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Association between *Helicobacter pylori* infection and type 2 diabetes mellitus: a retrospective cohort study and bioinformatics analysis

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Abstract

Purpose This study aimed to preliminarily investigate the association and possible mechanisms between *Helicobacter. pylori* (*H. pylori*) infection and type 2 diabetes mellitus (T2DM) through data collection, statistical analysis, and bioinformatics analysis.

Methods A retrospective cohort study, including a total of 4406 participants who attended annual health checkups at Xian GEM Flower Changqing Hospital, was conducted to explore the correlation between the incidence of T2DM and *H. pylori* infection. To uncover the potential mechanisms underlying the interaction between the two diseases, differentially expressed genes (DEGs) common to T2DM and *H. pylori* infection were identified using the GEO database and Venn diagrams. These DEGs were then analyzed through Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment, and protein-protein interaction (PPI) analysis.

Results In total, 2053 participants were classified into the *H. pylori*-positive group and 2353 into the *H. pylori*-negative group. *H. pylori* infection was associated with a higher risk of T2DM occurrence (adjusted HR 1.59; 95% CI 1.17–2.15, $P=0.003$). The average disease-free survival time was 34.81 months (95% CI 34.60-35.03 months) in the *H. pylori* positive group and 35.42 months (95% CI 35.28–35.56 months) in the *H. pylori* negative group. Multivariate analysis and subgroup analyses also showed that *H. pylori* infection increased the risk of developing T2DM. A total of 21 DEGs between T2DM and *H. pylori* infection were identified and enriched in 7 signaling pathways, indicating specific protein interactions.

Conclusions The prevalence of T2DM was associated with *H. pylori* infection. T2DM and *H. pylori* infection may interact with each other through metabolic and immune pathways.

Keywords *Helicobacter pylori*, Type 2 diabetes mellitus, Retrospective cohort study, Bioinformatics

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Introduction

Diabetes mellitus, particularly Type 2 diabetes mellitus (T2DM), is a major global health crisis with serious implications for public health. The prevalence of T2DM has markedly increased in China, reflecting a broader international trend [1]. T2DM is associated with severe complications affecting multiple organs and systems, including the cardiovascular, neurological, and renal systems. This highlights the urgent need for effective strategies to identify risk factors and manage the disease.

Helicobacter pylori (*H. pylori*) infection is prevalent worldwide, with an estimated 50% of the global population affected [2]. The infection rate ranges from 28.0 to 73.3% in different regions of China [3, 4]. While *H. pylori* commonly is well-known for causing gastrointestinal disorders such as chronic gastritis, peptic ulcer, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma, its potential role in extra gastrointestinal conditions, including metabolic disorders like T2DM, is increasingly recognized [5–7].

Several studies have investigated the association between *H. pylori* infection and T2DM. The link between *H. pylori* and diabetes was first reported by Simon et al. [8] in 1989, who suggested a potential correlation between the two conditions. Subsequent research has shown that *H. pylori* infection significantly affects glucose metabolism and immune function in patients with T2DM. Specifically, *H. pylori* infection has been associated with altered glucose metabolism, impaired immune responses, and increased susceptibility to infection [9]. Studies by Yao et al. have demonstrated that the efficacy of standard *H. pylori* eradication therapy was significantly reduced in T2DM patients compared to non-T2DM patients [10]. Furthermore, T2DM patients with *H. pylori* infection experienced more gastrointestinal side effects from metformin, a common antidiabetic medication, compared to those without the infection [11]. Animal studies have provided additional insights into the underlying mechanisms. For instance, *H. pylori* infection in animal models has been shown to induce chronic inflammation and insulin resistance, both of which are key factors in the development of T2DM [12]. Despite these findings, the exact mechanism by which *H. pylori* influences T2DM are not well understood, and the interaction between these conditions remains controversial.

Existing research has largely focused on isolated aspects of this interaction, often within specific clinical contexts or populations. A comprehensive analysis integrating genetic, molecular, and bioinformatics data to elucidate the underlying mechanisms is lacking. Therefore, this study aims to address these gaps by investigating the association between T2DM and *H. pylori* infection through a multifaceted approach that includes data collection, statistical analysis, and bioinformatics. We focus on elucidating the specific genes and signaling pathways involved in this interaction, providing a basis for future mechanistic research. Our goal is to inform strategies for the prevention and treatment of T2DM, providing new insights into how *H. pylori* infection may impact disease management and patient outcomes.

Methods

Study population

We initially recruited a total of 9989 individuals who underwent annual health checkups at Xian GEM Flower Changqing Hospital from January 2019 to December 2021. The study protocol was approved by the Institutional Review Board. Written informed consent was obtained for participation in this study.

Exclusion criteria were as follows: (1) Individuals with severe hepatic and renal diseases or cardiovascular and cerebral vascular diseases; (2) Individuals with a recent history of major surgeries or traumatic injuries; (3) Patients with malignant tumors or autoimmune diseases; (4) Patients with other endocrine disorders.

After excluding those with missing data or pre-existing T2DM, data allowing for the determination of *H. pylori*, diabetes mellitus status, general information, and relevant laboratory tests were available for 6,859 participants. Further, excluding those who met the exclusion criteria or had reversed *H. pylori* exposure status during follow-up, 4406 individuals were selected as the final cohort (Fig. 1).

