Research article

# A bioinformatics approach revealed the transcription factors of *Helicobacter pylori* pathogenic genes and their regulatory network nodes

Yu Bai [a], Wenliang Li [a], Guangyu Xu [b], Guihua Cui [a,*]

[a] *Pharmaceutical College, Jilin Medical University, Jilin, Jilin, 132011, China*
[b] *Pharmaceutical College, Beihua University, Jilin, Jilin, 132013, China*

## ARTICLE INFO

## ABSTRACT

*Background: Helicobacter pylori* is a chronic pathogenic bacteria that causes gastric mucosal damage through various host-related and pathogen-related factors. Thus, a single gene research cannot fully explain its pathogenicity. Purpose of the study: It is necessary to establish a *Helicobacter pylori* pathogenic gene transcription factor regulatory network (TFRN) and study its central nodes.
*Results:* The expression data of *Helicobacter pylori* pathogenic genes were obtained through GEO Datasets of NCBI. The genes were screened using linear model-empirical Bayesian statistics in R language Limma package combined with the conventional *t*-test; the results identified 1231 differentially expressed genes. The functional analysis (gene ontology-analysis) and signal pathway analysis (pathway-analysis) of differentially expressed genes were performed using the DAVID and KEGG databases, respectively. The pathogenic gene regulatory network was constructed by integrating transcriptional regulatory element database (TRED); the disease-related analysis of the pathogenic genes was conducted using the DAVID annotation tool. Five pathogenic genes (*Nos2*, *Il5*, *Colla1*, *Tnf*, and *Nfkb1*) and their transcription factors (Jun, Cebpa, Egrl, Ppara, and Il6) were found to suppress the host immune function and enhance the pathogenicity of *Helicobacter pylori* by regulating the host immune system.
*Conclusions:* This effect was largely mediated via three signaling pathways: Tnf pathway, PI3K Akt pathway, and Jak–STAT pathway. The pathogenicity of *Helicobacter pylori* is closely related to the body's immune and inflammatory system. A better understanding of the correlation of the pathogenic factors with the host immune and inflammatory factors may help to determine the precise pathogenic mechanism of *H. pylori* infection.
**How to cite:** Bai Y, Li W, Xu G, et al. A bioinformatics approach revealed the transcription factors of Helicobacter pylori pathogenic genes and their regulatory network nodes. Electron J Biotechnol 2020;45. https://doi.org/10.1016/j.ejbt.2020.04.002.

## 1. Introduction

*Helicobacter pylori* is a chronic pathogenic bacterium with a high rate of infection throughout the world. The organism has been implicated in the causation of chronic gastritis, peptic ulcer, gastric mucosa-associated lymphoid tissue (GALT) lymphoma, and gastric cancer [1,2]. Following infection, further damage to the gastric mucosa may be caused through various host-related (inflammation, impaired immunity, gastric acid secretion, and oxidative stress) and pathogen-related factors (virulence factor, cytokines, and free radical virulence genes) [3,4,5]. Thus, a single gene research model cannot explain the pathogenicity of *H. pylori* and may not be suitable for research on *H. pylori* infection-related diseases. Therefore, revealing the pathogenic genes along with their mutual network of regulation is a key imperative.

Transcription factor regulation network (TFRN) refers to the network formed by the interaction between mRNA and transcription factors in the cells [6]. This network fully reflects the interaction between all kinds of metabolites (genes, RNA, and protein) and the biochemical pathways involved in the pathogenesis of *H. pylori* infection [7]. Transcription factors perform different biological roles such as the regulation of gene expression via multiple regulatory pathways at multiple sites and network central sites, where the coupled genes may serve as potential therapeutic targets [8,9]. Thus, building a *H. pylori* pathogenic gene TFRN may facilitate a comprehensive understanding of its pathogenic mechanisms and help

* Corresponding author.
  *E-mail address:* baiyu218@163.com (Y. Bai).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

**Table 1**
*Helicobacter pylori* chip data.

| Dataset ID | Sample ID | Sample number | Control Sample number | Disease Sample number | Platfroms | Submission date | Manufacturer |
|---|---|---|---|---|---|---|---|
| GSE13873 | GSM349127–GSM349142 | 16 | 2 | 14 | GPL1261 | Dec 08, 2008 | Affymetrix |
| GSE16440 | GSM411428- GSM411439 | 18 | 12 | 6 | GPL1261 | Jun 04, 2009 | Affymetrix |
| | GSM411458- GSM411463 | | | | | | |
| GSE10262 | GSM259177- GSM259194 | 18 | 6 | 12 | GPL1261 | Jan 24, 2008 | Affymetrix |
| GSE21833 | GSM543406- GSM543411 | 6 | 3 | 3 | GPL1261 | May 14, 2010 | Affymetrix |

to identify potential therapeutic targets. This approach may lay a foundation for further research on the early clinical diagnosis of related diseases such as gastric cancer and gastritis, and the development of novel targeted therapies.

In the present study, we identified the differentially expressed pathogenic genes of *H. pylori* by screening GEO Datasets of NCBI database, the DAVID database (Database for Annotation, Visualization and Integrated Discovery), and the KEGG database (Kyoto Encyclopedia of Genes and Genomes). These genes were applied to the gene ontology (GO) and pathway-analysis. Further, the TRED (transcriptional regulatory elements database) was integrated and the pathogenic gene regulatory network of this bacterium was built based on the differential gene expression set. Analysis of the correlation of differentially expressed genes with the associated diseases was conducted online using the DAVID annotation tool, which revealed the related signaling pathways. Finally, the role of pathogenic factors in the regulation of immune system was investigated by analyzing the central nodes of TFRNs and the inter-relationship among the central nodes.

