

Original Article

Diversity of Toll-like Receptor Genes and *Helicobacter Pylori* Infections: A Meta-Analysis StudyHamid Vaez¹ Ph.D., Amirhossein Sahebkar^{2,3,4} Ph.D., Asad Mohammadi⁵ Ph.D., Mohsen Arzanlou⁶ Ph.D., Arshid Yousefi-Avarvand⁷ Ph.D., Farzad Khademi^{6*} Ph.D.¹Department of Microbiology, Faculty of Medicine, Zabol University of Medical Sciences, Zabol, Iran.²Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran.³Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.⁴Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.⁵Cellular and Molecular Research Center, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.⁶Department of Microbiology, Faculty of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.⁷Laboratory Sciences, School of Paramedical Sciences, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

A B S T R A C T

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Available online 30 May 2020**Key words***Helicobacter pylori*
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Toll-like receptors**Background and Aims:** Several studies have shown that host genetic factors can be associated with the risk of developing *Helicobacter pylori* infections. Therefore, we evaluated the most prevalent toll-like receptors (TLRs) polymorphisms in *Helicobacter pylori* positive subjects and their possible role in susceptibility to *Helicobacter pylori* infections.**Materials and Methods:** Using related keywords, an independent search in the electronic databases including PubMed, Scopus, Google Scholar and ISI web of knowledge was performed to collect studies evaluating, until January 15, 2019, polymorphisms in the TLR 1 to 13 genes and their association with susceptibility to *Helicobacter pylori* infection. A total of 18 articles met our inclusion criteria and thus were included in the meta-analysis.**Results:** In this meta-analysis, a significantly increased risk of *Helicobacter pylori* infection was observed in subjects carrying TLR2 rs3804099 (TT vs. CC: odds ratio = 2.209, 95% CI: 1.283-3.804), TLR4 rs4986790 (A allele vs. G allele: odds ratio = 2.987, 95% CI: 1.899-4.697), TLR4 rs4986791 (C allele vs. T allele: odds ratio = 5.469, 95% CI: 13.432-8.713), TLR4 rs4986791 (CC vs. TT: odds ratio = 7.974, 95% CI: 2.682-23.706), TLR4 rs10759932 (TT vs. CC: odds ratio = 3.180, 95% CI: 1.022-9.890), TLR4 rs1927914 (C allele vs. T allele: odds ratio = 8.831, 95% CI: 4.222-18.470), and TLR9 rs352140 (CC vs. CT: odds ratio = 1.878, 95% CI: 1.071-3.290) polymorphisms.**Conclusions:** This meta-analysis indicated that TLR2 rs3804099, TLR4 rs4986790, TLR4 rs4986791, TLR4 rs10759932, TLR4 rs1927914 and TLR9 rs352140 polymorphisms are associated with increased susceptibility to *Helicobacter pylori* infections.

Introduction

It has been estimated that gastric mucosa of more than half of the world's population is infected with *Helicobacter pylori* (*H. pylori*), ranging from 25-50% in the developed countries to more than even 80% in the developing world [1, 2]. The prevalence of infection in 10% of children in the industrialized countries and more than 50% in the developing countries shows that *H. pylori* infection is usually found in childhood and in most cases remains in the gastric mucosa for a lifetime unless treated [1-3]. Chronic inflammation in the mucosal epithelium due to persistent *H. pylori* infection is associated with a variety of clinical outcomes such as gastritis and gastric ulcers in 10–20% of the infected individuals, which are the most common type of gastric diseases related to *H. pylori* [3]. Additionally, it is well established that *H. pylori* infection, classified as category I carcinogen, plays a crucial role in the development of gastric cancer, and 1-2% of infected subjects develop this type of cancer [4]. In addition to bacterial and environmental factors that affect the individuals' susceptibility to *H. pylori* infection, host factors are also important [5]. Several polymorphisms in the genes encoding inflammatory proteins, cytokines, growth factors, chemokines and another immune response genes have been identified to be associated with the susceptibility to *H. pylori* infection [6, 7]. Cells involved in the innate immunity including mucosal epithelial cells and myeloid cells, macrophages and dendritic cells, are responsible for the initial response to

