Identification of key genes in Gram-positive and Gram-negative sepsis using stochastic perturbation

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Abstract. Sepsis is an inflammatory response to pathogens (such as Gram-positive and Gram-negative bacteria), which has high morbidity and mortality in critically ill patients. The present study aimed to identify the key genes in Gram-positive and Gram-negative sepsis. GSE6535 was downloaded from Gene Expression Omnibus, containing 17 control samples, 18 Gram-positive samples and 25 Gram-negative samples. Subsequently, the limma package in R was used to screen the differentially expressed genes (DEGs). Hierarchical clustering was conducted for the specific DEGs in Gram-negative and Gram-negative samples using cluster software and the TreeView software. To analyze the correlation of samples at the gene level, a similarity network was constructed using Cytoscape software. Functional and pathway enrichment analyses were conducted for the DEGs using DAVID. Finally, stochastic perturbation was used to determine the significantly differential functions between Gram-positive and Gram-negative samples. A total of 340 and 485 DEGs were obtained in Gram-positive and Gram-negative samples, respectively. Hierarchical clustering revealed that there were significant differences between control and sepsis samples. In Gram-positive and Gram-negative samples, myeloid cell leukemia sequence 1 was associated with apoptosis and programmed cell death. Additionally, NADH:ubiquinone oxidoreductase subunit S4 was associated with mitochondrial respiratory chain complex I assembly. Stochastic perturbation analysis revealed that NADH:ubiquinone oxidoreductase subunit B2 (NDUFB2), NDUFB8 and ubiquinol-cytochrome c reductase hinge protein (UQCRH) were associated with cellular respiration in Gram-negative samples, whereas large tumor suppressor kinase 2 (LATS2) was associated with Gi/S transition of the mitotic cell cycle in Gram-positive samples. NDUFB2, NDUFB8 and UQCRH may be biomarkers for Gram-negative sepsis, whereas LATS2 may be a biomarker for Gram-positive sepsis. These findings may promote the therapies of sepsis caused by Gram-positive and Gram-negative bacteria.

Introduction

Sepsis is a systemic and deleterious inflammatory response to noxious infection (1,2). Sepsis causes ~18 million new cases and millions of deaths worldwide annually; therefore, it is a major cause of morbidity and mortality globally in critically ill patients (3,4). The excessive activation of inflammation, complement and coagulation systems may damage the host's own tissues and organs, leading to multiple organ failure and death (5). In a group of patients diagnosed with sepsis, the most common causative agents are Gram-positive and Gram-negative bacteria (6,7).

Tang et al (8) used the microarray expression profile of GSE6535 to identify the differentially expressed genes (DEGs) between patients with Gram-positive and Gram-negative sepsis with univariate F test according to the cut-off criteria of false discovery rate (FDR) <0.05 and log fold-change (FC)>1.5 and determined that Gram-positive sepsis and Gram-negative sepsis had a common host response at the transcriptome level in critically ill patients (8). However, a previous study illustrated the different mechanisms of sepsis caused by Gram-positive bacteria and Gram-negative bacteria. Hypoxia-inducible factor 1α and Kruppel-like factor 2 have been identified to be involved in Gram-positive endotoxin-mediated sepsis by regulating cellular motility and proinflammatory gene expression in myeloid cells (9). In Gram-negative bacteria-induced sepsis, it has been determined that the inhibition of caspase-1 and defective interleukin 1β production are important immunological features (10). Additionally, α2-antiplasmin has been identified to be a protective mediator during Gram-negative sepsis by inhibiting bacterial growth, inflammation, tissue injury and coagulation (11). Furthermore, thrombomodulin-mediated protein C activation may contribute to protective immunity.
in severe Gram-negative sepsis by regulating inflammatory and procoagulant response (12). Despite the clinical importance of the disease and extensive research, no specific treatment is available for sepsis caused by Gram-positive and Gram-negative bacteria. Therefore, it is necessary to screen the biomarkers for sepsis.

The present study aimed to use the microarray data of Tang et al (8) to screen the DEGs in Gram-positive and Gram-negative samples compared with control samples using the limma package based on a wide range of thresholds (P<0.05 and llog-FC>0.8). In addition, specific genes were collected as biomarkers for sepsis caused by Gram-positive and Gram-negative bacteria. A previous study has proposed that analyses based on differential statistical tests may lead to different outcomes (13). Therefore, the findings of the present study may differ to those of Tang et al (8).

Materials and methods

Microarray data. The microarray dataset of GSE6535 (8) was downloaded from the database of gene expression omnibus (www.ncbi.nlm.nih.gov/geo), which was sequenced on the platform GPL4274 NHICU Human 19K version 1.0. Probe annotation information for mapping the probes into gene symbols was also downloaded. From GSE6535 dataset, 17 neutrophil samples from patients without sepsis, 18 neutrophil samples from patients with Gram-positive sepsis, and 25 neutrophil samples from patients with Gram-negative sepsis were selected. Tang et al (8) obtained whole blood samples from critically ill patients on admission to the intensive care unit of Nepean Hospital (Sydney, Australia). Using Ficoll-Paque density gradient separation, neutrophils were isolated from the whole blood. The patients with sepsis were diagnosed retrospectively according to their medical record. According to the criteria established by Calandra and Cohen (14), the patients with sepsis were divided into Gram-positive and Gram-negative sepsis groups through assessing various clinical features, including physical examination and history and microbiological cultures, such as bronchoalveolar washings, urine, blood and cerebrospinal fluid. GSE6535 was deposited by Tang et al (8). The study of Tang et al was approved by the Ethics Committee of Nepean Hospital and written informed consent was provided by the patients or their families (8).