## 2. Methods

### 2.1. Gene chip array data

The gene chip array data were retrieved from GEO Datasets of the National Center for Biotechnology Information (NCBI, USA) database using keywords "*H. pylori*". Data from Affymetrix gene chips from mouse infected with *H. pylori* and with the original CEL documents available were selected.

We chose gene expression profile of GSE13873 from GEO database, which is a publically available open-access database; GSE16440, which was based on Affymetrix GPL1261 and GPL6402 platform. The GSE10262 dataset included 18 samples (12 *H. pylori* samples and 6 healthy samples). We also downloaded the Series Matrix File of GSE21833 from the GEO database (Table 1).

### 2.2. Chip data processing

The Affymetrix Expression Console software tool was used for the background correction of the collected chip data and the conversion of probe fluorescence values into gene expression values. The Affymetrix Transcriptome Analysis Console was used for the logarithmic calculation and standardization of the chip data. The difference in mRNA expression between the chips in healthy people and those infected with *H. pylori* was compared by SAM, and the genes that exhibited a fold change of >2.0 or <-2.0 and p-value <0.05 were selected as significantly differentially expressed genes. These genes were further screened by Venn diagram (by considering the differences between the chip platforms); genes that overlapped with ≥3 three platforms were selected.

### 2.3. Construction of Transcription Factor (TF) mRNA gene network

Following the microarray data analysis and a search conducted on the Transcriptional Regulatory Element Database (TRED), we obtained some TFs and their target genes [10]. The TFs type we chose were experimentally validated. The networks were constructed using the

Cytoscape software. The yellow triangles in the TF-gene network represent transcription factors and the green circles represent target genes. The TFs and their target genes are joined by dotted lines with arrows indicating the direction from the source to the target.

### 2.4. Gene function annotation analysis

We used DAVID [11] and KEGG [12] to conduct the function analysis of the selected differential genes and pathway classification, respectively.

The DAVID database (http://david.abcc.ncifcrf.gov/) was opened, and 1213 genes were presented as the gene set of further analysis. At the same time, the corresponding gene identifier (the gene identifier corresponding to the gene name was OFFICIAL_GENE_SYMBOL) was selected; the whole genome of mice was ticked as the background gene, and "functional annotation tool" was then selected as the analytical tool, through which the results of GO and biological pathway enrichment analysis of differentially expressed genes could be obtained.

The KEGG pathway database (http://ftp.genome.jp/pub/kegg/) contains a collection of manually curated pathway maps representing the molecular interaction, reaction, and relation networks for genomes, biological pathways, diseases, drugs, and chemical substances. Based on KEGG databases, pathway analysis was conducted to reveal significant pathways from the differential expression of genes. Fisher's exact test was used to choose the significant pathways, and P ≤ 0.05 was considered as the significance threshold.

### 2.5. Network node statistics

The degree of network of each gene was calculated through the STRING [13] website, while the degree of relationship between TFs
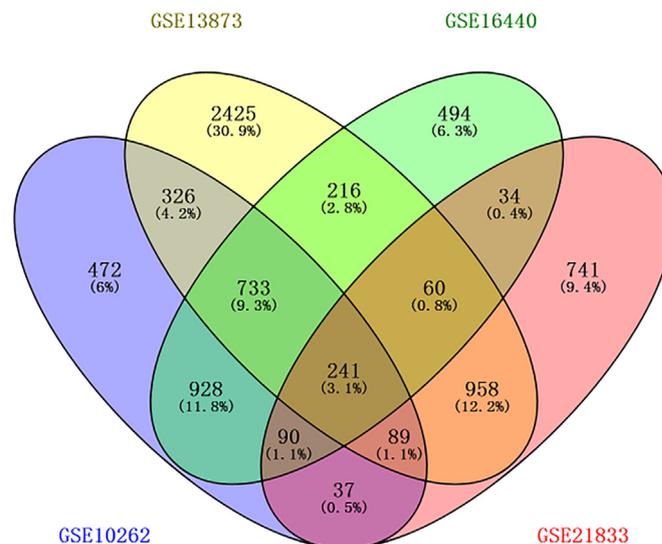


**Fig. 1.** Screening of differentially expressed genes of *Helicobacter pylori* in the four platforms. The blue, yellow, green, and pink colors represent the GSE10262, GSE13873, GSE16440, and GSE21833 platforms, respectively.