H. pylori components during infection which is mediated through the pattern recognition receptors (PRRs) such as toll-like receptors (TLRs). TLRs recognize preserved microbial structures, pathogen-dependent molecular patterns (PAMPs), such as flagellin, lipopolysaccharide (LPS) and peptidoglycan located on the surface of the bacteria, fungi, protozoa, and viruses. TLRs also recognize endogenous ligands such as damage-associated molecular patterns (DAMPs) e.g. heat shock proteins which are spontaneously released during cell stress [8]. The family of TLRs includes 13 members in mammals that are either located on the surface of the cell, such as TLRs 1, 2, 4, 5, 6 and 10, or are located on the endoplasmic reticulum membrane or on the endosomal/lysosomal membrane such as TLRs 3, 7, 8 and 9 [8-10]. The interaction of TLRs, expressed in most tissues and components of the envelope and nucleic acids of the infectious pathogen, results in the activation of intracellular signaling pathways that lead to inflammatory responses and activation of immune responses [8, 9]. Recently, several investigations have shown the role of genetic variations, including single nucleotide polymorphisms (SNPs), in the members of the TLR family in susceptibility to various infections, especially bacterial infections [9].

The purpose of this systematic review and meta-analysis was to evaluate the most prevalent TLR polymorphisms in *H. pylori*-positive subjects and the association of these polymorphisms with the susceptibility to *H. pylori* infections.

Materials and Methods

Literature search strategy

Three authors independently searched the electronic databases including PubMed, Scopus, Google Scholar and ISI web of knowledge to collect all case-control studies evaluating polymorphisms in the TLR 1 to 13 genes and their association with susceptibility to *H. pylori* infection. The last search was performed on January 15, 2019 using Medical Subject Headings (MeSH) terms including toll-like receptors, TLRs, polymorphisms, single-nucleotide polymorphisms, SNPs, mutations, variations and *H. pylori* infection. Hand searching of reference lists of the selected studies was performed to find any missed potential relevant article.

Study selection criteria

Eligible articles were selected based on the following criteria: 1) papers published in the English language, 2) case-control studies; case samples were infected individuals with *H. pylori* (*H. pylori* positive) and controls were *H. pylori* uninfected (*H. pylori* negative) and 3) articles investigating polymorphisms in the TLR 1 to 13 genes and their association with susceptibility to *H. pylori* infection. Articles with the following characteristics did not meet our inclusion criteria and were excluded from the meta-analysis: 1) articles evaluating the association between polymorphisms in the TLRs genes and susceptibility to other bacterial infection or other diseases, 2) articles evaluating other gene polymorphisms or other host genetic factors, 3) articles not comprising enough information on the prevalence of

polymorphisms in the TLRs genes, genotypes and alleles frequencies in *H. pylori* positive and negative individuals and 4) articles evaluating polymorphisms in the TLRs genes only in the *H. pylori* infected or *H. pylori* uninfected individuals.

Data extraction

Data from each study were extracted and tabulated in Table 1. Collected characteristics were as follows: 1) country of origin, 2) year of publication 3) ethnicity, 4) genotyping method, 5) number of *H. pylori* positive subjects, 6) number of *H. pylori* negative subjects, 7) type of disease, 8) type of polymorphism, 9) *H. pylori* detection method and 10) references.

Statistical analysis

We performed all statistical analyses using Comprehensive Meta-Analysis (CMA) software version 2.2 (Biostat, Englewood, NJ, USA). Using allelic and genotypic models, the association between TLRs polymorphisms and susceptibility to *H. pylori* infection was measured by ratio (ORs) with 95% confidence intervals (95% CIs). When the p value was less than 0.05, association was statistically significant. Additionally, the frequency of polymorphisms in the TLRs genotypes/alleles among *H. pylori* infection positive case and control subjects was expressed as percentage (%). Fixed-effects model was used to pooled data when there was no heterogeneity and Cochran Q test was statistically significant ($I^2=0-25\%$, $P<0.05$); however, in large heterogeneity ($I^2=25-100\%$, $P<0.05$), random effects model was used [11]. In the current meta-analysis, publication bias was evaluated using funnel plots.