Data preprocessing and differential expression analysis. Based on the probe annotation information, probe IDs were converted into their corresponding gene symbols. The average value of multiple probes (that were corresponding to the same gene) was used as the gene expression value. To eliminate inherent expression differences between genes, the gene expression values were performed with Z-score normalization as previously described (15). Subsequently, the limma package version 3.32.2 in R (16) was used to screen the DEGs in the Gram-positive and Gram-negative samples compared with the control samples. The P<0.05 and llog-FC>0.8 were used as the cut-off criteria for screening DEGs. Using the VennDiagram in R (17), the common DEGs between Gram-positive and Gram-negative samples, as well as the specific DEGs in Gram-positive samples or Gram-negative samples were identified. Gene Ontology (GO; www.geneontology.org) is a bioinformatics resource that may be used to classify gene product function using controlled, structured vocabularies (18). Using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (19), GO functional enrichment analysis was performed on the common DEGs. The hierarchical cluster analysis of the specific DEGs in Gram-positive or Gram-negative samples was conducted using cluster version 3.0 software (20) and then visualized using TreeView tool version 3 (21).

Similarity network construction. Pearson’s correlation coefficient (PCC) (22), which determines the correlation between two variables, was used to identify the positive or negative correlations among different samples, with the threshold of |PCC|>0.5. Using Cytoscape version 2.8 software (23), a similarity network was constructed for the Gram-positive, Gram-negative and control samples.

Functional and pathway enrichment analyses. Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.jp/kegg/), which integrates genomic, chemical and systemic functional information, is a useful resource for pathway mapping (24). Using the online tool DAVID (19), GO functional and KEGG pathway enrichment analyses were conducted for the DEGs. P<0.05 was used as the threshold.

Identification of significantly differential functions using stochastic perturbations. The average expression value in Gram-positive or Gram-negative samples was calculated for each gene enriched in the same term (GO functions or KEGG pathways). Euclidean distance (25) was used to calculate the difference between the levels of all the terms between Gram-positive and Gram-negative samples, according to the following equation:

\[
\text{distance} = \sqrt{\sum_{i=1}^{T} (\bar{X}_{pi} - \bar{X}_{ni})^2}
\]

Where distance represents the Euclidean distance between Gram-positive samples and Gram-negative samples; \( \bar{X}_{pi} \) stands for the average expression value of gene i in Gram-positive samples; \( \bar{X}_{ni} \) represents the average expression value of gene i in Gram-negative samples; and T indicates the gene number in each term.

Subsequently, stochastic perturbations were used (26) to determine the significance findings. The 18 Gram-positive and 25 Gram-negative samples were randomly selected. Subsequently, 18 samples were randomly selected and defined as Gram-positive samples and the remaining 25 samples were defined as Gram-negative samples. The Euclidean distance between the newly defined Gram-positive samples and Gram-negative samples was recalculated. This was repeated for 10,000 times and the Euclidean distance for 10,000 perturbations were sorted from small to large and used as the background distribution. The ranking order of the initial Euclidean distance in the background distribution was calculated and converted to a P-value. The terms with P<0.05 were considered significantly differential functions between Gram-positive and Gram-negative samples.
Results

DEGs analysis. The gene distribution of Gram-negative (Fig. 1A) and Gram-positive samples (Fig. 1B) are presented using a volcano plot. Using the P<0.05 and |log\(^2\)FC|>0.8 as thresholds, a total of 340 DEGs, including 181 upregulated genes, including large tumor suppressor kinase 2 (LATS2), NADH:ubiquinone oxidoreductase subunit S4 (NDUFS4) and 159 downregulated genes, including myeloid cell leukemia 1 (MCL1) and chitinase-like 1, were obtained in Gram-positive samples compared with control samples. A total of 485 DEGs were identified, 324 upregulated genes, including NDUFS4 and NADH:ubiquinone oxidoreductase subunit B2 (NDUFB2) and 161 downregulated genes, including MCL1 and ecotropic viral integration site 2B, were identified in Gram-negative samples compared with the control samples. The top 10 significantly upregulated genes and downregulated genes in the Gram-negative and Gram-positive samples are presented in Table I.

A total of 188 common DEGs, including 120 upregulated and 68 downregulated were identified between Gram-positive and Gram-negative samples. Additionally, 152 specific DEGs,
including 61 upregulated and 91 downregulated genes in the Gram-positive samples and 297 specific DEGs, including 204 upregulated and 93 downregulated genes in Gram-negative samples were also screened (Fig. 2). GO functional enrichment analysis was performed on the common DEGs, consisted of 120 upregulated and 68 downregulated genes in the Gram-positive and Gram-negative samples and the top 5 terms for each sample type were presented in Fig. 2. The findings revealed that the common upregulated genes were primarily associated with the regulation of apoptosis and cell death, whereas the common downregulated genes were primarily associated with cellular respiration (Fig. 2). Hierarchical cluster analysis of the specific DEGs revealed that there were significant differences between control and sepsis samples. However, no significant difference was identified between the Gram-positive and Gram-negative samples (Fig. 3).