**Table 2**

Eighteen transcription factors of *Helicobacter pylori* pathogenic genes and their corresponding target genes.

| TF | Target gene No. | Gene ID | Description |
|---|---|---|---|
| Jun | 120 | 16476 | jun proto-oncogene |
| Cebpa | 46 | 12606 | CCAAT/enhancer binding protein (C/EBP), alpha |
| Egr1 | 31 | 13653 | early growth response 1 |
| Ppara | 31 | 19013 | peroxisome proliferator activated receptor alpha |
| Il6 | 29 | 16193 | interleukin 6 |
| F3 | 25 | 14066 | coagulation factor III |
| Stat1 | 21 | 20846 | signal transducer and activator of transcription 1 |
| Bcl6 | 12 | 12053 | B cell leukemia/lymphoma 6 |
| Junb | 10 | 16477 | jun B proto-oncogene |
| Egr2 | 8 | 13654 | early growth response 2 |
| Dbp | 6 | 13170 | D site albumin promoter binding protein |
| Adh7 | 5 | 11529 | alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide |
| Cebpg | 4 | 12611 | CCAAT/enhancer binding protein (C/EBP), gamma |
| F5 | 3 | 14067 | coagulation factor V |
| Bcl3 | 3 | 12051 | B cell leukemia/lymphoma 3 |
| Ltf | 3 | 17002 | lactotransferrin |
| Arc | 3 | 11838 | activity regulated cytoskeletal-associated protein |
| Tst | 2 | 22117 | thiosulfate sulfurtransferase, mitochondrial |

and target genes was assessed using the cytoscape software. The gene signal transduction network was constructed based on the data of differentially expressed genes to show the core genes that played an important role in this network. The degree of each gene was added to obtain the number of network nodes. A higher degree indicated that the genes have a strong correlation with other genes, implying a more important role in the signaling network. We used the limma R package (version:3.36.5) and then obtained many differentially expressed genes (DEGs). We screened out key genes in the huge DEGs. The screening methods are diverse. The visualization of network was built by software Cytoscape.

## 3. Results

### 3.1. Chip data

The chip data were obtained from GEO DataSets NCBI Database. The Affymetrix chips with *H. pylori* and CEL original documents were selected, and ultimately four gene chip platforms (GSE13873, GSE16440, GSE10262, and GSE21833) were used for further analysis (Table 1). The data of each chip were divided into two groups: the normal and the pathogenic group.

### 3.2. Chip data processing

Transcriptome Analysis Console from Affymetrix Company was applied for the logarithmic calculation and standardization of the obtained data from the four chips. The differentially expressed mRNA between normal mice and those infected with *H. pylori* were compared using the SAM method; we obtained 241 differentially expressed genes in all the four platforms, 1213 in ≥3 platforms, and 3352 in ≥2 platforms (Fig. 1).
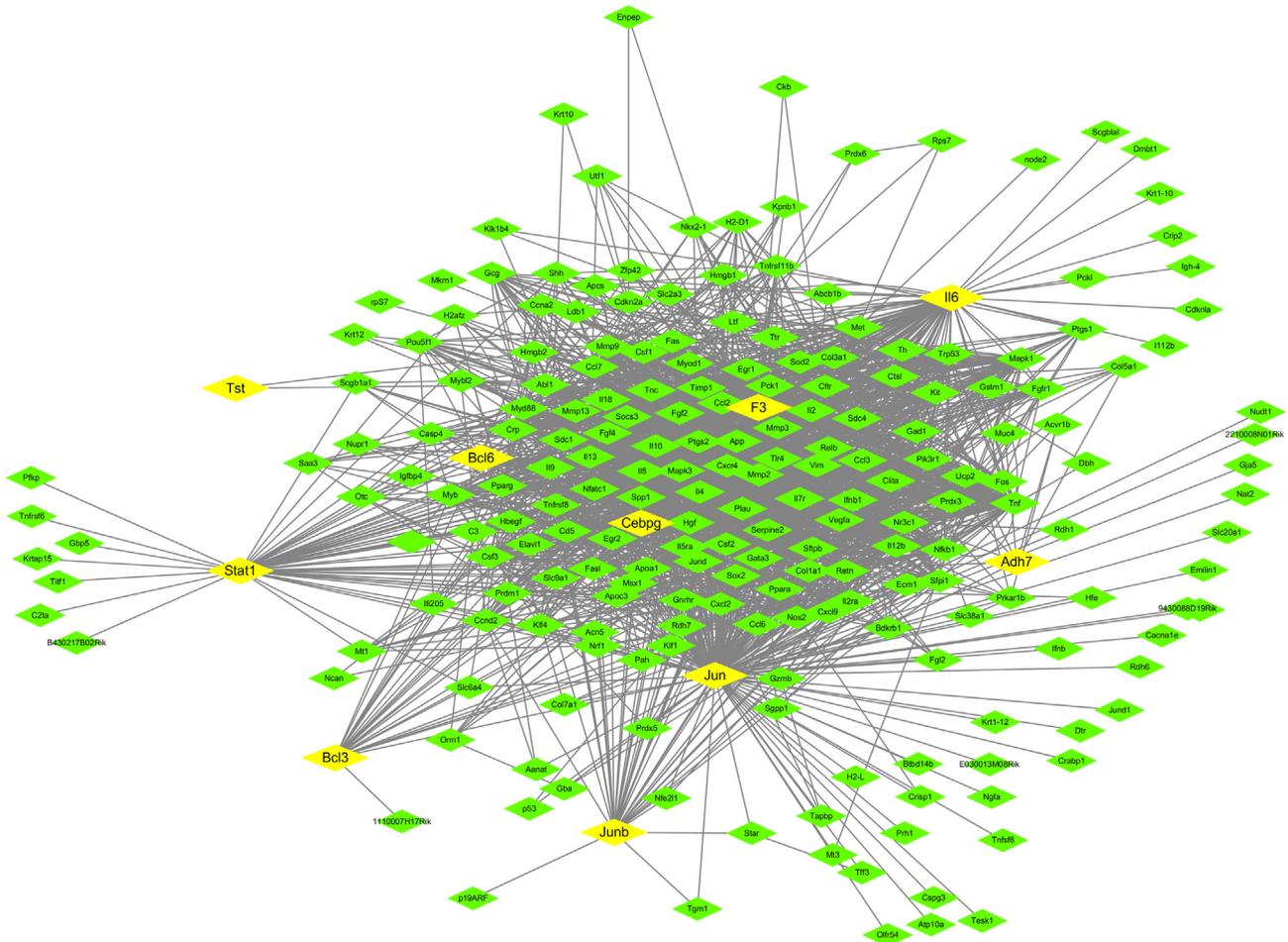