Study procedures

Participants underwent annual checkups at Xian GEM Flower Changqing Hospital for three years. General information collected included age, gender, weight, and height. Body mass index (BMI) was calculated as weight/height^2.

Laboratory tests included fasting blood glucose (FBG), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Blood samples were drawn from the median cubital vein early in the morning after a 12-hour fast and measured by the laboratory staff at Xian GEM Flower Changqing Hospital. Serum was separated by centrifugation at 3500 r/min for 10–15 min. FBG, TG, TC, HDL-C, and LDL-C levels were determined by an auto analyzer (AU5821, Beckmancoulter, USA). The standardization and quality control tests were performed before the assays, ensuring the results were within the specified range.

After 12 h of fasting, participants were required to take 45 mg of ^{13}C-urea (The Kit For 13 C-Urea Breath Test, Haiderun, Beijing, China) after providing an initial baseline breath sample. A second breath sample was collected after 15 min. ^{13}C-urea breath test analyzer (SHB-3000,

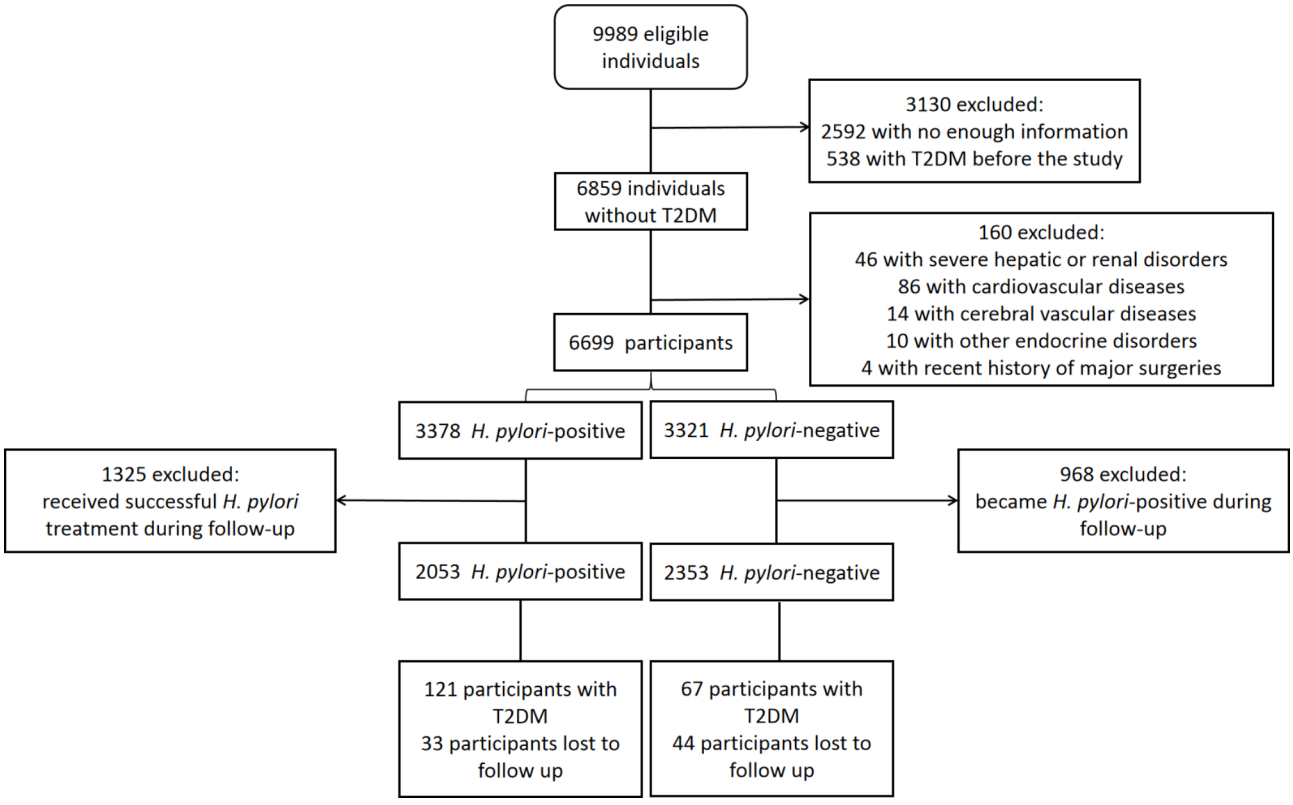

Fig. 1 Study flowchart of the retrospective cohort study. 9989 individuals who underwent annual health checkup from January 2019 to December 2021 were firstly recruited. After excluding those with missing data, T2DM before the study or meeting exclusion criteria, 4406 employees were selected as the final cohort. Abbreviations: *H. pylori*: *Helicobacter pylori*; T2DM: type 2 diabetes mellitus

Safe Heart, China) was used to detect the DOB value. DOB≥4.0 was applied to discriminate *H. pylori*-positive group from negative group. Participants who had used antibiotics, bismuth, or proton pump inhibitors within 1 month were excluded.

Outcome and definition of T2DM

Patients with T2DM met any of the following criteria: (1) the diagnostic criteria for T2DM established by the World Health Organization in 1999; (2) self-reported diagnosis of T2DM by a physician; (3) usage of medication for T2DM, specifically including but not limited to commonly prescribed anti-diabetic drugs such as metformin, sulfonylureas, insulin, DPP-4 inhibitors, SGLT2 inhibitors, and GLP-1 receptor agonists.