**Similarity network analysis.** In the similarity network, positive associations were identified between the majority of the control and sepsis samples. However, negative associations were also identified between the control and sepsis samples (Fig. 4).

**Functional and pathway enrichment analyses.** Functional enrichment analysis was performed on the upregulated and downregulated genes in the Gram-positive or Gram-negative samples separately. For the downregulated genes in the Gram-positive samples and Gram-negative samples, MCL1 was significantly associated with the functions of apoptosis and programmed cell death regulation. For the upregulated genes in the Gram-positive and Gram-negative samples, NDUFS4 was significantly associated with mitochondrial respiratory chain complex I assembly. Additionally, NDUFB2, NDUFB8 and ubiquinol-cytochrome c reductase hinge protein (UQCRH) were significantly enriched in the functions of cellular respiration, ATP synthesis coupled electron transport and respiratory electron transport chain in Gram-negative samples. LATS2 was significantly associated with the G1/S transition of the mitotic cell cycle in Gram-positive samples (Tables II and III). KEGG pathway enrichment analysis was also conducted for up and downregulated genes in Gram-positive and Gram-negative samples (Tables IV and V). NDUFS4 was significantly enriched in the pathway of oxidative phosphorylation.

**Significantly differential functions screening.** Based on the Euclidean distance of the biological functions, as well as the P-values of the 10,000 stochastic perturbations between Gram-positive samples and Gram-negative samples, a total of 10 significantly differential functions were obtained, including cellular respiration (P<1.00x10⁻⁸, Euclidean distance=1.156277), ATP synthesis coupled electron transport (P<1.00x10⁻⁸, Euclidean distance=1.156277) and G1/S transition of mitotic cell cycle (P=0.015, Euclidean distance = 0.554799; Table VI).

**Discussion**

In line with the results of Tang et al (8), the present study determined that there was no significant difference in the expression profile between Gram-positive and gram-negative samples from hierarchical clustering analysis. In the Gram-positive and Gram-negative samples, the GO functional enrichment analysis revealed that MCL1 was significantly associated with the regulation of apoptosis and programmed cell death. A previous study has determined that the apoptosis of T-cells may induce the breakdown of defense mechanisms resulting in sepsis (27). Additionally,
the inhibition of programmed cell death may reverse T-cell exhaustion and thus eradicate the invading pathogens which cause sepsis (28). Additionally, *MCL1* may also be associated with the reduction of apoptosis of neutrophils in patients with sepsis (29). Therefore, it is possible for *MCL1* to be involved in sepsis via the regulation of T-cell apoptosis and programmed T-cell death in both Gram-positive and Gram-negative sepsis.

Additionally, the present study also determined that *NDUFS4* was significantly associated with mitochondrial respiratory chain complex I assembly. Mitochondrial dysfunction may lead to oxidative stress and failure of...
energy production, which may result in organ dysfunction in sepsis (30). The KEGG pathway enrichment analysis revealed that \textit{NDUFS4} was significantly enriched in oxidative phosphorylation. Lee and Hüttemann (31) have determined that the inhibition of oxidative phosphorylation may lead to a reduction of the mitochondrial membrane potential, resulting in a lack of energy, which may cause organ failure and death in septic patients (31). \textit{NDUFS4} has been previously reported to be an important subunit of complex I which has a key role in oxidative phosphorylation (32). Additionally, \textit{NDUFS4} may participate in the regulation of sepsis induced by Gram-negative and Gram-positive bacteria through regulation of oxidative phosphorylation. However, the present study identified specific DEGs in Gram-positive and Gram-negative samples compared with normal samples. According to the Euclidean distance and the stochastic perturbations performed between Gram-positive and Gram-negative samples, \textit{NDUFB2}, \textit{NDUFB8} and \textit{UQCRH} were significantly upregulated in the Gram-negative samples, whereas they were not upregulated in the Gram-positive samples. In addition, functional annotation revealed that they were significantly associated with cellular respiration, ATP synthesis coupled electron transport and mitochondrial electron transport, ubiquinol to cytochrome c. \textit{NDUFB2} and \textit{NDUFB8} are parts of the multisubunit mitochondrial NADH ubiquinone oxidoreductase (complex I) which has an important role in mitochondrial functioning (33,34). A previous study determined that a dysfunction of respiratory chain complex I may be associated with reactive oxygen species (ROS) production (35). Additionally, previous studies reported that ROS are toxic oxygen-containing molecules that may damage the cells and the antioxidant defense system, which is the pathogenesis of sepsis (36,37). \textit{UQCRH}, which encodes the cytochrome b-cl complex subunit 6 of complexes III (cytochrome c-oxidoreductase), is involved in the mitochondrial oxidative phosphorylation and the dysfunction of \textit{UQCRH} may lead to breast and ovarian cancer by altering the function of the mitochondria (38,39). To the best of our knowledge, this is the first study investigating the functions of \textit{NDUFB2}, \textit{NDUFB8} and \textit{UQCRH} in Gram-negative bacteria-induced sepsis. The present study concluded that \textit{NDUFB2}, \textit{NDUFB8} and \textit{UQCRH} may be involved in the Gram-negative bacteria-induced sepsis by altering mitochondrial oxidative phosphorylation and may also be potential targets for the treatment of Gram-negative bacterial sepsis.