**Fig. 2.** Transcription factor regulatory network of *Helicobacter pylori* pathogenic genes. The yellow represents the transcription factors and the green represents the target gene corresponding to the transcription factors.

As the differentially expressed genes found in ≥3 platforms should have more significant difference, 1213 differential genes were cross-screened and were used for further analysis.

### 3.3. H. pylori transcription factor regulatory network

The TRED was used for the predictive analysis of the transcription factors of 1213 differentially expressed genes. It revealed 18 transcription factors and their corresponding 232 target genes (Table 2). In addition, the TFRN of the pathogenic genes with 18 transcription factors and their corresponding 232 target genes was drawn using Cytoscape software (Fig. 2).

Twenty-three target genes regulated by more than two transcription factors were found in this network (Table 3), of which the 3 target genes (namely *Nos2*, *Il5*, and *Col1a1*) were regulated by more number of transcription factors (3 transcription factors).

The TFRN contained 22 genes at more than 50 network nodes. Among these, the genes with the greater number of network nodes were *Jun* (216 nodes), *Il6* (131 nodes), and *Tnf* (96 nodes).

### 3.4. GO functional annotation analysis

GO functional annotation analysis of 1213 differentially expressed genes was conducted, in which 10 pathways with the most significant *P* values were selected (Table 5); these were closely associated with immune diseases. Among these four pathways, inflammatory bowel diseases (IBD), TNF signaling, PI3K-Akt signaling, and rheumatoid arthritis pathways were the most significant.

## 4. Discussion

*H. pylori* is recognized as the primary causative agent of several diseases of the digestive system, such as chronic active gastritis, gastroduodenal ulcer, GALT lymphoma, and gastric cancer. Hence, the eradication of *H. pylori* is an important step for the prevention and treatment of these diseases. In this study, NCBI GEO DataSets containing the expression arrays of normal mice and those infected with *H. pylori* were selected. Further, the Transcriptome Analysis Console of the Affymetrix Company was used for the logarithmic calculation and standardization of data obtained from these expression arrays; the results revealed 1213 differentially expressed pathogenic genes. Subsequently, the predictive analysis on the 1213 genes was conducted through the TRDE database and 18 transcription factors and 232 corresponding target genes were found. Thus, we could establish a transcription factor regulatory network of *H. pylori* pathogenic genes (Fig. 2). A series of analysis on the obtained TFRN revealed that several key factors among these were closely related to the mice immune system.

The TFRN (Fig. 2) contained 5 transcription factors (Table 2), namely: Jun (120 target genes), Cebpa (46 target genes), Egr1 (31 target genes), Ppara (31 target genes), and Il6 (29 target genes). The *Jun* gene is closely related to systemic lupus erythematosus (SLE), which is a typical autoimmune disease involving multiple organs and systems. Doniz-Padilla et al found that the expression level of *Jun* in the peripheral blood mononuclear cells (PBMC) of SLE patients was significantly higher than that in healthy controls [14], which suggests that *Jun* may be involved in the pathogenesis of SLE. The *Cebpa* gene is closely related to acute myeloid leukemia and is currently one of the diagnostic markers of acute myeloid leukemia [15]. *Egr1* is remotely expressed or its expression is very low in normal somatic cells [16]. Stimulation of cells by a variety of extracellular signals can activate the transcription of *Egr1* through one or more signal transduction pathways. The binding of activated Egr1 to the specific binding sites on the promoter of target gene may regulate the function of cells to exert various biological effects [17]. Egr1 plays an important role in the regulation of cell growth, differentiation, and proliferation, and in

**Table 3**
Target genes regulated by more than two transcription factors.

| Target genes | Number of transcription factors | Transcription factors | Network nodes |
|---|---|---|---|
| Nos2 | 3 | Stat1 Jun Il6 | |
| Il5 | 3 | Bcl6 Jun Junb | |
| Col1a1 | 3 | Il6 Jun Adh7 | |
| Ptgs2 | 2 | Il6 Jun | 68 |
| Ptgs1 | 2 | Jun Il6 | |
| Tnfrsf8 | 2 | Jun Stat1 | |
| Nfkb1 | 2 | Bcl6 Bcl3 | 74 |
| Il10 | 2 | Cebpg Il6 | 75 |
| Tnf | 2 | Bcl3 Jun | 96 |
| Pparg | 2 | Stat1 Il6 | |
| Il4 | 2 | Junb Jun | 74 |
| Il12b | 2 | Jun Junb | |
| Nrf1 | 2 | Jun Junb | |
| Alb1 | 2 | Tst F3 | |
| Mmp13 | 2 | Il6 Jun | |
| p53 | 2 | Il6 Jun | |
| Col5a1 | 2 | Jun Adh7 | |
| Tgm1 | 2 | Jun Junb | |
| Met | 2 | Il6 Jun | |
| Nfe2l1 | 2 | Jun Junb | |
| Spp1 | 2 | Jun F3 | |
| Hmgb2 | 2 | F3 Tst | |
| Cxcl9 | 2 | Stat1 F3 | |

