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Helicobacter pylori infection increases the risk of carotid plaque formation: Clinical samples combined with bioinformatics analysis

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ABSTRACT

Objective: Infection with *Helicobacter pylori* (*H. pylori*) may increase atherosclerosis, which can lead to carotid plaque formation. Our study examined the relationship between *H. pylori* infection and carotid plaque formation, and its underlying mechanisms.
Methods: A total of 36,470 people who underwent physical examination in Taizhou Hospital Health Examination Center from June 2017 to June 2022 were included in this study. All people participated in the urease test, neck ultrasound, blood pressure detection, anthropometric measurement and biochemical laboratory examination. In addition, the GSE27411 and GSE28829 datasets in the Gene Expression Omnibus (GEO) database were used to analyze the mechanism of *H. pylori* infection and atherosclerosis progression.
Results: H. pylori infection, sex, age, blood lipids, blood pressure, fasting blood glucose, glycated hemoglobin and body mass index were risk factors for carotid plaque formation. An independent risk factor was still evident in the multivariate logistic regression analysis, indicating *H. pylori* infection. Furthermore, after weighted gene coexpression network analysis (WGCNA), we discovered 555 genes linked to both *H. pylori* infection and the advancement of atherosclerosis. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses revealed a strong correlation between these genes and immunity, infection, and immune disorders. SsGSEA analysis showed that *H. pylori* infection and atherosclerosis included changes in the immune microenvironment. Finally, three genes MS4A6A, ADAMDEC1 and AQP9 were identified to be involved in the formation of atherosclerosis after *H. pylori* infection. Conclusion: Our research affirms that *H. pylori* is a unique contributor to the formation of carotid plaque, examines the immune microenvironment associated with *H. pylori* infection and advanced carotid atherosclerosis, and offers fresh perspectives on how *H. pylori* infection leads to atherosclerosis.

1. Introduction

The prevalence of *Helicobacter pylori* (*H. pylori*) infection is extensive on a global scale, impacting around 50% of the adult populace. This infection mainly resides in the gastrointestinal system and is notably more prevalent in developing countries [1]. It can colonize the mucosal surface and glands of the human stomach, produce a variety of adhesins and deliver virulence factors, which lead to changes in host signaling pathways and induce proinflammatory responses [2]. *H. pylori* has a close relationship with peptic ulcers,

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chronic gastritis, and even gastric cancer [3,4]. Increasing evidence indicates that infection with *H. pylori* is linked to diseases outside the stomach, including liver diseases, metabolic disorders, respiratory disorders, cardiovascular conditions, and even hyperemesis gravidarum [5–7].

Atherosclerosis is a pathological state marked by inflammation in the walls of arteries, resulting from abnormal lipid metabolism within the body [8]. It causes intimal thickening, arteriosclerosis and lumen stenosis, as well as pathological plaque formation [9]. Infection with *H. pylori* could potentially enhance the secretion of cytotoxin related gene A (Cag A), and further promote atherosclerosis and plaque formation through inflammatory reactions and immune responses [10,11]. In addition, *H. pylori* infection may aggravate disorders of lipid and lipoprotein metabolism and further aggravate atherosclerosis [10]. Nevertheless, the primary focus of these investigations was the examination of clinical data, with limited exploration of the genetic aspect regarding the association between *H. pylori* infection and carotid plaque formation.

The advancement of bioinformatics analysis technology using microarrays in recent times has facilitated a deeper comprehension of the genetic aspects of diseases [12]. Weighted gene coexpression network analysis (WGCNA), a technique for identifying crucial genes, involves partitioning genes into distinct modules based on their similarity in expression. It helps facilitate a systematic understanding of the function of genes at the molecular level [13,14]. In this research, our objective was to examine the association between *H. pylori* and carotid plaque formation, while also exploring the underlying mechanism using clinical samples and genomic data obtained from publicly available databases.

2. Materials and methods

2.1. Data collection

Individuals who received physical examinations at Taizhou Hospital's health examination center from June 2017 to June 2022 were included as participants in this study. Patients were included according to the following criteria: patients with complete data on age, sex, laboratory parameters, urea breath test results, blood pressure, body mass index (BMI) and neck ultrasound data. Laboratory parameters included triglycerides (TGs), low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), fasting blood glucose (FBG), and glycated hemoglobin A1c (HbA1c). Patients with thyroid function problems, gastrointestinal surgery, malignancies, pregnancy, minors or incomplete clinical information were excluded from this research. A total of 36,470 patients were retrospectively analyzed as part of this study. The Ethics Committee of Taizhou Hospital (K20220790) has granted approval for this study. This study was a retrospective analysis of clinical data obtained during the course of the study, and the written informed consent requirement was waived by the ethics committee of Taizhou Hospital. In addition, we obtained datasets GSE27411 and GSE28829 from the Gene Expression Omnibus (GEO) database. The GSE27411 dataset included transcriptome sequencing data of 6 H. pylori positive patients and 6 H. pylori negative patients, and the GSE28829 dataset contained transcriptome sequencing data of 13 early carotid atherosclerotic (EA) plaque samples and 16 advanced carotid atherosclerotic (AA) plaque samples.