Statistical analysis

Data were analyzed using IBM SPSS Statistics 26.0 for Windows (IBM Corp., Armonk, NY, USA). Continuous variables were shown as mean±standard deviation (SD), and comparison between groups was made using the student's *t*-test. Categorical variables were expressed as number of cases (percentage) [*n*, (%)], and comparisons between groups were made using the chi-square test. Hazard ratio (HR) and corresponding 95% CI were determined using a Cox proportional hazard model. Univariate and multivariate analyses were performed to evaluate the impact of *H. pylori* infection on T2DM. Survival curves were generated using Kaplan-Meier estimates. Confounding variables considered in the multivariate analysis included age, gender, BMI, FBG, TG, TC, HDL-C, and LDL-C. The difference was considered statistically significant at two-sided $P<0.05$.

Bioinformatics analysis

Using "type 2 diabetes" and "*Helicobacter pylori*" as keywords, we searched for differentially expressed genes (DEGs) ($P<0.05$ and $|\mathrm{Log_2FoldChange}|\geq 1$) in T2DM and *H. pylori* infection in the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), and identified the genes shared by both diseases through Venn diagram. Two datasets were retrieved from the GEO database, including one gene expression profiles related to *H. pylor*i (GSE27411) and one dataset related to T2DM (GSE26168). For the GEO search, we used the following keyword combinations: "type 2 diabetes" AND "*Helicobacter pylori*". This syntax ensures that the search results include studies related to both T2DM and *H. pylori*.

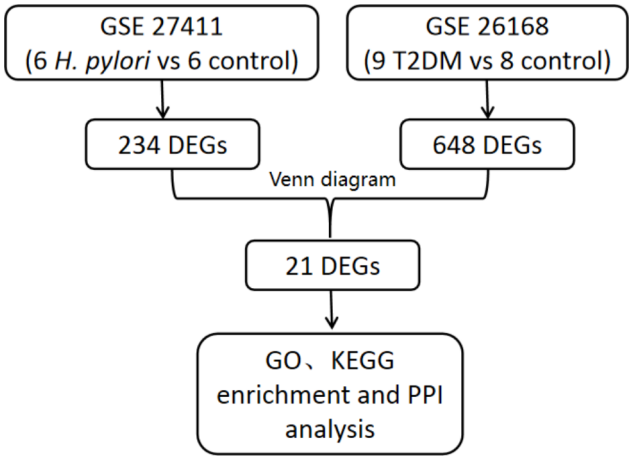

Fig. 2 Study flowchart of bioinformatics analysis of T2DM and *H. pylori* infection. Two categories of samples (T2DM and *H. pylori* infection) were collected from the GSE26168 dataset and GSE27411 dataset, respectively. The common DEGs of both datasets were identified. GO enrichment analysis, KEGG pathway analysis and PPI network construction were performed on the common DEGs. Abbreviations: *H. pylori*: *Helicobacter pylori*; T2DM: type 2 diabetes mellitus; DEG: differentially expressed gene; PPI: protein-protein interaction

Table 1 Baseline characteristics of the participants in the study

Variant	Group	H. pylori-positive	H. pylori-negative	t/χ^2	*P*-Value
Gender	Male	1330 (64.78)	1423 (60.48)	8.677	0.003
	Female	723 (35.22)	930 (39.52)		
Age/Years		48.85 ± 15.4	47.17 ± 15.47	3.60	<0.001
BMI/(kg/m^2)		24.38 ± 3.49	24.13 ± 3.52	2.35	0.019
FBG/(mmol/L)		5.43 ± 1.34	5.23 ± 0.93	5.66	<0.001
TG/(mmol/L)		1.71 ± 1.35	1.58 ± 1.13	3.35	0.001
TC/(mmol/L)		4.49 ± 0.88	4.42 ± 0.86	2.94	0.003
HDL-C/(mmol/L)		1.34 ± 0.3	1.35 ± 0.29	-1.02	0.308
LDL-C/(mmol/L)		2.8 ± 0.74	2.73 ± 0.74	3.11	0.002

Abbreviations: *H. pylori*: *Helicobacter pylori*; BMI: body mass index; FBG: fasting blood glucose; TG: triglycerides; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol

DAVID database was used to analyze the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment of the DEGs to determine the molecular functions, cellular components, biological processes and related signaling pathways. Additionally, the STRING database was used to analyze the protein-protein interaction (PPI) of the common DEGs to construct the protein-interaction network. In the STRING database, we used a specific analysis model with the following settings: a confidence score cutoff of 0.7 to ensure high-confidence interactions and an MCL clustering algorithm to identify significant clusters of interacting proteins.

The results of DEGs, GO and KEGG enrichment analysis were visualized using the online website (https://cloud.oebiotech.com/task/). The specific steps are shown in Fig. 2.

Results

Participants

After excluding the participants who received successful *H. pylori* treatment during the follow-up, 2053 participants were considered *H. pylori* positive, while 2353 were *H. pylori* negative. Patient demographics and baseline characteristics are shown in Table 1. More than half of the participants were male. Except for HDL-C, all the other variants were statistically different between the two groups ($P<0.05$). Therefore, we considered these variants as confounding variables in the subsequent multivariate analysis.