the initiation and amplification of inflammatory reaction [18]. The *Ppara* gene was shown to be involved in the pathogenesis of peripheral neuropathy in type 2 diabetes mellitus [19]. Overexpression of RAGE in the endoneurial and epineurial microvasculature and its binding to the sedimentary carboxymethyl cellulose was shown to activate NF-KB and increase the release of inflammatory cytokines such as interleukin 6 [20]. The resultant inflammatory reaction may interfere with the normal function of blood vessels and lead to vasculitic neuropathy. Interleukin 6 can act on multiple cells, such as B cells, liver cells, hybridoma cells and plasma cells, and strengthen the body's defense through its pro-inflammatory activity and influence other cytokines [21,22,23]. To sum up, these five transcription factors that can regulate their target genes were all related to immune inflammation. In addition, Il6, Egr1, and Jun are directly involved in immune inflammation.

**Table 4**
Network node statistics table.

| Gene | Network node | Number of transcription factors | Number of corresponding target genes |
|---|---|---|---|
| Jun | 216 | | 120 |
| Il6 | 131 | | 29 |
| Tnf | 96 | 2 | |
| Trp53 | 81 | | |
| Stat1 | 80 | | 21 |
| Fos | 78 | | |
| Il10 | 75 | 2 | |
| Nfkb1 | 74 | 2 | |
| Il4 | 74 | 2 | |
| Il2 | 70 | | |
| Csf2 | 68 | | |
| Ptgs2 | 68 | 2 | |
| Ccl2 | 66 | | |
| Mmp9 | 66 | | |
| Vegfa | 59 | | |
| Fgf2 | 57 | | |
| Tlr4 | 57 | | |
| Networkk1 | 56 | | |
| Kit | 56 | | |
| Pik3r1 | 51 | | |
| Hgf | 51 | | |
| F3 | 50 | | 25 |

**Table 5**
GO functional annotation table of *Helicobacter pylori* pathogenic genes.

| Pathway | Gene NO. | P-value | Benjamini |
|---|---|---|---|
| Cytokine-cytokine receptor interaction | 26 | 4.8E-15 | 8.9E-13 |
| Leishmaniasis | 15 | 5.2E-13 | 4.8E-11 |
| Inflammatory bowel disease (IBD) | 14 | 8.2E-12 | 5.1E-10 |
| TNF signaling pathway | 17 | 1.0E-11 | 4.7E-10 |
| PI3K-Akt signaling pathway | 26 | 3.4E-11 | 1.3E-9 |
| Malaria | 12 | 3.9E-10 | 1.2E-8 |
| Jak–STAT signaling pathway | 17 | 5.5E-10 | 1.5E-8 |
| Chagas disease (American trypanosomiasis) | 15 | 1.2E-9 | 2.8E-8 |
| Pertussis | 13 | 1.3E-9 | 2.8E-8 |
| Hematopoietic cell lineage | 13 | 4.0E-9 | 7.4E-8 |
| Rheumatoid arthritis | 13 | 9.3E-9 | 1.6E-7 |

Further, we found that the target genes regulated by the TFRN were *Nos2*, *Il5*, and *Col1a1* (Table 3), and these were regulated by more than three transcription factors. Nos2 and Il5 have been shown to be closely related to the immune system. Nitric oxide synthase (NOS) is an isozyme with three subtypes: Nos1, Nos2, and Nos3, corresponding to nNos, iNos, and eNos, respectively [24,25]. Nitric oxide synthase 2 (NOS2) plays a role after stimulation and activation of cells, resulting in the production of a large amount of NO. Studies have shown that NOS2 plays an important role in chronic neurodegenerative diseases, inflammation, obstructive lesions, tumors, transplantation/implantation, injury, and parasitic infection [25]. IL-5 [also referred to as the T cell replacement factor (TRF)] has been found to have no significant role in the stimulation of B cells [26]. Its main function is to stimulate the proliferation, differentiation, and activation of eosinophils [27]. IL-5 not only increases the number of eosinophils but also enhances their function. The increase in eosinophils during worm infestation and allergic diseases is mainly caused by IL-5. Human IL-5 can also promote the release of inflammatory mediators such as histamine and leukotriene by basophils, which improves the activity of basophils [28]. To date, Col1a1 has only been found to be associated with bone injury-related diseases, and there is no direct experimental evidence to show its correlation with immune inflammation. Hence it could be speculated that the Col1a1 gene may be a potential inflammation factor, which needs to be further studied [29].

Although *Nfkb1* is regulated by only two transcription factors, it has 74 network nodes, which suggests that it could be a very important regulatory factor. *Nfkb1* gene is a member of Nf-κB family. The Nf-κB family is a widespread transcription factor and can specifically bind to the κB sites on the gene promoters or enhancers such as several cytokines and cell adhesion factors required for the regulation of immune response, inflammation, cell growth, differentiation, cell adhesion, and apoptosis. Being a transcription factor, it initiates and regulates the transcription of these genes, which play an important role in the immune response and inflammation as well as in cell growth and development [30].