2.2. Measurement of carotid plaque

The subjects were instructed to lie in a low occipital supine position, with their heads tilted back and inclined to the non-examination side to fully expose the neck. The intima-media thickness (IMT) in the neck vessels was measured by an experienced sonographer using a color Doppler ultrasound instrument. Plaques were defined as distinct areas with an IMT >50% of that of surrounding areas.

2.3. Collection of clinical indicators

Trained nurses gathered information regarding the patient's age and sex, and performed measurements of height and weight. Additionally, sitting diastolic blood pressure (DBP) and systolic blood pressure (SBP) were recorded. After a period of 8 h of fasting during the night, the individuals undergoing the physical examination had their venous blood samples taken and then sent to Taizhou Hospital's laboratory for additional analysis. The laboratory testing involved the use of an automatic biochemical analyzer to determine parameters such as TG, TC, HDL, LDL, and FBG. The level of HbA1c was measured using a glycosylated hemoglobin analyzer.

2.3.1. Identifying Helicobacter pylori

The detection of *H. pylori* was established through the utilization of either a ^{13}C or ^{14}C urease breath examination. The process for the ^{13}C breath test involved the following steps: (a) collecting the first breath sample from the patient while they were fasting, (b) ingesting ^{13}C labeled capsules with warm boiled water, (c) waiting for a duration of 30 min, (d) gathering respiratory samples subsequent to the ingestion of the medication, and (e) analyzing the two samples using an instrument. The process for the ^{14}C breath test consisted of the following steps: (a) taking a ^{14}C urea capsule orally, (b) following by a wait for 15–20 min, (c) exhaling evenly into the device for 3 min, and (d) placing a collection bottle into the apparatus to extract the results.

2.3.2. Building the network of coexpression

The WGCNA package in R was utilized to conduct coexpression network analysis [15]. WGCNA aims to discover the relationship between gene modules and phenotypes. The hierarchical clustering tree is constructed using the correlation matrix, which consists of

correlation coefficients between gene pairs, and is based on Pearson coefficients. Different branches on the tree represent distinct gene modules, with each module assigned a specific color. The heatmap displays the outcomes of the correlation analysis between gene modules and disease traits. The module demonstrating the highest correlation with the traits of interest is then selected for further analysis.

2.4. Analysis of functional enrichment

The Gene Ontology (GO) project serves as an extensive source for functional genomics. GO annotations are categorized into three categories, namely, molecular function (MF), biological process (BP) and cellular component (CC) [16]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) is an extensive repository that combines genomic, chemical, and systemic functional data [17]. In particular, the KEGG pathway functions as a storage for genetic pathway data across various organisms. To clarify the biological roles of the target genes, we performed enrichment analyses for both GO and KEGG.

2.4.1. Gene differential expression analysis

The R package "limma" was utilized to detect dissimilarly expressed mRNAs among distinct groups [18]. The screening threshold was established as $|\log2\text{-fold change (FC)}| > 1$ and $P < 0.05$.

2.4.2. Estimating the abundance of immune cells

Single sample gene set enrichment analysis (ssGSEA) was used to quantitatively estimate the infiltration of 28 immune cell types [19]. The relative infiltration abundance of immune cells in different groups was estimated using ssGSEA algorithm in the GSVA package of R software.

2.5. Statistical analysis

The *t*-test was used to express data on continuous variables that followed a normal distribution as the mean ± standard deviation (SD). Chi-square tests were used to express categorical variables as counts and percentages. After adjusting for confounding factors by multicollinearity tests, the relationship between *H. pylori* infection and carotid plaque formation was analyzed by multivariate logistic regression. The correlation between gene expression and immune cells was tested by the Spearman correlation test. Statistical analysis was performed in SPSS software (Windows Version 25.0, Armonk, NY, USA), and R (4.1.2) software, and $P < 0.05$ was considered significant.