In addition, the function of the G1/S transition of the mitotic cell cycle was also determined to be significantly different between the Gram-positive and Gram-negative samples. \textit{LATS2} was enriched in this function and was significantly upregulated in patients with Gram-positive sepsis, whereas it was not significantly expressed in Gram-negative patients. \textit{LATS2}, encoding serine/threonine-protein kinase, has been identified to inhibit the G1/S transition in the cell cycle of tumor cells (40). Additionally, G1 cell cycle arrest may be important for the initiation of kidney injury in sepsis (41). Therefore, \textit{LATS2} may be associated with Gram-negative bacterial sepsis by the modulation of G1/S transition in cell cycle.

In conclusion, \textit{MCL1}, \textit{NDUFS5} and \textit{NDUFS4} may be potential target genes for the treatment of Gram-positive and Gram-negative bacterial sepsis. Additionally, \textit{NDUFB2}, \textit{NDUFB8} and \textit{UQCRH} may also be associated with Gram-negative bacterial sepsis. \textit{LATS2} may contribute to the progression of Gram-negative bacterial sepsis. However, further studies are still required in order to elucidate their action mechanisms in sepsis.
Table II. Top 10 enriched GO terms for the upregulated and the downregulated genes in Gram-positive samples.

A. Upregulated genes

| Term | Function | Count | P-value | Gene symbol |
|------|----------|-------|---------|-------------|
| GO:0006412 | Translation | 22 | 3.52x10^{-11} | AIMP1, EEF1A2, GARS, MRPS10, RPS27L, MRPS18C, MRPL15, MRPS18A, RPL22, MRPL27, EEF1E1, NARS2, EIF2S2, HARS, MRPL19, MRPL36, RPL10, RPL11, RPL22, EEFSEC, MRPL33 |
| GO:0006120 | Mitochondrial electron transport, NADH to ubiquinone | 7 | 4.78x10^{-6} | NDUF4A4, NDUF6S, NDUF5S, NDUF5S4, NDUF8A, NDUF9A, NDUFCC2 |
| GO:0042773 | ATP synthesis coupled electron transport | 7 | 2.62x10^{-5} | NDUF4A4, NDUF6S, NDUF5S, NDUF5S4, NDUF8A, NDUF9A, NDUFCC2 |
| GO:0022904 | Respiratory electron transport chain | 7 | 5.65x10^{-5} | NDUF4A4, NDUF6S, NDUF5S, NDUF5S4, NDUF8A, NDUF9A, NDUFCC2 |
| GO:0045333 | Cellular respiration | 8 | 7.34x10^{-5} | NDUF4A4, NDUF6S, NDUF5S, NDUF5S4, NDUF8A, NDUF9A, NDUFCC2, MDH1 |
| GO:0044267 | Cellular protein metabolic process | 44 | 1.09x10^{-4} | FASTK, FKBP3, PRDX4, MRPS10, RPS27L, CANX, LATS2, VRK1, PSMB7, PSMB6, MRPL15, PLOD2, NARS2, MRPL36, B3GNT1, MRPL19, RPL10, RPL11, RPL24D1, MRPL32, LOXL1, FGF2, MRPL33, HSP90AA1, AIMP1, EEF1A2, GARS, SOD1, LRPAP1, IKBKE, MAST4, HSP90B1, PPIH, MRPS18C, PSMA5, MRPL27, RPL22, RPL18A, EEF1E1, EIF2S2, HARS, DSP, EEFSEC, FKBP2 |
| GO:0016310 | Phosphorylation | 19 | 1.87x10^{-3} | NDUF4A4, NDUF6S, MVD, NDUF9A, FASTK, HK2, NDUFCC2, PRDX4, SOD1, LATS2, NDUF5S, IKBKE, MAST4, VRK1, NDUF5S4, ATP5C1, ATP5A1, FGF2 |
| GO:0000079 | Regulation of cyclin-dependent protein kinase activity | 5 | 2.51x10^{-3} | GTPBP4, CDKN2C, CK52, CDKN3, LATS2 |
| GO:0033365 | Protein localization in organelle | 7 | 4.53x10^{-3} | PPIH, MYO2, NDUF13A, FGF2, TIMM44, SEC61G, NR5A1 |
| GO:0010257 | NADH dehydrogenase complex assembly | 3 | 4.73x10^{-3} | NDUF4A4, NDUF5S, NDUF5S4 |