Results

Study and populations characteristics

After comprehensive literature search in the electronic databases including PubMed, Scopus, Google Scholar and ISI web of knowledge until January 15, 2019, a total of 3157 articles were collected. As shown in Figure 1, 18 qualified studies were included for final analysis after meeting the inclusion criteria, including 5 studies from Caucasian ethnicity, 3 studies from Iranian ethnicity, 2 studies from Chinese ethnicity, 2 studies from Japanese ethnicity, 2 studies from Thai ethnicity, 1 study from Malaysian ethnicity, 1 study from Indian ethnicity, 1 studies from Kashmiri ethnicity and 1 study from Brazilian ethnicity. The main characteristics of included studies in the meta-analysis are shown in table 1. In the present study, included studies were reported from China, Japan, Thailand, Malaysia, India, Iran, Germany, Scotland, Lithuania, Latvia and Brazil. Main TLRs genes polymorphisms in included studies which are more common among *H. pylori* infection positive individuals were shown in the different ethnicities (Table 1). Polymerase chain reaction (PCR)-based methods were the most frequent techniques used for determining the TLRs genotypes/alleles. Additionally, various studies have used different detection methods to identify *H. pylori* positive subjects. Patients had different *H. pylori*-related diseases including gastric cancer, non-ulcer dyspepsia, peptic ulcer disease, gastric atrophy, mucosa-associated lymphoid tissue lymphoma and gastritis. Table 2 displays distribution of

genotypes and alleles frequencies of TLRs genes polymorphisms in *H. pylori* positive and negative individuals. Additionally, Table 3 reveals association between TLRs genes polymorphisms, genotypes/ alleles, and *H. pylori* infection.

Association between TLR1 polymorphisms and susceptibility to *H. pylori* infections

In the present meta-analysis, we evaluated possible associations between TLR1 rs4833095 and TLR1 rs5743618 polymorphisms and susceptibility to *H. pylori* infections (Table 2).

The TLR1 rs4833095 variants were CC, CT and TT that occurred in 896 (45.8%), 687 (35.2%) and 370 (19%) of *H. pylori* infection-positive cases and in 416 (29%), 871 (60.5%) and 151 (10.5%) *H. pylori* infection-negative controls. Frequency of mutant genotypes, CT and TT, were 1057 (54.1%) and 1022 (71%) in *H. pylori* infection-positive and -negative individuals, respectively. In the Chinese, Thai, and Malaysian populations, genotype frequency of variant genotypes, CT and TT, were 859 (44%), 164 (8.3%) and 34 (1.7%), respectively, in *H. pylori* infection-positive individuals and 639 (44.4%), 364 (25.3%) and 19 (1.3%), in *H. pylori* infection-negative individuals, respectively. Overall, in the genotypic model, there was no significant association between TLR1 rs4833095 polymorphism and susceptibility to *H. pylori* infections (Table 3). For the TLR1 rs5743618 polymorphism, genotypes, II, IS and SS, were 14 (6.2%), 72 (31.4%) and 143 (62.4%) in *H. pylori* infection-positive cases and 4 (7.5%), 17 (32%) and 32 (60.5%) in *H. pylori* infection-negative controls. Additionally, in Caucasian population,

frequency of mutant genotypes, IS and SS, were 215 (93.8%) and 49 (92.4%) in *H. pylori* infection-positive and -negative individuals, respectively. Such as TLR1 rs4833095 polymorphism, there was no significant association between TLR1 rs5743618 polymorphism and susceptibility to *H. pylori* infections (Table 3).

Association between TLR2 polymorphisms with susceptibility to *H. pylori* infections

Several studies have reported association between six TLR2 SNPs (-196 to -174 (ins→del), rs3804099 (T→C), rs3804100 (T→C), +2251 (G→A), rs121917864 (Arg677Trp), and rs5743708 (Arg753Gln)) with susceptibility to *H. pylori* infections.

