUB B1357;TUB B1358;TUB B1359;TUB B1360;TUB B1361;TUB B1362;TUB B1363;TUB B1364;TUB B1365;TUB B1366;TUB B1367;TUB B1368;TUB B1369;TUB B1370;TUB B1371;TUB B1372;TUB B1373;TUB B1374;TUB B1375;TUB B1376;TUB B1377;TUB B1378;TUB B1379;TUB B1380;TUB B1381;TUB B1382;TUB B1383;TUB B1384;TUB B1385;TUB B1386;TUB B1387;TUB B1388;TUB B1389;TUB B1390;TUB B1391;TUB B1392;TUB B1393;TUB B1394;TUB B1395;TUB B1396;TUB B1397;TUB B1398;TUB B1399;TUB B1400;TUB B1401;TUB B1402;TUB B1403;TUB B1404;TUB B1405;TUB B1406;TUB B1

Table 1: Contd...

Pathway	Number	Member
Graft-versus-host disease	35	P2;CALR;CANX;FCAR;FCGR1A;FCGR2A;FCGR2B;FCGR3B;C1R;ITGAM;ITGB2;C3;COLEC11;COLEC12;SFTPD;ITGAV;ITGA5;ITGA5;ITGB1;ITGB3;ITGB5;THBS1;COMP;THBS2;THBS3;THBS4;TLR2;TLR6;TLR4;CD14;PLA2R1;MRCl;CD209;CLEC7A;MSR1;MARCO;OLR1;SCARBI;CD36;CYBA;CYBB;RAC1;NCF1;NCF2;NCF4;IL6;IL1A;IL1B;TNF;HLA-DMA;HLA-DMB;HLA-DQA1;HLA-DQB1;HLA-DPA1;HLA-DPB1;HLA-DQA2;HLA-DQA2;HLA-DQA2;HLA-DQA2;HLA-DQB1;HLA-DQB1;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;KLRCl;GAD1;PTPRN;PTPRN2;CPE;HSPD1;HLA-DMA;HLA-DMB;HLA-DOA;HLA-DOB;HLA-DPA1;HLA-DPB1;HLA-DQA1;HLA-DQA2;HLA-DQB1;HLA-DQA;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;JCA1
Type 1 diabetes mellitus	37	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;D8A;CD3D;CD3E;CD3G;MME;CD9;CD19;CD22;CD24;MS4A1;CR2;CD37;FCER2;CRI;CSF2RA;IL3RA;CD33;IL4R;IL6R;FCGR1A;CSF1R;ANPEP;ITGAM;CD14;IL1R1;IL1R2;CSF3R;IL5;RA;EPOR;CD36;CD55;CD59;IL11RA;ITGB3;GPIBA;ITGA1;ITGA2;ITGA3;ITGA4;ITGA5;ITGA6;HLA-DRA;HLA-DRB1;HLA-DRB3;HLA-DRB4;HLA-DRB5;CD38
Hematopoietic cell lineage	74	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;STAT1;TBX21;IL12RB1;STAT4;IL18;IL18R1;IL18RAP;JUN;TNF;IL6;IL1A;IL1B;TGFB2;TGFB3;S;MAD2;SMAD3;STAT3;IL2IR;IL23A;RORC;RORA;IL17F;IL4R;IL2RG;STAT6;IL10;MAF;NFATC1
Inflammatory bowel disease (IBD)	51	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;IFNG;IFNGR1;IFNGR2;STAT1;TBX21;IL12RB1;STAT4;IL18;IL18R1;IL18RAP;JUN;TNF;IL6;IL1A;IL1B;TGFB2;TGFB3;S;MAD2;SMAD3;STAT3;IL2IR;IL23A;RORC;RORA;IL17F;IL4R;IL2RG;STAT6;IL10;MAF;NFATC1
Intestinal immune network for IgA production	38	HLA-A;HLA-B;HLA-C;HLA-F;HLA-G;HLA-E;KIR3DL1;KIR2DL3;KIR2DL4;KLRCl;KLRD1;PTPN6;PTPN11;ICAM1;ICA M2;ITGAL;ITGB2;PTK2B;VAV3;VAV1;VAV2;RAC1;RAC2;RAC3;PAK1;MAP2K1;MAP2K2;MAPK1;MAPK3;TNF;CSF2;IFNG;KIR2DS3;TYROBP;LCK;FCGR3B;FCER1G;CD247;ZAP
Leishmaniasis	66	HLA-A;HLA-B;HLA-C;HLA-F;HLA-G;HLA-E;KIR3DL1;KIR2DL3;KIR2DL4;KLRCl;KLRD1;PTPN6;PTPN11;ICAM1;ICA M2;ITGAL;ITGB2;PTK2B;VAV3;VAV1;VAV2;RAC1;RAC2;RAC3;PAK1;MAP2K1;MAP2K2;MAPK1;MAPK3;TNF;CSF2;IFNG;KIR2DS3;TYROBP;LCK;FCGR3B;FCER1G;CD247;ZAP
Natural killer cell mediated cytotoxicity	98	HLA-A;HLA-B;HLA-C;HLA-F;HLA-G;HLA-E;KIR3DL1;KIR2DL3;KIR2DL4;KLRCl;KLRD1;PTPN6;PTPN11;ICAM1;ICA M2;ITGAL;ITGB2;PTK2B;VAV3;VAV1;VAV2;RAC1;RAC2;RAC3;PAK1;MAP2K1;MAP2K2;MAPK1;MAPK3;TNF;CSF2;IFNG;KIR2DS3;TYROBP;LCK;FCGR3B;FCER1G;CD247;ZAP