3. Result

3.1. Clinical analysis

Table 1 displayed the demographic and laboratory features of all populations. Univariate analysis showed that gender, age, TC, HDL, LDL, *H. pylori* infection, DBP, SDP, FBG, HbA1c and BMI were risk factors for carotid plaque (Table 2). In the multicollinearity test, lipids, blood pressure values, FBG, and HbA1c were filtered out, and these variables were not included in the adjusted analysis except for lipids judged according to clinical significance, Table 3.

3.1.1. WGCNA analysis

The GSE27411 and GSE28829 date were analyzed by WGCNA to build a coexpression network, and the genes associated with

Table 1
Baseline characteristics of all physical examination populations.

Variables	*H. pylori*-negative (n = 22718)	*H. pylori*-positive (n = 13752)	P value
Gender (n, %)			0.022
Female	7606 (33.5)	4444 (32.3)	
Male	15112 (66.5)	9308 (67.7)	
Age (year)	51.25 ± 9.57	52.20 ± 9.25	<0.001
Triglycerides (mmol/L)	1.95 ± 1.68	2.01 ± 1.73	0.004
Total cholesterol (mmol/L)	5.14 ± 0.98	5.13 ± 0.97	0.631
High density lipoprotein (mmol/L)	1.40 ± 0.32	1.39 ± 0.31	<0.001
Low density lipoprotein (mmol/L)	2.83 ± 0.75	2.79 ± 0.74	<0.001
Arterial plaque (n, %)			<0.001
No	14589 (64.2)	8572 (62.3)	
Yes	8129 (35.8)	5180 (37.7)	
Diastolic blood pressure (mmHg)	77.38 ± 11.72	78.01 ± 11.85	<0.001
Systolic blood pressure (mmHg)	128.40 ± 17.54	129.42 ± 18.12	<0.001
Fasting blood glucose (mmol/L)	5.59 ± 1.55	5.65 ± 1.70	<0.001
Glycated hemoglobin A1c (%)	5.93 ± 0.94	6.01 ± 1.03	<0.001
Body mass index (kg/m^2)	24.65 ± 3.15	24.83 ± 3.11	<0.001

Table 2
Univariate analysis of risk factors for carotid plaque.

Variables	OR (95%CI)	P value
Male (%)	1.579 (1.507–1.655)	<0.001
Age (>50)	5.372 (5.117–5.640)	<0.001
Triglycerides (mmol/L)	1.012 (1.000–1.025)	0.060
Total cholesterol (mmol/L)	1.216 (1.190–1.243)	<0.001
High density lipoprotein (mmol/L)	0.879 (0.821–0.941)	<0.001
Low density lipoprotein (mmol/L)	1.394 (1.354–1.435)	<0.001
H. pylori (+)	1.085 (1.038–1.133)	<0.001
Diastolic blood pressure (mmHg)	1.025 (1.024–1.027)	<0.001
Systolic blood pressure (mmHg)	1.031 (1.029–1.032)	<0.001
Fasting blood glucose (mmol/L)	1.244 (1.225–1.263)	<0.001
Glycated hemoglobin A1c (%)	1.555 (1.516–1.595)	<0.001
Body mass index (kg/m^2)	1.041 (1.034–1.048)	<0.001

Table 3
Multivariate logistic analysis of risk factors for carotid plaque.

Variables	OR (95%CI)	P value
Male (%)	1.659 (1.573–1.749)	<0.001
Age (>50)	5.57 (5.298–5.855)	<0.001
Triglycerides (mmol/L)	1.042 (1.015–1.070)	0.002
Total cholesterol (mmol/L)	0.831 (0.756–0.914)	<0.001
Low density lipoprotein (mmol/L)	1.678 (1.505–1.870)	<0.001
Body mass index (kg/m^2)	1.017 (1.009–1.025)	<0.001
H. pylori (+)	1.055 (1.006–1.107)	0.029

H. pylori infection and arterial plaque formation were identified. In GSE27411, the optimal soft threshold was set to 9 to construct a scale-free network (Fig. 1A). Using the dynamic clustering tree cutting approach, a grand total of 16 modules were identified (Fig. 1C). Among them, the black module gene was most significantly associated with *H. pylori* infection, including 1097 genes, with a correlation coefficient of 0.9, $P < 0.01$ (Fig. 1E). Similarly, in the GSE28829 dataset, the optimal soft threshold was set to 14 (Fig. 1B), and 11 modules were identified (Fig. 1D). Among them, the turquoise module genes were significantly correlated with plaque progression, including 555 genes, with a correlation coefficient of 0.83, $P < 0.01$ (Fig. 1F).

3.1.2. Enrichment analysis

Gene enrichment analysis was performed on the module genes that were significantly related to *H. pylori* infection and plaque progression (Fig. 2A). GO enrichment analysis indicated a strong association between these genes and immune regulation, immune activation of various cells, extracellular matrix formation and immune receptor activity (Fig. 2B). Fig. 2C showed that the KEGG enrichment analysis revealed a strong correlation between these genes and both bacterial infection as well as various immune disorders.