H. pylori infection was associated with T2DM occurrence

Participants in the *H. pylori* positive group had significantly shorter survival time compared with those in the *H. pylori* negative group considering T2DM occurrence (HR 2.08; 95% CI 1.55–2.81; log-rank $P<0.001$). Average disease free survival time was 34.81 months (95% CI 34.60-35.03 months) in the *H. pylori* positive group and 35.42 months (95% CI 35.28–35.57 months) in the *H. pylori* negative group (Fig. 3). Although the difference in average disease-free survival time between the groups was minimal, the HR value indicated that *H. pylori* infection significantly increased the risk of developing T2DM.

In multivariate analysis, after adjusting confounding variables such as age, gender, and BMI, the increased risk of T2DM associated with *H. pylori* infection remained significant (adjusted HR 1.59; 95% CI 1.17–2.15, $P=0.003$) (Table 2), suggesting that the association between H. pylori infection and T2DM was robust and not merely due to these confounders.

Furthermore, subgroup analyses used diagnostic thresholds to divide participants into different subgroups, which showed that *H. pylori* infection increased the risk of developing T2DM across different demographics and clinical parameters, reinforcing the primary finding (Table 3).

Bioinformatics analysis of *H. pylori* infection and T2DM

To reveal the underlying molecular mechanisms of the association between T2DM and *H. pylori* infection, we identified common DEGs and performed GO enrichment and KEGG pathway analysis. The GEO database showed 648 DEGs in T2DM, of which 355 were up-regulated and 293 were down-regulated (Fig. 4A). For *H. pylori* infection, 234 DEGs were identified, with 174 up-regulated

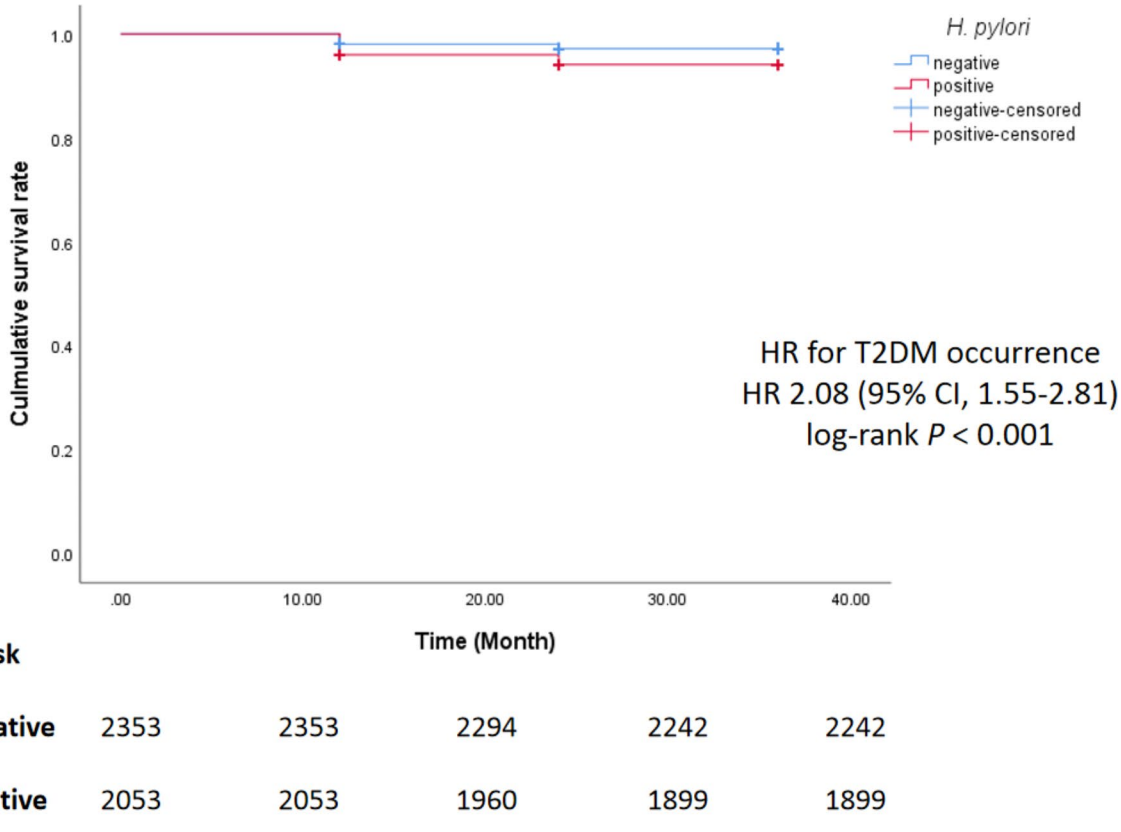

Fig. 3 Kaplan-Meier curve for time to T2DM non-occurrence in the two groups. Abbreviations: *H. pylori*: *Helicobacter pylori*; T2DM: type 2 diabetes mellitus; HR: hazard ratio

Table 2 Multivariate Cox regression analyses

Variant	B	S.E.	Wals	*P*-Value	Adjusted HR	95.0% CI	
						Lower Limit	Upper Limit
H.pylori infection	0.46	0.16	8.78	0.003	1.59	1.17	2.15
Gender	0.06	0.20	0.09	0.768	1.06	0.72	1.58
Age/Years	0.06	0.01	97.91	<0.001	1.06	1.05	1.07
BMI/(kg/m^2)	0.13	0.02	37.29	<0.001	1.14	1.10	1.19
FBG/(mmol/L)	0.31	0.02	330.36	<0.001	1.37	1.32	1.41
TG/(mmol/L)	0.09	0.06	2.60	0.107	1.10	0.98	1.23
TC/(mmol/L)	-0.32	0.26	1.56	0.211	0.73	0.44	1.20
HDL-C/(mmol/L)	0.18	0.34	0.26	0.608	1.19	0.61	2.32
LDL-C/(mmol/L)	0.24	0.27	0.78	0.378	1.27	0.75	2.16