We counted the network nodes in the TFRN and found 3 genes at more than 90 nodes, namely *Jun*, *il6*, and *Tnf* (Table 4). As discussed
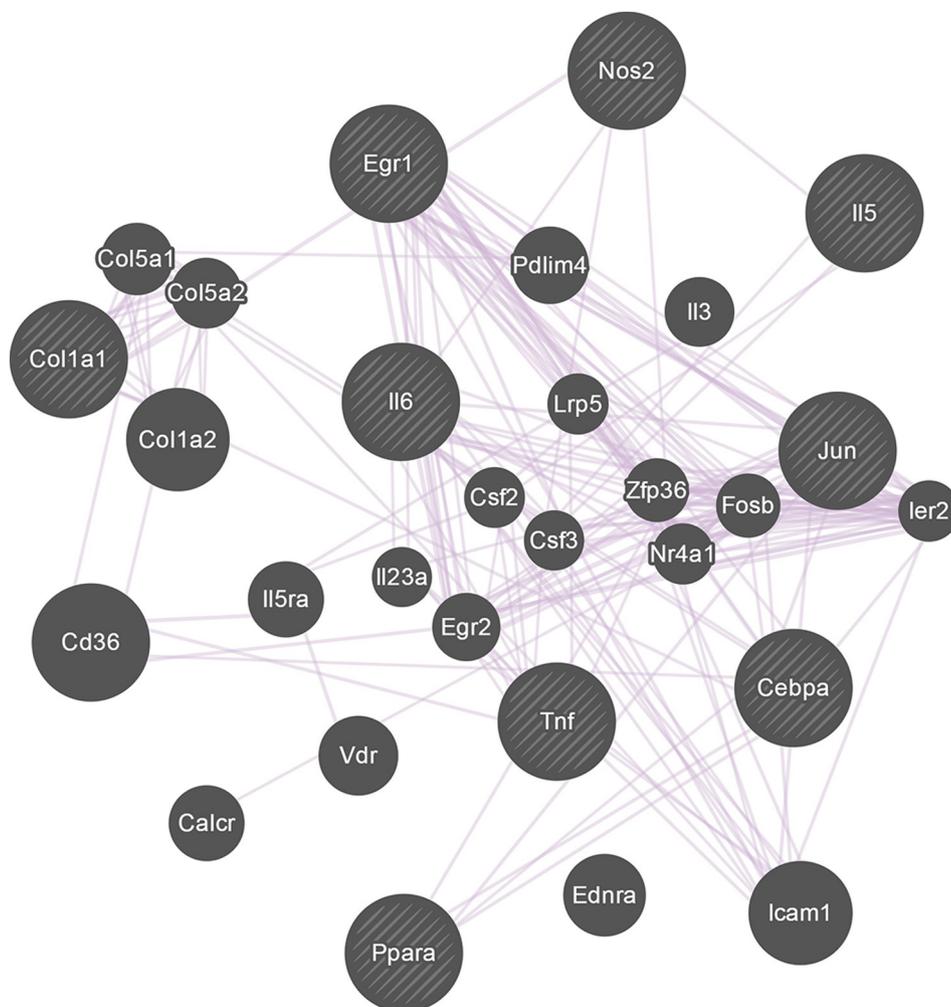


**Fig. 3.** Regulatory network of the 10 important pathogenic genes. The large circles represent 10 important pathogenic genes and small circles represent other genes related to these pathogenic genes.

earlier, both Jun and Il6 are transcription factors. Inflammation factor (Tnf), the most important inflammatory mediator that first appears in the process of inflammation, can activate neutrophils and lymphocytes, increase the permeability of vascular endothelial cells, regulate the metabolic activity of other tissues, and stimulate the synthesis and release of other cytokines [31].

We obtained significant results in the form of five transcription factors, namely Jun, Cebpa, Egrl, Ppara, and Il6 (which regulated most of the target genes); three target genes, namely *Nos2, Il5,* and *Colla1* (which were regulated by most of the transcription factors); three genes, namely *Jun*, *Il6,* and *Tnf* (found at most of the network nodes); and the *Nfkb1* gene. Thus, we used a total of 10 genes for the establishment and analysis of the network. The results showed that these genes were closely related to Csf2, Csf3, and Egr2 and that these could regulate each other. Four genes (*Tnf, Jun, Il6,* and *Egrl*) were in the central position of regulation (Fig. 3). In addition, these genes were also found to be involved in the regulation of immune system and centered in some signaling pathways, such as the Jak–STAT signaling pathway.

In this study, the screened 1213 genes were analyzed using GO functional annotation. The results showed that there were 10 related pathways that mainly included three signaling pathways (TNF signaling pathway, PI3K Akt signaling pathway, and Jak–STAT signaling pathway). The TNF signaling pathway may indirectly regulate inflammatory reaction and promote apoptosis through interaction with other cytokines or effector cells; in addition, both Tnf and Il6 genes are associated wth this pathway. PI3K signaling plays a key role in regulating the growth of cells by regulating the downstream molecular mTOR. Significant mutations of key regulatory factors in the PI3K signaling pathway have been identified in a majority of human tumors. Multiple genes are closely related to the PI3K signaling pathway; for example, the *Pik3r1* gene is also an important network node (Table 4). The Jak–STAT signaling pathway, a signal transduction pathway stimulated by cytokines, is involved in many important biological processes such as the proliferation, differentiation and apoptosis of cells and immune regulation. Our study showed that Stat1 was part of this pathway, and Stat1 was not only a transcription factor (Table 3) but also corresponded to 80 network nodes (Table 4); this suggests that it may play an important role in the regulation of the complete network [32].