Table 1: Contd...

Pathway	Number	Member
Influenza A	146	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 PRSS3;PRSS2; TMPRSS2; TMPRSS4;OAS1;OAS2; OAS3;RNASEL; DNAJB1;DNAJC3; HSPA8; HSPA 1A;HSPA2;HSPAL1;H SPA1B;EIF2AK1; EIF2AK2;EIF2AK3; EIF2AK4;EIF2S1; MAP2K3; MAPK11;MAPK12;MAPK13;MAPK14;M AP2K4; MAP2K7; MAPK8 ;MAPK10;MAPK9;JUN; ATF2;DDX58;IFIH1; MAVS;NLRX1;IKBK; NFKB1B; NFKB1A; 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;ICAMI ;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- 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;KPN1;KPN2;XPO1;A GFG1; RAF1; MAP2KI; M AP2K2; MAPK1;M APK3;RSAD2;FDP3;ACTB;ACTG1;IV NS1ABP; CPSF4; NXF1; NXF3; NXT1; NXT2;RAE1;HNRNPUL1; NUP98;FURIN;TMPRSS13; SLC25A6; PRKCA; PRKCB IFNG;TNF;PSME1;PS ME2;PSME3;HSPA8; HSPA1A;HSPA2;HSPA1L; HSPA1B; HSPA4; HSP90A1; HSP90AB1;HL A-A;HL A-B;HL A-C;HL A-F;HL A-G;HL A-E; CANX;B2M;CALR;TAPBP;TAPI;T AP2;CD8A; KIR3DL1; 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 LCK;ZAP70;LAT;PLCG1; PRKCQ;SYK;LYN; BTK;PLCG2;CARD11; BCL10; MALTI;IL1B;IL1RI;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; IKBK;G;CHUK;IKBK;PIAS4;UBE2I;ATM;PIDD1; ERCL1; NFKB1A; NFKB1; RELA;CFLAR;XIAP;BCL 2L1;BCL2; TRAF1; BCL2A1; NFKB2;CXCL8; TNFAIP3;PTGS2;CCL4;VCAMI ;PLAU;CSNK2A1; CSNK2A2; CSNK2B; RELB;CCL13;CCL19; CCL21;CXCL12;ICAMI; PARP1; CXCL2; GADD45B; PRKCB ITGA5;ITGB1;ITGAM;ITGB2;CALML3 ;CALM2;CALM3; CALM1;CALML5; RHOA;CFL1;CFL2;CASP3; CASP7;CASP1;PYCA RD;NLRP3;IL1B;CIQA; CIQB;CIQC; CIR-C1S;C2;C 4A;C3;C5;SERPING1;C4BPA ;LY96;TLR4; CD14;TIRAP; MYD88;IRAK4; 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
Antigen processing and presentation	62	
NF-kappa B signaling pathway	84	
Pertussis	67	





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;COL11A1;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;GNAI;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;SER PINB6; SERPINB9; SERPINB13; CTSG;IL10;TGFB2; TGFB3;C8A;CXCL1; CD1D

concrete results are presented in Supplement Table 1.