Abbreviations: HR: hazard ratio; CI: confidence interval; *H. pylori*: *Helicobacter pylori*; BMI: body mass index; FBG: fasting blood glucose; TG: triglycerides; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol

and 60 down-regulated (Fig. 4B). The intersection of the DEGs from both diseases was determined using a Venn diagram, resulting in 21 common DEGs (Fig. 4C).

GO and KEGG enrichment analysis of the 21 common DEGs were performed using the DAVID database, and PPI analysis was constructed using the STRING database. In biological process (BP), these common genes were primarily involved in chemotaxis, chemokine-mediated signaling pathway, neutrophil chemotaxis, inflammatory response, signal transduction, immune response, killing of cells of other organism, antimicrobial humoral immune response mediated by antimicrobial peptide, and negative regulation of cysteine-type endopeptidase activity involved in the apoptotic signaling pathway. In cellular component (CC), these common genes were mostly enriched in the extracellular region, extracellular space, tertiary granule lumen, specific granule lumen, cell surface, and external side of plasma membrane. In molecular function (MF), these common genes were mainly involved in chemokine activity (Table 4; Fig. 5A).

KEGG analysis showed that these common DEGs were involved in viral protein interaction with cytokine and

Table 3 Subgroup Univariate Cox regression analyses

		N	HR	95.0% CI	Interaction *P*
Gender	Male	2753	2.18	1.55–3.06	0.034
	Female	1653	1.52	0.80–2.90	
Age/Years	<60	3138	3.01	1.62–5.60	<0.001
	≥60	1268	1.68	1.19–2.36	
BMI/(kg/m^2)	<24.00	2171	2.41	1.24–4.69	<0.001
	≥24.00	2235	1.95	1.39–2.72	
FBG/(mmol/L)	<6.11	3943	1.80	1.00-3.22	<0.001
	≥6.11	463	1.35	0.95–1.92	
TG/(mmol/L)	<1.70	2953	2.58	1.71–3.89	<0.001
	≥1.70	1453	1.51	0.97–2.34	
TC/(mmol/L)	<5.20	3598	1.78	1.27–2.48	<0.001
	≥5.20	808	3.89	1.87–8.11	
HDL-C/(mmol/L)	<1.29	2081	1.61	1.10–2.36	0.061
	≥1.29	2325	2.95	1.82–4.80	
LDL-C/(mmol/L)	<3.12	3099	2.07	1.43–3.01	<0.001
	≥3.12	1307	2.04	1.24–3.37	

Abbreviations: HR: hazard ratio; CI: confidence interval; BMI: body mass index; FBG: fasting blood glucose; TG: triglycerides; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol

cytokine receptor, IL-17 signaling pathway, cytokine-cytokine receptor interaction, chemokine signaling pathway, rheumatoid arthritis, TNF signaling pathway, and lipid and atherosclerosis signaling pathways (Table 5; Fig. 5B). The PPI network revealed extensive interactions among the proteins encoded by the 21 DEGs (Fig. 5C). These results suggested that inflammatory response and metabolism played a crucial role in the progression of T2DM and *H. pylori* infection.

Discussion

Several studies have investigated the correlation between T2DM and *H. pylori* infection, although the findings remain controversial. The link between diabetes and *H. pylori* was first reported by Simon et al. in 1989 [8]. Subsequent research, such as that by Chen et al. [13], identified a strong association between *H. pylori* infection and impaired glucose tolerance, particularly in individuals with elevated BMI. Other studies have supported these findings, noting that *H. pylori* infection is associated with