## 5. Conclusion

In the present study, we identified 1213 differentially expressed genes and constructed the transcriptional factor regulatory network. Statistical analysis of transcription factors and nodes of the regulatory network indicated that the genes *Nos2, Il5, Colla1, Tnf,* and *Nfkb1* and the transcription factors Jun, Cebpa, Egrl, Ppara, and Il6 collectively regulate the body's immune function through three main signaling pathways: Tnf pathway, PI3K Akt pathway, and Jak–STAT pathway. The *Csf2, Csf3, and Egr2* genes were also involved in the coordinated regulation. Overall, our study revealed that the pathogenicity of *H. pylori* is closely related to the body's immune and inflammatory system.

## Data availability statement

All datasets collected by the authors from public database (GEO database) in this study are available at https://www.ncbi.nlm.nih.gov/gds/.

## Conflict of interest

The authors declare that they have no competing interests.

## References

[1] Sokic-Milutinovic A, Alempijevic T, Milosavljevic T. Role of *Helicobacter pylori* infection in gastric carcinogenesis: Current knowledge and future directions. World J Gastroenterol 2015;21(41):11654–72. https://doi.org/10.3748/wjg.v21.i41.11654 PMid: 26556993.

[2] Pawlowicz R, Panaszek B. [Pathogenic and clinical significance of Helicobacter pylori infection in chronic urticaria and angioedema] (in Polish). Pol Merkur Lekarski 2004;17(100):399–402. [PMid: 15690714].

[3] Kersulyte D, Mukhopadhyay AK, Velapatiño B, et al. Differences in genotypes of *Helicobacter pylori* from different human populations. J Bacteriol 2000;182(11):3210–8. https://doi.org/10.1128/JB.182.11.3210-3218.2000 PMid: 10809702.

[4] Pejin B, Ciric A, Glamoclija J, et al. *In vitro* anti-quorum sensing activity of phytol. Nat Prod Res 2015;29(4):374–7. https://doi.org/10.1080/14786419.2014.945088 PMid: 25103916.

[5] Pejin B, Savic A, Sokovic M, et al. Further *in vitro* evaluation of antiradical and antimicrobial activities of phytol. Nat Prod Res 2014;28(6):372–6. https://doi.org/10.1080/14786419.2013.869692 PMid: 24422895.

[6] Pajares M, Cuadrado A, Rojo AI. Modulation of proteostasis by transcription factor NRF2 and impact in neurodegenerative diseases. Redox Biol 2017;11:543–53. https://doi.org/10.1016/j.redox.2017.01.006 PMid: 28104575.

[7] Jin HF, Dai JF, Meng LN, et al. *Curcuma wenyujin* Y. H. Chen et C. Ling n-Butyl alcohol extract inhibits AGS cell *Helicobacter pylori*CagA+VacA+ promoted invasiveness by down-regulating caudal type homeobox transcription factor and claudin-2 expression. Chin J Integr Med 2020;26:122–9. https://doi.org/10.1007/s11655-017-2958-y PMid: 28819779.

[8] Melzer R, Theissen G. MADS and more: Transcription factors that shape the plant. In: Yuan L, Perry S, editors. Plant transcription factors, 754. Methods in Molecular Biology (Methods and Protocols), Humana Press Methods; 2011. p. 3–18. https://doi.org/10.1007/978-1-61779-154-3_1 PMid: 21720944.

[9] Zhang Y, Yu J, Lee C, et al. Genomic binding and regulation of gene expression by the thyroid carcinoma-associated PAX8-PPARG fusion protein. Oncotarget 2015;6(38):40418–32. https://doi.org/10.18632/oncotarget.6340.

[10] Zhao F, Xuan Z, Liu L, et al. TRED: A transcriptional regulatory element database and a platform for *in silico* gene regulation studies. Nucleic Acids Res 2005;33(Suppl1):D103–7. https://doi.org/10.1093/nar/gki004 PMid: 15608156.

[11] Huang DW, Sherman BT, Tan Q, et al. The DAVID gene functional classification tool: a novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol 2007;8(9):R183. https://doi.org/10.1186/gb-2007-8-9-r183 PMid: 17784955.

[12] Xu G, Ni Z, Shi Y, et al. Screening essential genes of *Mycobacterium tuberculosis* with the pathway enrichment method. Mol Biol Rep 2014;41(11):7639–44. https://doi.org/10.1007/s11033-014-3654-z PMid: 25098602.

[13] Szklarczyk D, Morris JH, Cook H, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 2016;45(D1):D362–8. https://doi.org/10.1093/nar/gkw937 PMid: 27924014.

[14] Doniz-Padilla L, Martínez-Jimenez V, Niño-Moreno P, et al. Expression and function of Cbl-b in T cells from patients with systemic lupus erythematosus, and detection of the 2126 A/G *Cblb* gene polymorphism in the Mexican mestizo population. Lupus 2011;20(6):628–35. https://doi.org/10.1177/0961203310394896 PMid: 21558139.