For TLR2 -196 to -174 SNP, genotype and allele frequencies were as follows: ins/ins 541 (46.2%), ins/del 504 (43.2%), del/del 124 (10.6%), ins 345 (70.5%), and del 145 (29.5%) in *H. pylori* infection-positive individuals and ins/ins 376 (46%), ins/del 355 (43.6%) and del/del 85 (10.4%), ins 198 (72.7%), del 74 (27.3%) in *H. pylori* infection-negative individuals. Frequency of variant allele, del, and variant genotypes, ins/del and del/del, in different populations are illustrated in table 2. Overall, no association was found between TLR2 -196 to -174 polymorphism with susceptibility to *H. pylori* infections in genotypic and allelic models (Table 3). For TLR2 rs3804099 SNP, TT, TC and CC genotypes frequency were 126 (61.7%), 27 (13.3%) and 51 (25%) in *H. pylori* infection-positive individuals and 131 (66.8%), 41 (21%) and 24 (12.2%) in *H. pylori* infection-negative individuals. In addition, genotype frequency of mutant

heterozygote and homozygote, TC and CC, were 78 (38.2%) and 65 (33.1%) in *H. pylori* infection-positive and -negative individuals, respectively. Individuals with the CC mutant homozygote genotype for TLR2 rs3804099 SNP had a significantly increased risk of *H. pylori* infection (Table 3 and Fig. 2). For TLR2 rs3804100 SNP, TT, TC and CC genotypes frequency were 147 (72%), 49 (24%), and 8 (4%) in *H. pylori* infection-positive individuals and 149 (76%), 43 (22%), and 4 (2%) in *H. pylori* infection-negative individuals. Genotype frequency of mutant heterozygote and homozygote, TC and CC, were 57 (28%) and 47 (24%) in *H. pylori* infection-positive and -negative individuals, respectively. The results indicated that the TLR2 rs3804100 TC and CC genotypes variant failed to have any association with increased risk of *H. pylori* infection in Thai population (Table 3). For TLR2 +2251 (G→A) SNP, GG, GA and AA genotypes frequency were 225 (99.1%), 2 (0.9%) and 0 (0%) in *H. pylori* infection-positive individuals and 252 (98.4%), 4 (1.6%) and 0 (0%) in *H. pylori* infection-negative individuals. Genotype frequency of mutant genotypes, GA and AA, were 2 (9%) and 4 (1.6%) in *H. pylori* infection-positive and -negative individuals, respectively. In Brazilian population, the TLR2 +2251 GG and GA genotypes variant did not show any association with increased risk of *H. pylori* infection (Table 3).

Association between TLR4 polymorphisms and susceptibility to *H. pylori* infections

In the current meta-analysis, association between five TLR4 SNPs (rs4986790, rs4986791, rs11536889, rs10759932, and

rs1927914) and susceptibility to *H. pylori* infections was evaluated. For TLR4 *rs4986790* SNP, frequency of genotypes amounted to AA 1104 (84.5%), AG 192 (14.7%) and GG 9 (0.8%) in *H. pylori* infection-positive cases and 981 (85.5%), 157 (13.6%) and 9 (0.9%) in *H. pylori* infection-negative controls. Frequency of mutant genotypes, AG and GG, were 201 (15.4%) and 166 (14.4%) in *H. pylori* infection-positive and -negative individuals, respectively. Additionally, frequency of mutant allele, G, was 45 (17%) and 47 (8.5%) in *H. pylori* infection-positive and -negative individuals, respectively. Overall, no association was found between TLR4 *rs4986790* polymorphism with susceptibility to *H. pylori* infections in genotypic model; however, there was a significant association in allelic model (Table 3 and Figure 2). For TLR4 *rs4986791* SNP, CC, CT and TT genotypes frequency were 131 (72.4%), 41 (22.6%), and 9 (5%) in *H. pylori* infection-positive individuals and 223 (87.1%), 27 (10.5%), and 6 (2.4%) in *H. pylori* infection-negative individuals. Frequency of variant allele, T, and variant genotypes, CT and TT, were as follows: 57 (21.4%) and 50 (27.6%) in *H. pylori* infection-positive individuals, and 36 (6.5%) and 33 (12.8%) in *H. pylori* infection-negative individuals. Both mutant homozygote genotype, TT, and mutant allele, T, have shown highly significant association with susceptibility to *H. pylori* infections (Table 3 and Fig. 2). For TLR4 *rs11536889* SNP, GG, GC and CC genotypes frequency were 1016 (59.4%), 587 (34.2%), and 109 (6.4%) in *H. pylori* infection-positive individuals and 431 (59.8%), 242 (33.6%), and 47 (6.6%) in *H. pylori* infection-