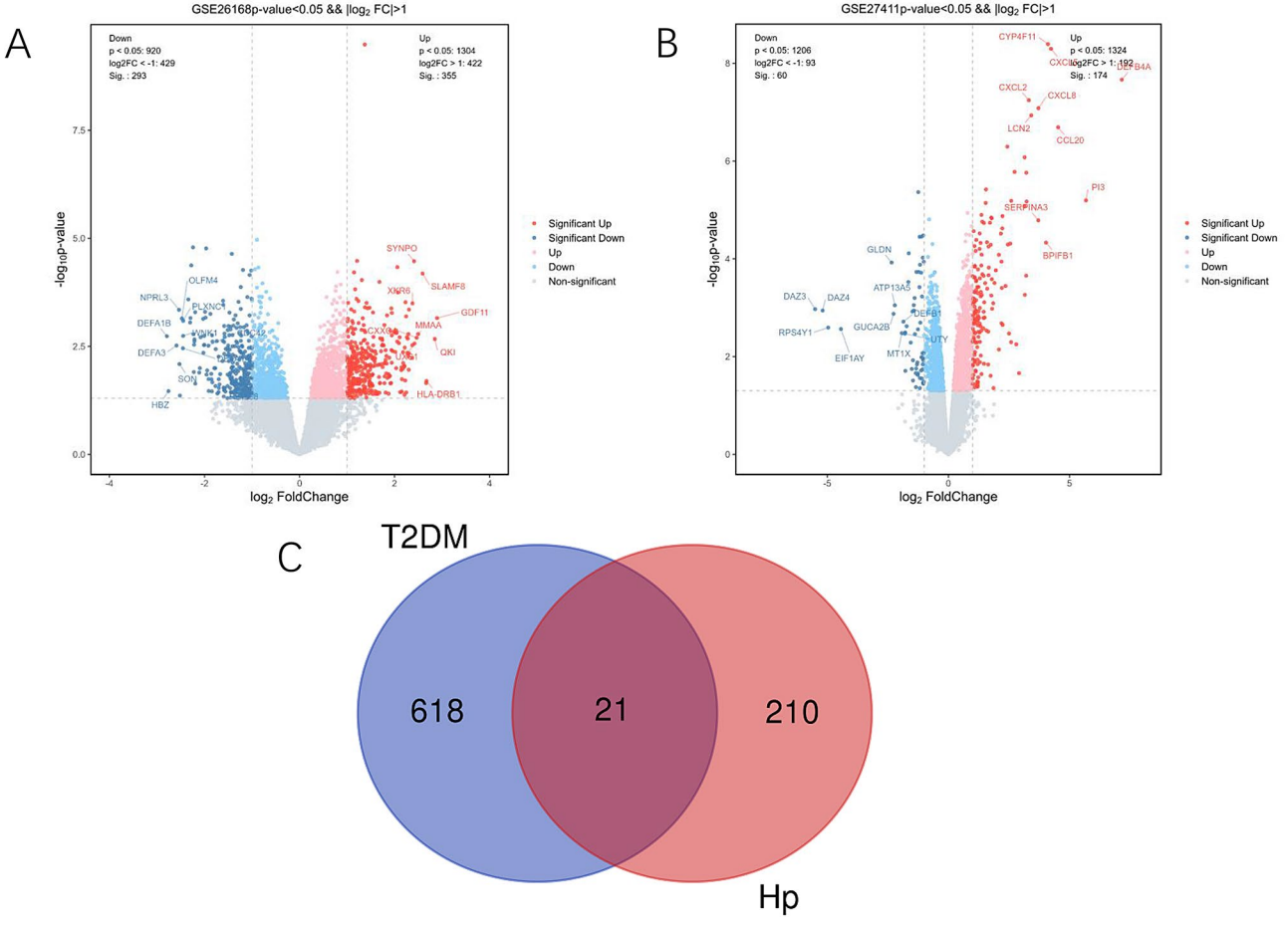

Fig. 4 The common differentially expressed genes between T2DM and *H. pylori* infection. (**A**) Volcano plot of DEGs in the T2DM-related GSE26168 dataset. (**B**) Volcano plot of DEGs in the *H. pylori* infection-related GSE27411 dataset. Red dots represent up-regulated DEGs, blue dots represent down-regulated DEGs, and gray dots represent genes that were not significantly different between the two groups. (**C**) Common DEGs in the GSE26168 dataset (blue) and GSE27411 dataset (red) were represented by venn diagrams. Abbreviations: T2DM: type 2 diabetes mellitus; Hp: *Helicobacter pylori*

Table 4 GO enrichment analysis of the common DEGs of *H. Pylori* infection and T2DM

GO Term	Category	-Log10 (*P*Value)	Count
extracellular region	Cellular Component	6.17	12
extracellular space	Cellular Component	5.56	11
chemotaxis	Biological Process	5.23	5
tertiary granule lumen	Cellular Component	4.75	4
specific granule lumen	Cellular Component	4.60	4
chemokine-mediated signaling pathway	Biological Process	4.38	4
neutrophil chemotaxis	Biological Process	4.16	4
inflammatory response	Biological Process	3.13	5
chemokine activity	Molecular Function	2.95	3
signal transduction	Biological Process	2.94	7
immune response	Biological Process	2.88	5
cell surface	Cellular Component	2.57	5
killing of cells of other organism	Biological Process	2.44	3
antimicrobial humoral immune response mediated by antimicrobial peptide	Biological Process	2.23	3
negative regulation of cysteine-type endopeptidase activity involved in apoptotic signaling pathway	Biological Process	2.17	2
external side of plasma membrane	Cellular Component	2.03	4

Abbreviations: *H. pylori*: *Helicobacter pylori*; T2DM: type 2 diabetes mellitus; DEGs: differentially expressed genes

increased diabetes incidence and complications [14–18]. These studies have confirmed that the two diseases play an important role in each other's occurrence and progression.

However, some studies using methods like histological examination, mucosal biopsy, and fecal antigen testing have found no significant interaction between *H. pylori* infection and T2DM [19–24]. For instance, a large cohort study by Pyo et al. found that *H. pylori* infection was not associated with diabetes mellitus, impaired glycemic control or impaired glucose tolerance [25]. This inconsistency highlights the need for a deeper investigation into the mechanisms underlying the relationship between the two conditions.

To explore the correlation between these two diseases, our study statistically analyzed data from 4406 individuals who had completed medical history diagnosis, general condition measurements, and relevant laboratory tests at Xian GEM Flower Changqing Hospital from 2019 to 2021. Our findings indicated that *H. pylori* infection significantly increased the incidence of T2DM, thus confirming the association between the two diseases. Differences in conclusions among the studies can be attributed to variations in sample size, population characteristics, T2DM diagnosis criteria, *H. pylori* detection methods, and *H. pylori* strains. Large, heterogeneous interventional studies are needed to evaluate the long-term association between T2DM and *H. pylori* infection.