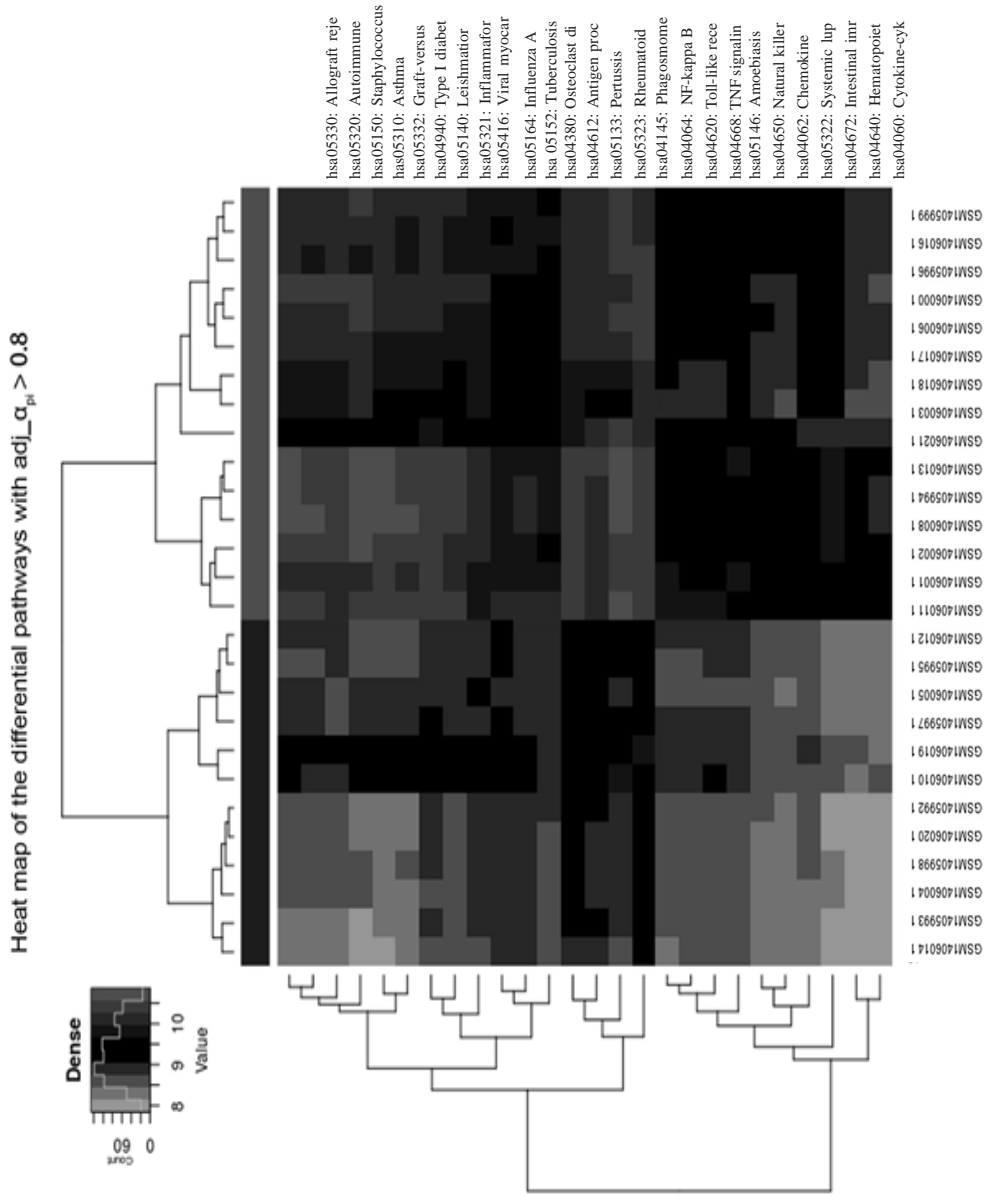
**Hub Genes Identification**

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  $adj\_alpha_{pi} > 0.8$ , 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- $\alpha$ , 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; Green: lower expression level.**  
 Source: Author