B. Downregulated genes

| Term | Function | Count | P-value | Gene symbol |
|------|----------|-------|---------|-------------|
| GO:0010942 | Positive regulation of cell death | 17 | 1.51x10^{-6} | PTGS2, PREX1, STK17B, RRAGA, PRKDC, NLRP3, NLRP3, SERINC3, NOTCH1, E24, SSTR3, CASP4, DUSP1, CASP8, BNIP3L, FGD3, KALRN |
| GO:0043065 | Positive regulation of apoptosis | 16 | 6.31x10^{-6} | PTGS2, PREX1, STK17B, PRKDC, NLRP3, NLRP1, SERINC3, NOTCH1, E24, SSTR3, CASP4, DUSP1, CASP8, BNIP3L, FGD3, KALRN |
| Term          | Function                                      | Count | P-value         | Gene symbol                                                                 |
|--------------|-----------------------------------------------|-------|----------------|-----------------------------------------------------------------------------|
| GO:0043068   | Positive regulation of programmed cell death  | 16    | 6.86x10^-6     | PTGS2, PREX1, STK17B, PRKDC, NLRP3, NLRP1, SERINC3, NOTCH1, EI24, SSTR3,    |
|              |                                               |       |                | CASP4, DUSP1, CASP8, BNIP3L, FGD3, KALRN                                   |
| GO:0042981   | Regulation of apoptosis                       | 20    | 8.53x10^-3     | PTGS2, MCL1, PREX1, STK17B, PRKDC, PIM2, NLRP3, NLRP1, SERINC3, NOTCH1,    |
|              |                                               |       |                | EI24, SSTR3, CASP4, DUSP1, IGF2R, BNIP3L, CASP8, DLG5, FGD3, KALRN         |
| GO:0043067   | Regulation of programmed cell death           | 20    | 9.73x10^-5     | PTGS2, MCL1, PREX1, STK17B, PRKDC, PIM2, NLRP3, NLRP1, SERINC3, NOTCH1,    |
|              |                                               |       |                | EI24, SSTR3, CASP4, DUSP1, IGF2R, BNIP3L, CASP8, DLG5, FGD3, KALRN         |
| GO:0012502   | Induction of programmed cell death            | 12    | 1.38x10^-4     | SERINC3, EI24, CASP4, SSTR3, PREX1, BNIP3L, CASP8, STK17B, NLRP3, NLRP1,  |
|              |                                               |       |                | FGD3, KALRN                                                                 |
| GO:0009966   | Regulation of signal transduction             | 20    | 2.68x10^-4     | LITAF, PREX1, KLK5, CYTH4, RABGAP1L, PIM2, TBC1D22A, OSM, ECE1, CXCR4,     |
|              |                                               |       |                | SOSTDC1, CASP8, GPSM1, RAMP1, RAPGEF1, RUNX2, ARAP1, FGD3, GNG7, KALRN     |
| GO:0008277   | Regulation of G-protein coupled receptor protein signaling pathway | 5     | 1.10x10^-3     | ECE1, KLK5, GPSM1, RAMP1, GNG7                                              |
| GO:0051056   | Regulation of small GTPase mediated signal transduction | 8     | 7.45x10^-3     | PREX1, CYTH4, RABGAP1L, RAPGEF1, FGD3, ARAP1, TBC1D22A, KALRN              |
| GO:0010647   | Positive regulation of cell communication     | 8     | 2.82x10^-2     | OSM, LAMA2, ECE1, PTGS2, LITAF, KLK5, CASP8, PIM2                           |

GO, gene ontology.
Table III. Top 10 enriched GO terms for the upregulated and the downregulated genes in Gram-negative samples.

**A. Upregulated genes**

| Term          | Function                                         | Count | P-value         | Gene symbol                  |
|---------------|--------------------------------------------------|-------|-----------------|------------------------------|
| GO:0045333    | Cellular respiration                             | 15    | 8.97x10^{-9}    | UQRC2, NDUFA8, NDUFB8, NDUFA6, NDUFA7, CYCS, NDUFC2, NDUFB2, NDUFS6, UQCR10, NDUFS5, NDUFS4, UQCRH, UQCRB, MDH1 |
| GO:0042773    | ATP synthesis coupled electron transport         | 12    | 1.31x10^{-8}    | NDUFS6, NDUFS5, UQCR10, NDUFS4, NDUFA8, UQCRH, NDUFB8, NDUFA6, NDUFA7, NDUFC2, UQCRB, NDUFB2 |
| GO:0022904    | Respiratory electron transport chain             | 12    | 5.68x10^{-8}    | NDUFS6, NDUFS5, UQCR10, NDUFS4, NDUFA8, UQCRH, NDUFB8, NDUFA6, NDUFA7, NDUFC2, UQCRB, NDUFB2 |
| GO:0006412    | Translation                                      | 24    | 2.78x10^{-7}    | MRPL3, RPL19, EEF1B2, EEF1A2, HBS1L, RPL15, MRPS10, RPL27, RPS27L, RPL22L1, IARS2, RPS7, MRPS18C, MRPL15, MRPS18A, RPL22, MRPL27, EEF1E1, MRPL19, RPL11, RSL24D1, MRPL32, MRPL33, RPS23 |
| GO:0006457    | Protein folding                                  | 17    | 6.33x10^{-7}    | HSP90AA1, FKB5F, FKB4F, FKB3, TTC9, PDI1, CCT3, LMAN1, CANX, LRPAP1, CCT7, PPIH, HSP90B1, SIL1, RUVBL2, HSPD1, FKB2 |
| GO:0006120    | Mitochondrial electron transport, NADH to ubiquinone | 9     | 1.70x10^{-6}    | NDUFS6, NDUFS5, NDUFS4, NDUFA8, NDUFB8, NDUFA6, NDUFA7, NDUFC2, NDUFB2 |
| GO:0051437    | Positive regulation of ubiquitin-protein ligase activity during mitotic cell cycle | 9     | 6.75x10^{-5}    | CDK1, PSMB7, PSMD14, PSMB6, PSMA6, PSMA5, PSMC2, PSMA4, PSMD1 |
| GO:0051351    | Positive regulation of ligase activity           | 9     | 1.12x10^{-4}    | CDK1, PSMB7, PSMD14, PSMB6, PSMA6, PSMA5, PSMC2, PSMA4, PSMD1 |
| GO:0051438    | Regulation of ubiquitin-protein ligase activity | 9     | 1.80x10^{-4}    | CDK1, PSMB7, PSMD14, PSMB6, PSMA6, PSMA5, PSMC2, PSMA4, PSMD1 |
| GO:0051436    | Negative regulation of ubiquitin-protein ligase activity during mitotic cell cycle | 8     | 3.37x10^{-4}    | PSMB7, PSMD14, PSMB6, PSMA6, PSMA5, PSMC2, PSMA4, PSMD1 |