Despite the conflicting results, the correlation between *H. pylori* infection and T2DM is biologically plausible. It has been reported that *H. pylori* infection is involved in the development of T2DM through several mechanisms: (1) It induces the secretion of inflammatory factors such as IL-1, IL-6, IL-8, IL-17, IL-18, IL-21, IL-22, TGF-β, TNF-α, and IFN-γ, which contribute to host inflammatory states [26–28] ; (2) *H. pylori* infection causes DNA damage and thus plays an important role in T2DM [29]; (3) During infection, gastric neuroendocrine cells secrete hormones like gastrin, leptin, and somatostatin, affecting the host's metabolic state; (4) *H. pylori* infection can cause abnormalities in glucose metabolism and insulin tolerance through signaling pathways such as NF-κB, c-Jun/miR-203/SOCS3, leading to T2DM [30]. Conversely, T2DM increases the susceptibility to *H. pylori* infection by (1) causing gastrointestinal motility disorders, decreased gastric acid secretion, and microangiopathy, which facilitate *H. pylori* colonization [17]; (2) impairing immune cell functions due to abnormal glucose metabolism, facilitating *H. pylori* infection; (3) altering chemical production in the gastric mucosa, promoting bacterial colonization [31]. The interaction between T2DM and *H. pylori* infection has been confirmed by several studies through the collection and analysis of clinical data from study subjects [18, 32, 33].

In the present study, we innovatively identified 21 differentially expressed genes shared by the two diseases through bioinformatics analysis, and found that the main pathways involved are viral protein interaction with cytokine and cytokine receptor, IL-17 signaling pathway, cytokine-cytokine receptor interaction, chemokine signaling pathway, rheumatoid arthritis, TNF signaling pathway, lipid and atherosclerosis signaling pathway. Specifically, the IL-17 signaling pathway is known to play a crucial role in inflammatory response. Elevated levels of IL-17 have been implicated in the chronic inflammation observed in both *H. pylori* infection and T2DM [34, 35]. Additionally, the TNF signaling pathway is another critical pathway where TNF-α, a pro-inflammatory cytokine