Identification of genes between differentially expressed genes and coexpression modules.

According to the screening threshold, in the GSE27411 dataset, a total of 67 differential genes were screened for differential expression in normal samples and *H. pylori* infected samples. Out of these genes, 11 were found to be downregulated while 56 were upregulated (Fig. 3A). In the dataset GSE28829, there were 177 genes that showed differential expression between EA and AA. Among these genes, 23 were found to be downregulated while 154 were upregulated, as depicted in Fig. 3B. The overlapping genes between the differentially expressed genes and the coexpression module genes were assumed to be the hub genes, and three hub genes, MS4A6A, ADAMDEC1 and AQP9, were identified (Fig. 3C). The expression of these genes was significantly elevated in patients infected with *H. pylori* and patients diagnosed with AA (Fig. 4A–B).

3.1.3. Immune infiltration analysis

Based on the GSE27411 dataset, we used the ssGSEA method to compare 28 immune cell subsets in *H. pylori* infected and -uninfected patients. We found that there were differences in 10 immune cells, encompassing activated dendritic cells, activated CD4 T cells and gamma delta T cells (Fig. 5A–B). In the GSE28829 dataset, we observed significant differences in the expression of 23 immune cells, including activated CD8 T cells, activated CD4 T cells and activated B cells (Fig. 5C–D). Among them, 8 types of immune cells, including activated CD4 T cells, activated dendritic cells, gamma delta T cells, immature dendritic cells, myeloid-derived suppressor cells (MDSCs), macrophages, monocytes, and regulatory T cells, were differentially expressed in *H. pylori* infection and plaque progression.

3.1.4. The correlation between hub genes and immune infiltration

We used the Spearman correlation test to compare the relationship between hub genes and various immune cells during *H. pylori* infection and plaque progression. In the GSE27411 dataset, MS4A6A, ADAMDEC1 and AQP9 were significantly correlated with a