[15] Wang H, Chu TT, Han SY, et al. *FLT3*-ITD and *CEBPA* mutations predict prognosis in acute myeloid leukemia irrespective of hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2018;25(5):941–8. https://doi.org/10.1016/j.bbmt.2018.11.031 PMid: 30503388.

[16] Huh S, Song HR, Jeong GR, et al. Suppression of the ERK-SRF axis facilitates somatic cell reprogramming. Exp Mol Med 2018;50(2):e448. https://doi.org/10.1038/emm.2017.279 PMid: 29472703.

[17] Boyle KB, Hadaschik D, Virtue S, et al. The transcription factors Egr1 and Egr2 have opposing influences on adipocyte differentiation. Cell Death Differ 2009;16(5):782–9. https://doi.org/10.1038/cdd.2009.11 PMid: 19229250.

[18] Palladino MA, Fasano GA, Patel D, et al. Effects of lipopolysaccharide-induced inflammation on hypoxia and inflammatory gene expression pathways of the rat testis. Basic Clin Androl 2018;28:14. https://doi.org/10.1186/s12610-018-0079-x PMid: 30473791.

[19] Yilmaz-Aydogan H, Kurnaz O, Kucukhuseyin O, et al. Different effects of PPARA, PPARG and ApoE SNPs on serum lipids in patients with coronary heart disease based on the presence of diabetes. Gene 2013;523(1):20–6. https://doi.org/10.1016/j.gene.2013.03.136 PMid: 23583468.

[20] Mizwicki MT, Fiala M, Magpantay L, et al. Tocilizumab attenuates inflammation in ALS patients through inhibition of IL6 receptor signaling. Am J Neurodegener Dis 2012;1(3):305–15. [PMid: 23383400].

[21] Song XF, Hu TT, Lei Y, et al. Activation of intrahepatic CD4$^+$CXCR5$^+$ T and CD19$^+$ B cells is associated with viral clearance in a mouse model of acute hepatitis B virus infection. Oncotarget 2016;7(32):50952–62. https://doi.org/10.18632/oncotarget.10688.

[22] Lv H, Pan Z, Hu S, et al. [Relationship between CD4 + CD25 + Treg cells, Th17 cells and IL-6 and the prognosis of hepatitis B virus-related acute-on-chronic liver failure: A meta-analysis] (in Chinese). Zhonghua Gan Zang Bing Za Zhi 2014;22(7):493–8. [PMid: 25203799].

[23] Behnke K, Zhuang Y, Xu HC, et al. B cell-mediated maintenance of cluster of differentiation 169-positive cells is critical for liver regeneration. Hepatology 2018;68(6): 2348–61. https://doi.org/10.1002/hep.30088 PMid: 29742809.

[24] Mungrue IN, Husain M, Stewart DJ. The role of NOS in heart failure: Lessons from murine genetic models. Heart Fail Rev 2002;7(4):407–22. https://doi.org/10.1023/A:1020762401408 PMid: 12379825.

[25] Jeong YH, Kim Y, Song H, et al. Anti-inflammatory effects of α-galactosylceramide analogs in activated microglia: involvement of the p38 MAPK signaling pathway. PLoS One 2014;9(2):e87030. https://doi.org/10.1371/journal.pone.0087030 PMid: 24523867.

[26] Takatsu K, Tominaga A, Harada N, et al. T cell-replacing factor (TRF)/interleukin 5 (IL-5): Molecular and functional properties. Immunol Rev 1988;102:107–35.

[27] Mizel SB. The interleukins. FASEB J 1989;3(12):2379–88. https://doi.org/10.1096/fasebj.3.12.2676681 PMid: 2676681.

[28] Sá-Nunes A, Rogerio AP, Medeiros AI, et al. Modulation of eosinophil generation and migration by *Mangifera indica* L. extract (Vimang). Int Immunopharmacol 2006;6 (9):1515–23. https://doi.org/10.1016/j.intimp.2006.04.008 PMid: 16846846.

[29] Chen Z, Xue J, Shen T, et al. Curcumin alleviates glucocorticoid-induced osteoporosis through the regulation of the Wnt signaling pathway. Int J Mol Med 2015;37(2): 329–38. https://doi.org/10.3892/ijmm.2015.2432 PMid: 26677102.

[30] Bottex-Gauthier C, Pollet S, Favier A, et al. [The Rel/NF-kappa-B transcription factors: Complex role in cell regulation](in French). Pathol Biol (Paris) 2002;50(3):204–11. https://doi.org/10.1016/S0369-8114(02)00289-4.

[31] Fraser SD, Sadofsky LR, Kaye PM, et al. Reduced expression of monocyte CD200R is associated with enhanced proinflammatory cytokine production in sarcoidosis. Sci Rep 2016;6:38689. https://doi.org/10.1038/srep38689 PMid: 27929051.

[32] Park JW, Lee HS, Lim Y, et al. *Rhododendron album* Blume extract inhibits TNF-α/IFN-γ-induced chemokine production via blockade of NF-κB and JAK/STAT activation in human epidermal keratinocytes. Int J Mol Med 2018;41(6):3642–52. https://doi.org/10.3892/ijmm.2018.3556.