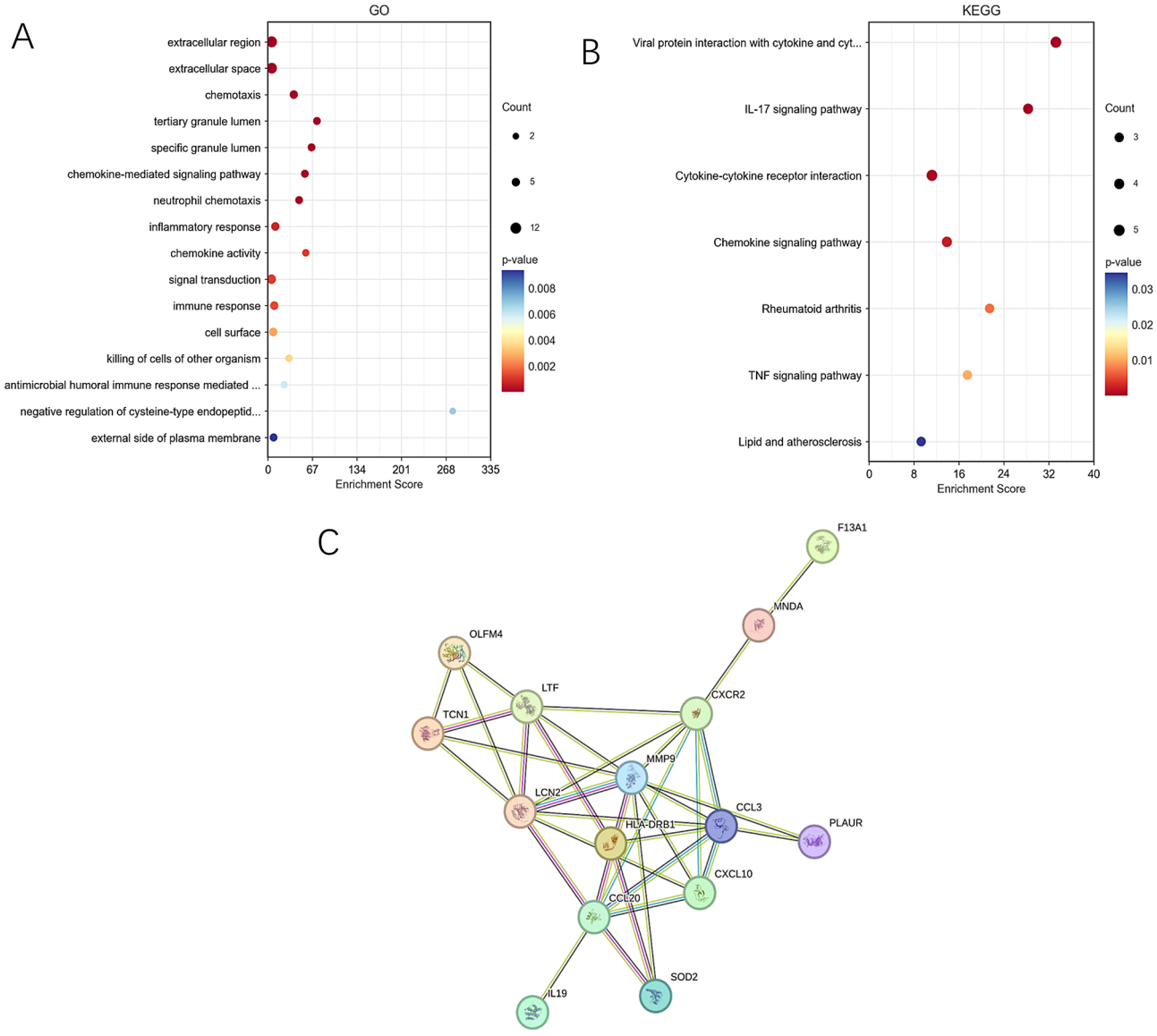

Fig. 5 GO, KEGG and PPI analysis of common DEGs. (**A**) GO enrichment analysis of the 21 common DEGs identified in T2DM and *H. pylori* infection. (**B**) KEGG enrichment analysis of the 21 common DEGs identified in T2DM and *H. pylori* infection. (**C**) PPI network for common DEGs that are shared by T2DM and *H. pylori* infection. The straight line (edge) between the nodes of the circle in the protein-interaction network represents the relationship between the two proteins connected by the line

Table 5 KEGG enrichment analysis of the common DEGs of *H. Pylori* infection and T2DM

Enriched signaling pathways	-Log10 (*P*Value)	Count
Viral protein interaction with cytokine and cytokine receptor	5.11	5
IL-17 signaling pathway	3.59	4
Cytokine-cytokine receptor interaction	3.26	5
Chemokine signaling pathway	2.69	4
Rheumatoid arthritis	2.15	3
TNF signaling pathway	1.98	3
Lipid and atherosclerosis	1.46	3

Abbreviations: *H. pylori*: *Helicobacter pylori*; T2DM: type 2 diabetes mellitus; DEGs: differentially expressed genes

induced by *H. pylori* infection, is known to interfere with insulin signaling, potentially leading to insulin resistance and T2DM [36]. Furthermore, the PPI analysis indicated extensive interactions between the proteins encoded by these genes. These results reveal potential mechanisms involved in the interaction between T2DM and *H. pylori* infection, providing insights into how these diseases may influence each other at a molecular level.

The strengths of this study include its focus on a single Chinese population, filling a gap in regional research, and the use of bioinformatics analysis to explore the dual-disease mechanisms. The relatively large sample size due to

the annual check-up program enhances the robustness of our conclusions.

However, several limitations should be considered. Firstly, this study involved a single center and was retrospective, relying on electronic medical records, potentially introducing information bias. Additionally, key indicators such as insulin data, hormone or inflammation levels, and T2DM complications were not available. Moreover, data collection was limited to annual check-ups, potentially affecting the accuracy of information such as disease-free survival time. Furthermore, it would be essential to perform a reciprocal study including a T2DM-free control group to examine the incidence of *H. pylori* infection, which is crucial for a comprehensive understanding of the bidirectional relationship between *H. pylori* infection and T2DM. Lastly, further studies including cell and animal experiments and larger-scale prospective studies are needed to validate our findings.

It is worth mentioning that the decision to merge the cohort research and bioinformatics analysis into one study is based on the complementary nature of the findings. The cohort study provided clinical evidence of the association between T2DM and *H. pylori* infection, while the bioinformatics analysis offered a molecular perspective by identifying the differentially expressed genes and elucidating the underlying biological mechanisms. Combining these two approaches offers a comprehensive understanding of the relationship, bridging clinical observations with molecular insights.

Taken together, this study builds on existing research that have tentatively reveals a correlation between *H. pylori* infection and T2DM prevalence through data collection, statistical analysis, and bioinformatics analysis. Our results emphasize the importance of considering *H. pylori* infection in the health management of patients with diabetes. In addition, this study provides a theoretical basis for future research directions and the selection of targets for disease prevention or treatment.

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Author contributions
Conceptualization: Lianxu Jia, Jiaqi Li, Wenjie Yuan, Jing Liu, Bowei Yang. Methodology: Jiaqi Li, Wenjie Yuan, Jing Liu. Investigation: Jiaqi Li, Wenjie Yuan, Jing Liu, Bowei Yang, Xiao Xu, Xiaoxia Ren. Statistical analysis: Jiaqi Li, Wenjie Yuan. Writing-Original Draft: Jiaqi Li, Wenjie Yuan. Writing-Reviewing & Editing: Jiaqi Li, Wenjie Yuan, Bowei Yang, Jing Liu, Xiao Xu, Xiaoxia Ren, Lianxu Jia. Supervision: Lianxu Jia.

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Data availability
All relevant data are within the paper. The data are not publicly available due to privacy or ethical restrictions. Further information is available from the corresponding authors on request. Reference datasets, such as the NCBI GEO datasets (at https://www.ncbi.nlm.nih.gov/gds), are provided in the "Bioinformatics analysis" section.

Declarations

Statement of ethics
All procedures in studies involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study received ethical approval by the Ethics Committee of Xian GEM Flower Changqing Hospital, Xian, China (XACHQ-YJ-23-008). Written informed consent was obtained for participation in this study.

Patient consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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