**B. Downregulated genes**

| Term          | Function                                          | Count | P-value          | Gene symbol                  |
|---------------|---------------------------------------------------|-------|------------------|------------------------------|
| GO:0043067    | Regulation of programmed cell death               | 22    | 3.47x10^{-6}     | IFIH1, PTGS2, MCL1, PREX1, TGFBR1, BCL2A1, STK17B, IFI16, NLRP3, NLRP1, TNFRSF9, CASP4, DUSP1, BTG2, IGF2R, BNIP3L, CHST11, CASP8, LRRK2, MX1, IFI16, KALRN |
Table III. Continued.

B. Downregulated genes

| Term | Function | Count | P-value    | Gene symbol                                      |
|------|----------|-------|------------|-------------------------------------------------|
| GO:0010942 | Positive regulation of cell death | 16    | 3.57x10^{-6} | PTGS2, PREX1, TGFBR1, STK17B, RRAGA, IFI16, NLRP3, NLRP1, TNFRSF9, CASP4, DUSP1, CASP8, BNIP3L, MX1, LRRK2, KALRN |
| GO:0042981 | Regulation of apoptosis | 21    | 1.10x10^{-5} | IFIH1, PTGS2, MCL1, PREX1, TGFBR1, BCL2A1, STK17B, IFI16, NLRP3, NLRP1, TNFRSF9, CASP4, DUSP1, BTG2, IGF2R, BNIP3L, CHST11, CASP8, MX1, IFI6, KALRN |
| GO:0043068 | Positive regulation of programmed cell death | 15    | 1.61x10^{-5} | PTGS2, PREX1, TGFBR1, STK17B, IFI16, NLRP3, NLRP1, TNFRSF9, CASP4, DUSP1, CASP8, BNIP3L, MX1, LRRK2, KALRN |
| GO:0043065 | Positive regulation of apoptosis | 14    | 6.62x10^{-5} | PTGS2, TGFBR1, PREX1, STK17B, IFI16, NLRP3, NLRP1, TNFRSF9, CASP4, DUSP1, CASP8, BNIP3L, MX1, KALRN |
| GO:0012502 | Induction of programmed cell death | 12    | 8.32x10^{-5} | TNFRSF9, CASP4, PREX1, TGFBR1, BNIP3L, CASP8, STK17B, IFI16, MX1, NLRP3, NLRP1, KALRN |
| GO:0031401 | Positive regulation of protein modification process | 7     | 5.07x10^{-3} | OSM, CCND3, TGFBR1, CD4, RICTOR, UBE2D1, LRRK2 |
| GO:0010562 | Positive regulation of phosphorus metabolic process | 5     | 1.02x10^{-2} | OSM, CCND3, TGFBR1, CD4, RICTOR |
| GO:0045937 | Positive regulation of phosphate metabolic process | 5     | 1.02x10^{-2} | OSM, CCND3, TGFBR1, CD4, RICTOR |
| GO:0019048 | Virus-host interaction | 3     | 1.22x10^{-2} | IRF7, RRAGA, CD4 |
Table IV. Enriched pathways for the upregulated and the downregulated genes in Gram-negative samples.