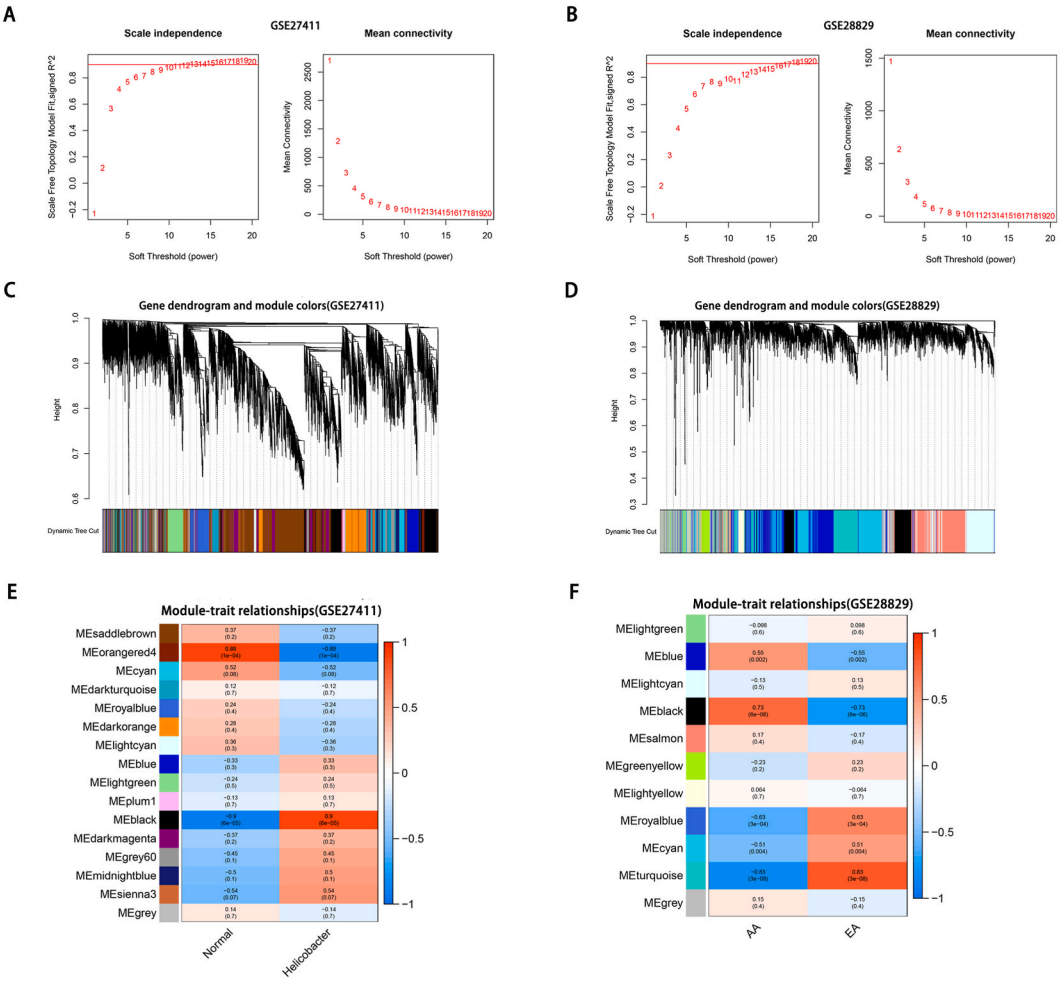

Fig. 1. Construction of coexpression network of *H. pylori* infection and arterial plaque progression. (A, B) Selection of the optimal soft threshold in GSE27411 and GSE28829 datasets. (C, D) Division of different modules in GSE27411 and GSE28829 datasets. (E, F) Correlation between modules and diseases in GSE27411 and GSE28829 datasets.

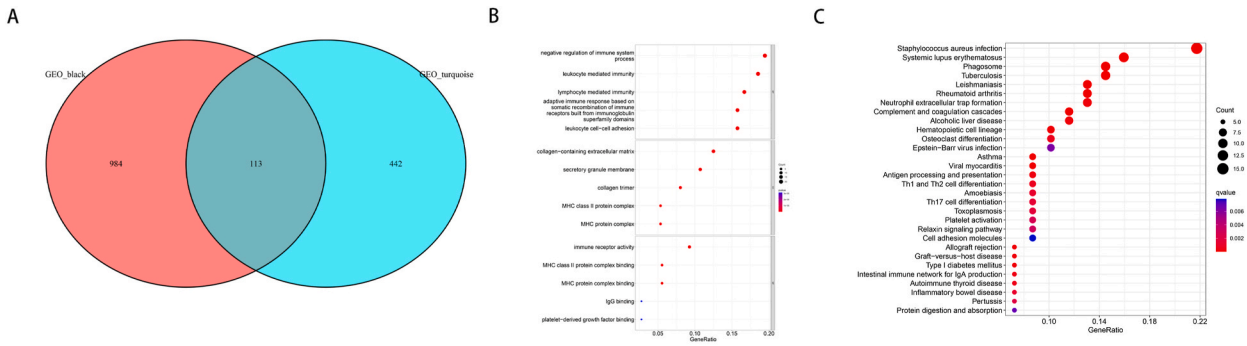

Fig. 2. Enrichment analysis of genes related to *H. pylori* infection and plaque progression. (A) Intersection gene of black module and turquoise module. (B) Go enrichment analysis of intersection genes. (C) KEGG enrichment analysis of intersection genes.

variety of immune cells, among which neutrophils were significantly correlated with the three hub genes (Fig. 6A). Similarly, in the GSE28829 dataset, MS4A6A, ADAMDEC1 and AQP9 were also related to a variety of immune cells, among which MDSCs and hub genes were closely related (Fig. 6B). In the process of *H. pylori* infection and plaque progression, ADAMDEC1 was significantly correlated with differentially expressed activated CD4 T cells, and AQP9 was closely correlated with differentially expressed regulatory

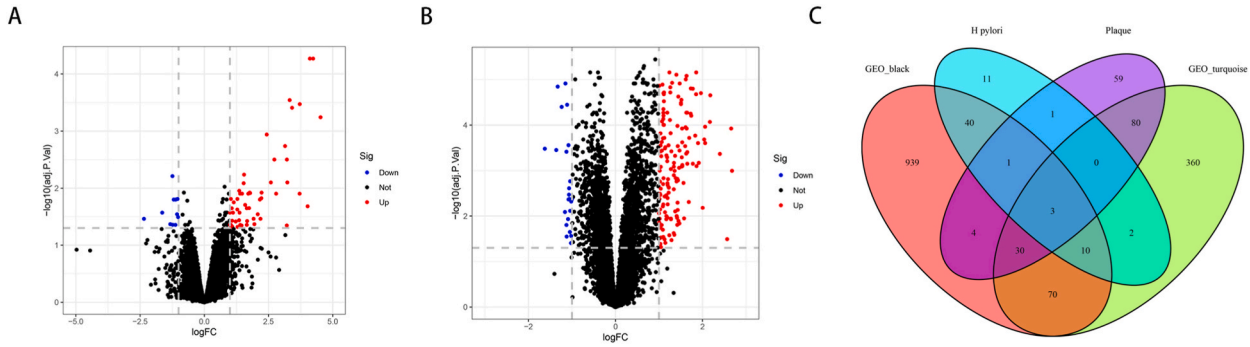

Fig. 3. Identification of differential genes and hub genes. (A) Differential genes in GSE27411 dataset. (B) Differential genes in GSE28829 dataset. (C) Overlapping genes of modular genes and differential genes.