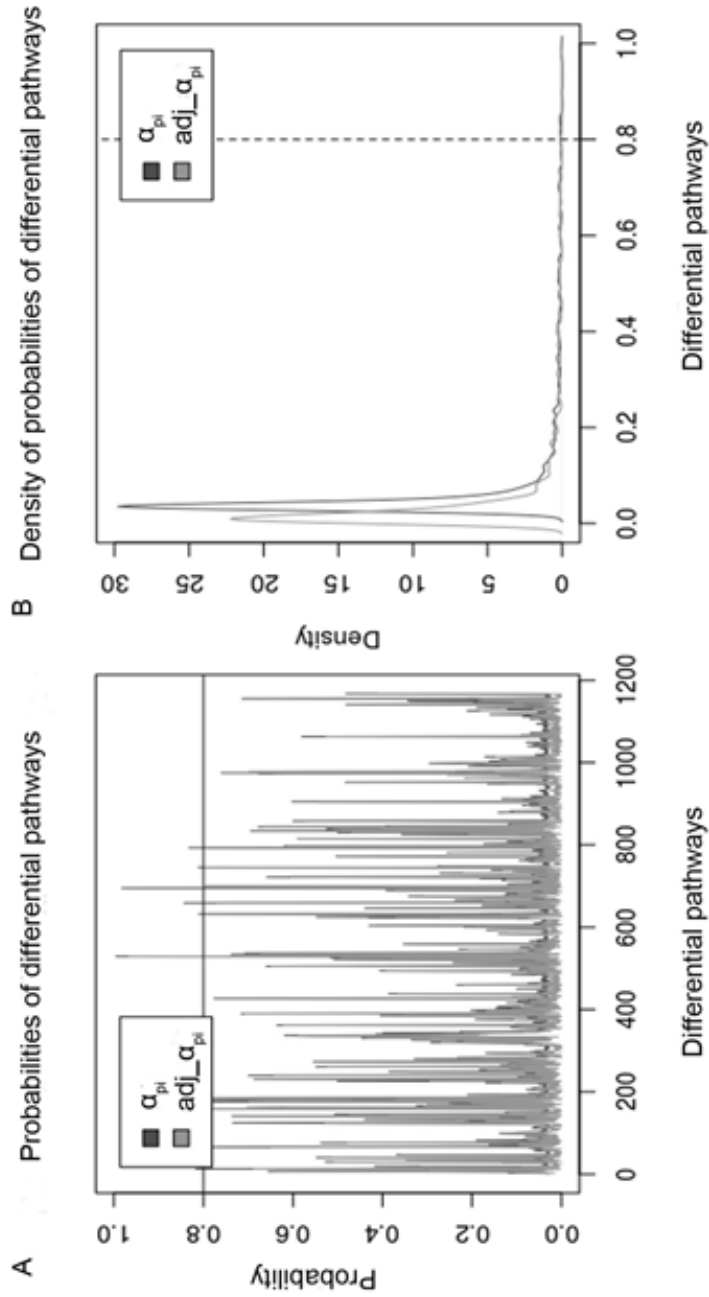
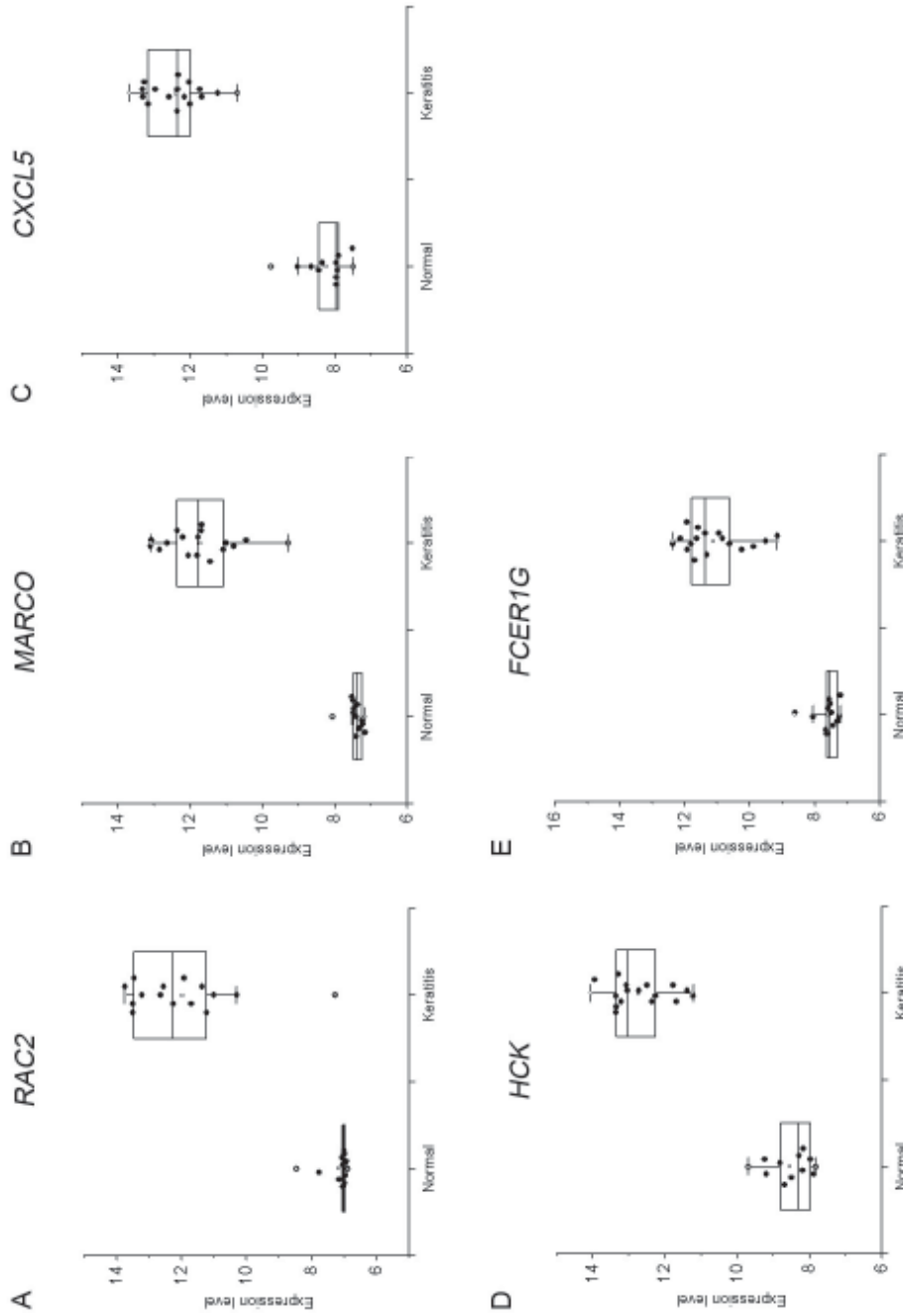
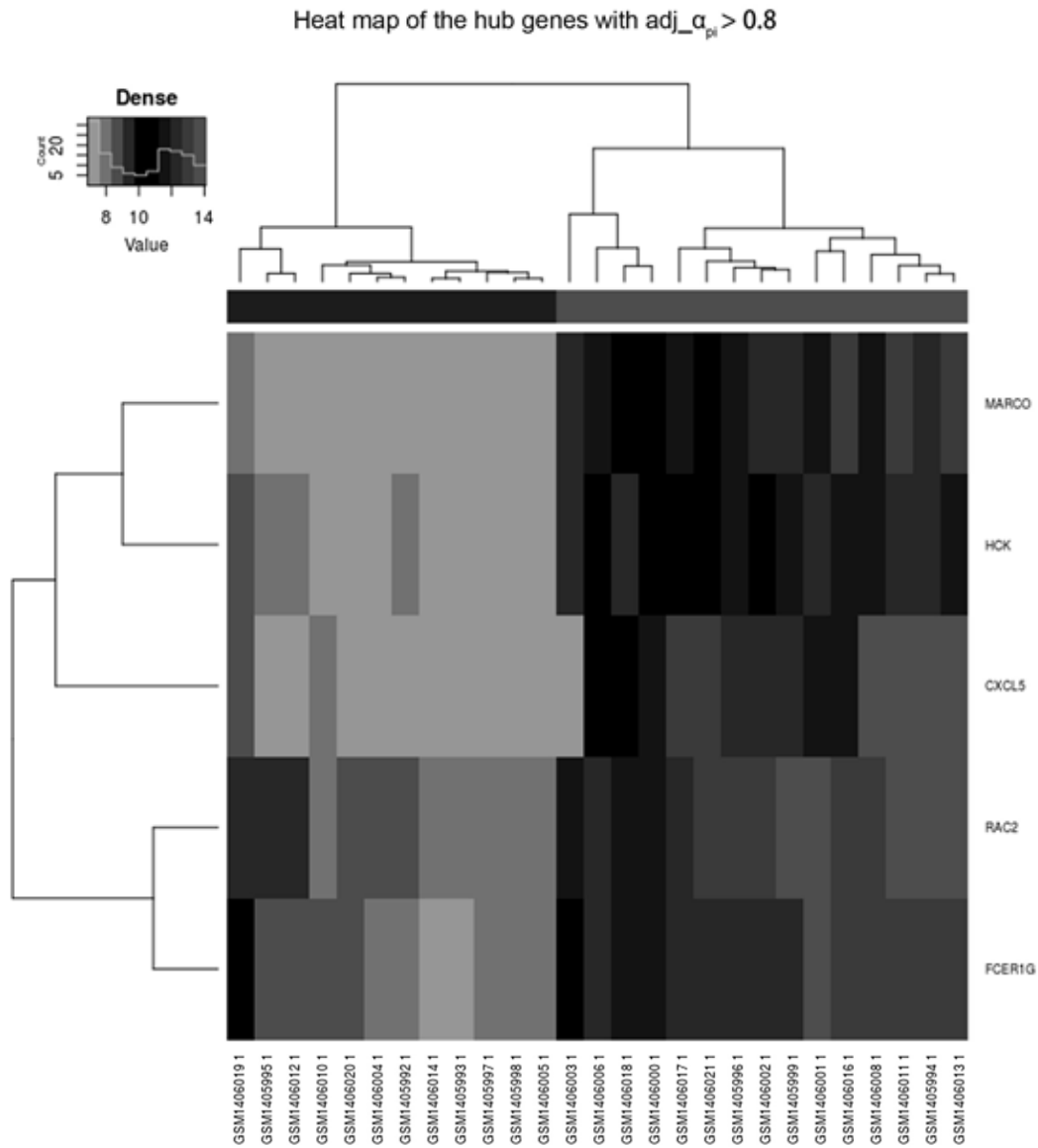


Fig. 3. The probabilities distribution of 1167 differential pathway gene set. The X axis denoted the differential pathway gene set, and the Y axis denoted the posterior value of the differential pathway genes. A. The adjusted posterior value distribution of differential pathway gene set. B. The density of differential pathway genes posterior value distribution. Differential pathway genes were considered as hub gene judging by  $adj\_ \alpha_{pi} > 0.8$ .  
 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; Green: 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- $\alpha$  and IL-6 was reported to facilitate corneal lymphangiogenesis during acute HSV-1 infection (Bryant-Hudson et al. 2014). Con-

sistent 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-1 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 pro-inflammatory 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  $\gamma$ -chain (Fcr1g) 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.

arkers 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.

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