### A. Upregulated genes

| Term                       | Function                        | Count | P-value    | Gene symbol                                      |
|----------------------------|---------------------------------|-------|------------|--------------------------------------------------|
| hsa05012 Parkinson’s disease |                                 | 19    | 9.00x10^{-9} | UQCR2, NDUFA8, SLC25A5, NDUFA4L2, NDUF8, SLC25A6, NDUFA6, COX7B, NDUFA7, CYCS, NDUFC2, NDUF8, NDUFS6, UQCR10, NDUFS5, NDUFS4, UQCRH, ATP5A1, UQCRB |
| hsa05016 Huntington’s disease  |                                 | 21    | 7.73x10^{-8} | UQCR2, NDUFA8, SLC25A5, NDUFA4L2, NDUF8, POLR2K, SLC25A6, NDUFA6, CYCS, COX7B, NDUFA7, NDUFC2, SOD1, NDUF8, NDUFS6, UQCR10, NDUFS5, NDUFS4, UQCRH, ATP5A1, UQCRB |
| hsa00190 Oxidative phosphorylation |                                      | 17    | 4.25x10^{-7} | UQCR2, NDUFA8, NDUFA4L2, NDUF8, NDUFA6, COX7B, NDUFA7, NDUFC2, NDUF8, NDUFS6, UQCR10, NDUFS5, NDUFS4, UQCRH, ATP5A1, ATP5I, UQCRB |
| hsa05010 Alzheimer’s disease |                                 | 18    | 1.96x10^{-6} | UQCR2, NDUFA8, NDUFA4L2, NDUF8, NDUFA6, CYCS, NDUFC2, NAE1, NDUFB2, NDUFS6, UQCR10, NDUFS5, NDUFS4, UQCRH, ATP5A1, UQCRB, PSMB7, PSMD14, PSMB6, PSMA6, PSMA5, PSMC2, PSMA4, SHFM1, PSMD1 |
| hsa03050 Proteasome         |                                 | 9     | 3.61x10^{-5} | PSMB7, PSMD14, PSMB6, PSMA6, PSMA5, PSMC2, PSMA4, SHFM1, PSMD1 |
| hsa03010 Ribosome           |                                 | 10    | 6.12x10^{-4} | RPL19, RPL22, RPL15, RPL27, RPS27L, RPL11, RSL24D1, RPL22L1, RPS23, RPS7 |
| hsa00620 Pyruvate metabolism |                                 | 6     | 4.60x10^{-3} | LDHA, ACYP1, GLD1, ACAT2, PCK1, MDH1 |
| hsa04260 Cardiac muscle contraction |                                 | 7     | 2.05x10^{-2} | UQCR2, UQCR10, UQCRH, COX7B, ATP1A2, TNN13, UQCRB |
| hsa04110 Cell cycle         |                                 | 9     | 2.21x10^{-2} | CDK1, YWHAG, CDKN2C, YWHAC, TFDP2, PCNA, CDK6, GADD45A, SMC3 |
| hsa04115 p53 signaling pathway |                                 | 6     | 3.94x10^{-2} | CDK1, CYCS, CDK6, PERP, IGFBP3, GADD45A |

### B. Downregulated genes

| Term                       | Function                        | Count | P-value    | Gene symbol                                      |
|----------------------------|---------------------------------|-------|------------|--------------------------------------------------|
| hsa04622 RIG-I-like receptor signaling pathway  |                                 | 5     | 5.32x10^{-3} | IFIG1, ISG15, IRF7, CASP8, IFNA8 |
| hsa04612 Antigen processing and presentation |                                 | 5     | 9.21x10^{-3} | HSPA6, CD4, IFNA8, CTSS, HLA-F |
| hsa04620 Toll-like receptor signaling pathway |                                 | 4     | 1.99x10^{-2} | IRF7, CASP8, IFNA8, CD14 |
| hsa04660 T cell receptor signaling pathway  |                                 | 4     | 2.33x10^{-2} | PTPN6, RAF1, CD4, MAP3K14 |
Table V. Enriched pathways for the upregulated and the downregulated genes in Gram-positive samples.

### A. Upregulated genes

| Term              | Function                                      | Count | P-value     | Gene symbol                                                                 |
|-------------------|-----------------------------------------------|-------|-------------|----------------------------------------------------------------------------|
| hsa05012          | Parkinson's disease                           | 10    | $3.37 \times 10^{-5}$ | NDUFA4, NDUFS6, NDUFS5, NDUFS4, NDUFA8, SLC25A5, NDUFA9, NDUFC2, ATP5C1, ATP5A1 |
| hsa05016          | Huntington's disease                          | 11    | $9.07 \times 10^{-5}$ | NDUFA4, NDUFS6, NDUFS5, NDUFS4, NDUFA8, SLC25A5, NDUFA9, NDUFC2, ATP5C1, ATP5A1 |
| hsa00190          | Oxidative phosphorylation                     | 9     | $2.43 \times 10^{-4}$ | NDUFA4, NDUFS6, NDUFS5, NDUFS4, NDUFA8, NDUFA9, NDUFC2, ATP5C1, ATP5A1, SOD1 |
| hsa05010          | Alzheimer's disease                           | 9     | $1.11 \times 10^{-3}$ | NDUFA4, NDUFS6, NDUFS5, NDUFS4, NDUFA8, NDUFA9, NDUFC2, ATP5C1, ATP5A1 |
| hsa03010          | Ribosome                                      | 5     | $2.58 \times 10^{-2}$ | RPL22, RPL10, RPS27L, RPL11, RSL24D1                                      |
| hsa04612          | Antigen processing and presentation           | 4     | $9.23 \times 10^{-2}$ | HSP90AA1, IFI130, HSPA4, CANX                                            |
| hsa00970:         | Aminoacyl-tRNA biosynthesis                   | 3     | $9.85 \times 10^{-2}$ | NARS2, HARS, GARS                                                        |