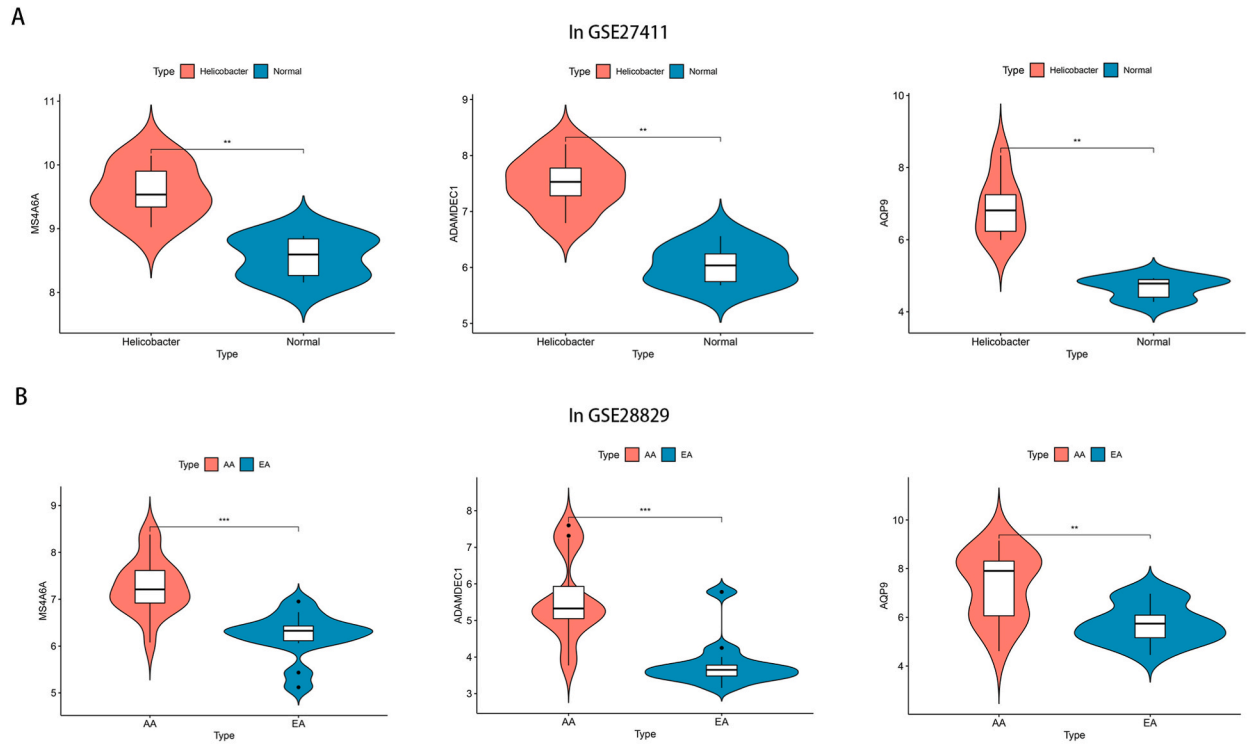

Fig. 4. Expression of hub gene in *H. pylori* infected patients and patients with advanced plaque formation. (A) Expression of hub gene in GSE27411 dataset. (B) Expression of hub gene in GSE28829 dataset.

T cells and MDSCs.

4. Discussion

To date, an increasing number of studies have confirmed that *H. pylori* infection is closely related to cardiovascular events. According to reports, infection with *H. pylori* may elevate the likelihood of recurring cardiovascular incidents in individuals diagnosed with acute coronary syndrome (ACS) [20]. In a meta-analysis involving 230,288 participants, it was discovered that the presence of *H. pylori* was associated with an elevated risk of cardiovascular disease [21]. In addition, infection with *H. pylori* can elevate the risk of cardiovascular events by 3–4 times [22]. However, the relationship between *H. pylori* and arterial plaque formation remains controversial. Some studies have verified that infection with *H. pylori* can elevate the likelihood of carotid atherosclerosis in individuals below the age of 50 [23]. In another study involving 14588 healthy people, *H. pylori* was not associated with increased carotid intima thickness [24]. However, in most studies, *H. pylori* infection was consistent with increased CIMT [25,26]. In our study, it was further confirmed that *H. pylori* infection increased the risk of carotid plaque formation.

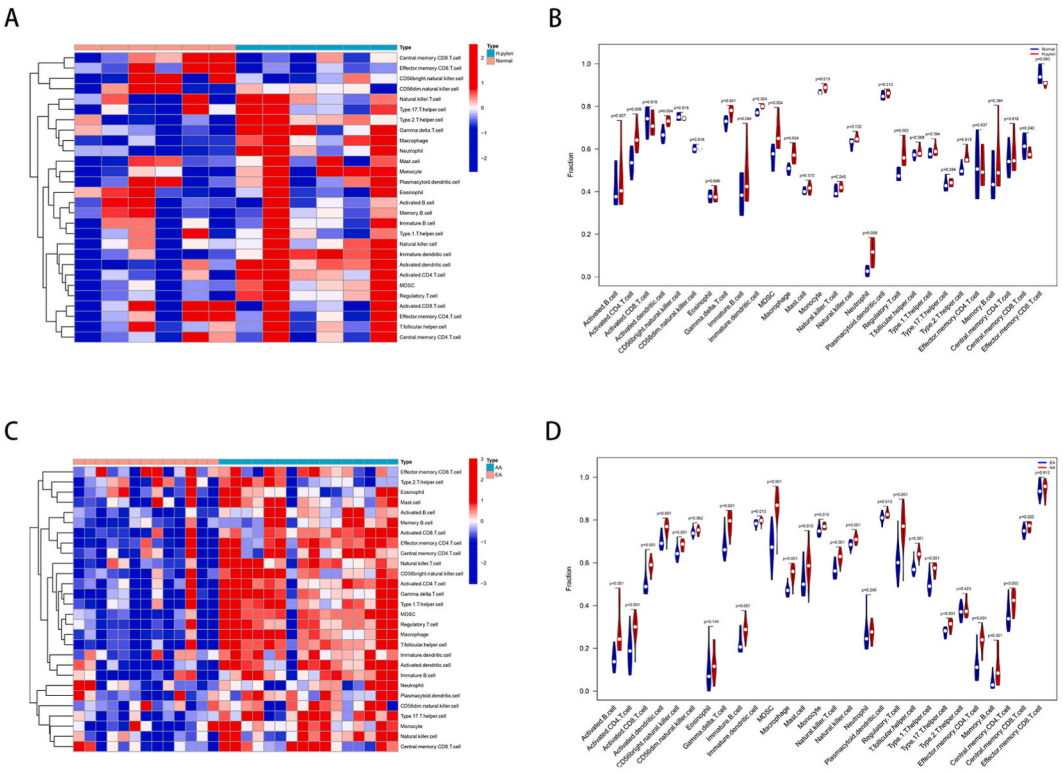

Fig. 5. Immune cell infiltration among different samples. (A) Heatmap of distribution of different immune cells in GSE27411 dataset. (B) Difference of immune cells between *H. pylori* infected patients and normal people. (C) Heatmap of distribution of different immune cells in GSE28829 dataset. (D) Difference of immune cells between EA patients and AA patients.

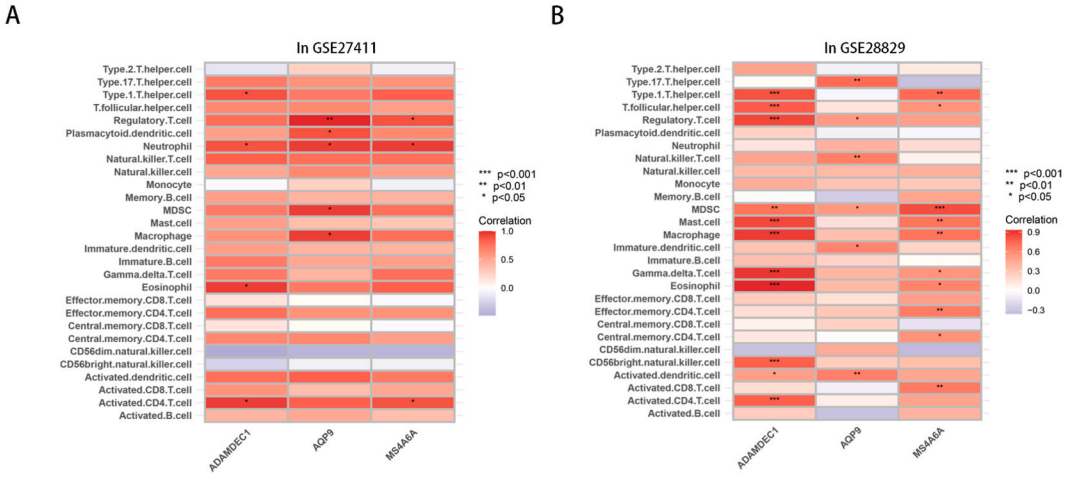

Fig. 6. Relationship between hub gene and immune cells. (A) Relationship between hub gene and immune cells in GSE27411 dataset. (B) Relationship between hub gene and immune cells in GSE28829 dataset.