### B. Downregulated genes

| Term              | Function                                      | Count | P-value     | Gene symbol                                                                 |
|-------------------|-----------------------------------------------|-------|-------------|----------------------------------------------------------------------------|
| hsa04650          | Natural killer cell mediated cytotoxicity     | 6     | $9.37 \times 10^{-3}$ | PTPN6, ICAM2, RAF1, IFNA8, NFATC2, KLRD1                                   |
| hsa04621          | NOD-like receptor signaling pathway           | 4     | $2.25 \times 10^{-2}$ | IL8, CASP8, NLRP3, NLRP1                                                   |
| hsa04660          | T cell receptor signaling pathway             | 4     | $2.91 \times 10^{-2}$ | PTPN6, RAF1, NFATC2, MAP3K14                                               |
Table VI. Top 10 significant differential functions between Gram-negative samples and Gram-positive samples.

| GO ID         | Term                                                                 | Euclidean distance | P-value          | Gene symbols                                      |
|---------------|----------------------------------------------------------------------|--------------------|------------------|--------------------------------------------------|
| GO:0006120    | Mitochondrial electron transport, NADH to ubiquinone                | 1.156277           | <1.00x10⁻⁸       | NDUFB8, NDUFB2, NDUF6, NDUF7, NDUF9, NDUF4        |
| GO:0042773    | ATP synthesis coupled electron transport                             | 1.156277           | <1.00x10⁻⁸       | UQCRH, NDUFB8, NDUF6, UQCRB, NDUF6, UQCR10, NDUF9, NDUF4 |
| GO:002904     | Respiratory electron transport chain                                  | 1.156277           | <1.00x10⁻⁸       | UQCRH, NDUFB8, NDUF6, UQCRB, NDUF6, UQCR10, NDUF9, NDUF4 |
| GO:0045333    | Cellular respiration                                                  | 1.156277           | <1.00x10⁻⁸       | UQCRH, NDUFB8, UQCR2, UQCRB, NDUF6, UQCR10, CYCS, NDUF6, NDUF9 |
| GO:0016310    | Phosphorylation                                                       | 1.413364           | 1.00x10⁻⁴        | UQCRH, PAK4, FGFR1, NDUF8, NDUF6, CDK1, CDK6, UQCR2, GHR, IGFBP3, UQCRB, NDUF6, UQCR10, MET, ATP5I, MVD, NDUF9, LATS2, MAST4, NDUF4, IKBKE, ATP5C1 |
| GO:0042981    | Regulation of apoptosis                                              | 1.508092           | 2.70x10⁻³        | IFI16, TNFRSF9, IFI1H, MX1, BTG2, CHST11, TGFBR1, BCL2A1, IFI6, LGALS1, PRDX1, PHLDA1, HSPD1, SORT1, MAL, DHCR24, GLO1, ITGB3BP, CDK1, GHR, SERPINB2, NQO1, ANXA1, IGFBP3, SMO, CADM1, CD44, KRT18, CYCS, NAE1, PERP, DG5, EI24, PRKDC, NOTCH1, SERINC3, FGD3, PIM2, SSTR3 |
| GO:0043067    | Regulation of programmed cell death                                   | 1.517301           | 3.60x10⁻⁴        | IFI16, TNFRSF9, IFI1H, MX1, BTG2, CHST11, LRRK2, TGFBR1, BC12A1, IFI6, LGALS1, PRDX1, PHLDA1, HSPD1, SORT1, MAL, DHCR24, GLO1, ITGB3BP, CDK1, GHR, SERPINB2, NQO1, ANXA1, IGFBP3, SMO, CADM1, CD44, KRT18, CYCS, NAE1, PERP, DG5, EI24, PRKDC, NOTCH1, SERINC3, FGD3, PIM2, SSTR3 |
| GO:0000082    | G1/S transition of mitotic cell cycle                                | 0.554799           | 1.50x10⁻²        | LATS2                                            |
| GO:0044267    | Cellular protein metabolic process                                    | 2.267426           | 2.37x10⁻²        | RPS23, IARS2, HSPD1, PAK4, FGFR1, SEC11A, HEXB, FKB5P, PSMA4, MPRL3, RPL27, PSMC2, SIL1, SUP3H, RPL27, RPS7, RPL19, FKBP4, LMN1, TPTP1, CDK1, CDK6, GHR, RPL22L1, DIA5, CCT7, FBX07, ANXA1, IGFBP3, PSMA6, PSMD1, CCT3, HERC3, TFC9, MET, NAE1, PSMD14, HBS1L, RABGTTB, EEF1B2, RPL15, RUVB12, AMP1, LATS2, MAST4, EEFSEC, LOXL1, NARS2, HARS, B3GNT1, MRPL36, IKBKE, EIF252, DSP, RPL10, GARS |
| GO:0007517    | Muscle organ development                                             | 1.050407           | 4.15x10⁻²        | FAM65B, LAMA2, ANKRD2, FOXP1, CACNB4              |

GO, gene ontology.
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