Dyslipidemia, such as elevated TC and LDL, is an important risk factor for arterial plaque formation [27,28]. In our study, TC, LDL and *H. pylori* infection were observed as independent risk factors associated with the formation of carotid plaques. In a study of male subjects in Japan, *H. pylori* infection was significantly correlated with low HDL and high LDL [29]. Our study confirmed that in all populations, the HDL level of *H. pylori* infected patients was reduced, but LDL was also reduced. This result indicates that *H. pylori* infection may further affect the changes in lipid mass spectra [30].

Nevertheless, additional investigation is required to understand the process through which *H. pylori* infection contributes to the formation of atherosclerosis. Research has shown that *H. pylori* infection leads to chronic inflammation, triggering the release of

various chemical mediators, such as tumor necrosis factor (TNF-α) and a variety of interleukins [31,32]. The activation of these inflammatory agents can trigger inflammatory reactions, either directly or indirectly causing harm to the blood vessel lining, ultimately resulting in the development of atherosclerosis. Furthermore, *H. pylori* additionally triggers an immune response through the activation of cyclooxygenase-2 (COX-2), leading to an elevation in the synthesis of prostaglandins and nitric oxide (NO), consequently enhancing the likelihood of developing atherosclerosis [33]. Infection with *H. pylori* that is positive for CagA can enhance the development of foam cells and accelerate the growth of arterial plaques [34]. In our study, we found that *H. pylori* infection and advanced arteriosclerosis were related to immunity, inflammation and bacterial infection.

There are different immune microenvironments in patients with *H. pylori* infection and advanced arteriosclerosis. Infection with *H. pylori* can cause a variety of T-cell immune responses, such as gamma delta T cells, regulatory T cells, activated CD4 T cells [35–37]. Furthermore, the activation of macrophages, dendritic cells and B cells contributes to the production of proinflammatory cytokines and chemokines [38]. Likewise, in atherosclerosis, there are also a variety of T cells, macrophages, dendritic cells and monocytes, which may participate in the formation of atherosclerotic plaques [39–41]. The findings of our research were in line with this. We further screened three genes related to *H. pylori* infection and advanced atherosclerosis, MS4A6A, ADAMDEC1 and AQP9. In patients with Alzheimer's disease, MS4A6A was confirmed to be associated with dysregulation of lipid metabolism [42]. In addition, ADAMDEC1 has been reported to be associated with susceptibility to atherosclerosis [43]. Based on whole genome sequencing, AQP9 was confirmed as a candidate gene for atherosclerosis [44]. Similarly, these genes were related to immunity, inflammation and bacterial infection [45–47]. Nevertheless, additional verification is required to establish the connection between these genes and *H. pylori* infection.

Our research has certain limitations. This study was retrospective, and it is possible that there may be some bias. In addition, the immune microenvironment and genes analyzed by bioinformatics still require further exploration by experimental research. The study also lacked other risk factors that may influence carotid plaque, such as socioeconomic status, dietary intakes, obesity, and insulin resistance [48–50]. *H. pylori* infection may further influence risk factors such as insulin resistance and obesity [51]. In the future, more well-designed intervention trials will be conducted to further exclude the influence of confounding factors on the study.

5. Conclusion

The findings of our research validated that the presence of *H. pylori* increased the risk of carotid plaque formation. Furthermore, we further explored the potential mechanism by which *H. pylori* infection causes carotid plaques, and screened three genes that may be involved in its regulation. This study provides new insight into the advanced carotid atherosclerosis caused by *H. pylori* infection.

Funding

None.

Data availability statement

The public data set of this study can be found in the GEO database (https://www.ncbi.nlm.nih.gov/geo/) (Accessions: GSE27411, GSE28829). Other detailed data may be obtained from the corresponding author upon reasonable request.

Authorship

Jinshun Zhang: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data. Yi Chen: Performed the experiments, Analyzed and interpreted the data, Wrote the paper. Ningning You: Analyzed and interpreted the data, Wrote the paper. Chaoyu Yang: Wrote the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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