negative individuals. Also, G and C alleles frequency were 984 (85%) and 170 (15%) in *H. pylori* infection-positive individuals and 614 (85%) and 112 (15%) in *H. pylori* infection-negative individuals. Frequency of variant allele, C, and variant genotypes, GC and CC, were as follows: 170 (14.7%) and 696 (40.6%) in *H. pylori* infection-positive individuals, and 112 (15.4%) and 289 (40.1%) in *H. pylori* infection-negative individuals. Overall, no association was found between TLR4 *rs11536889* polymorphism with susceptibility to *H. pylori* infections in genotypic and allelic models (Table 3). For TLR4 *rs10759932* SNP, TT, TC and CC genotypes frequency leveled at 153 (75%), 37 (18.2%), and 14 (6.8%) in *H. pylori* infection-positive individuals and 139 (71%), 53 (27%), and 4 (2%) in *H. pylori* infection-negative individuals. We found a significant association between TLR4 *rs10759932* CC mutant genotype with susceptibility to *H. pylori* infections (Table 3 and Fig. 2). Our results revealed a significant association between TLR4 *rs1927914* T mutant allele with susceptibility to *H. pylori* infections (Table 3 and Figure 2).

Association between TLR5 polymorphisms and susceptibility to *H. pylori* infections

Among four TLR5 SNPs (*rs5744174*, *rs5744168*, *rs1640827* and *rs17163737*) which were evaluated in terms of their susceptibility to *H. pylori* infections, only one polymorphism, TLR5 *rs5744168*, yielded enough information. Genotype frequency of CC, CT, and TT were 206 (91.6%), 19 (8.4%), 0 (0%) in *H. pylori* infection-positive individuals and 239 (93.7%), 16 (6.3%), and 0 (0%) in *H. pylori* infection-

negative individuals. Frequency of variant genotypes, CT and TT, were 19 (8.4%) in *H. pylori* infection-positive individuals, and 16 (6.2%) in *H. pylori* infection-negative individuals. Overall, no association was found between TLR5 rs5744168 polymorphism and susceptibility to *H. pylori* infections in genotypic model (Table 3).

Association between TLR9 polymorphisms with susceptibility to *H. pylori* infections

We evaluated the role of four TLR9 SNPs (*rs352140*, *rs34399053*, *rs150459369* and *rs5743836*) in susceptibility to *H. pylori* infections. For TLR9 rs352140 SNP, genotype frequency of CC, CT, and TT were 25 (32.5%), 46 (59.7%), and 6 (7.8%) in *H. pylori* infection-positive individuals and 100 (43.4%), 98 (42.6%), and 32 (14%) in *H. pylori* infection-negative individuals. In addition, C and T alleles frequencies were 96 (62.3%) and 58 (37.7%), in *H. pylori* infection-positive individuals and 298 (64.8%) and 162 (35.2%) in *H. pylori* infection-negative individuals. Frequency of variant allele, T, and variant genotypes, CT and TT reached as follows: 58 (37.7%) and 52 (67.5%) in *H. pylori* infection-positive individuals, and 162 (35.2%) and 130 (56.5%) in *H. pylori* infection-negative individuals. Our results demonstrated a significant association between TLR9 rs352140 CT mutant genotype with susceptibility to *H. pylori* infections (Table 3 and Fig. 2).

For TLR9 *rs34399053* SNP, genotype frequency of CC, CT, and TT were 26 (33.7%), 51 (66.3%), and 0 (0%) in *H. pylori* infection-positive individuals and 79 (34.3%), 150 (65.2%), and 1 (0.5 %) in *H. pylori* infection-

negative individuals. Frequency of variant allele, T, and variant genotypes, CT and TT, were as follows: 51 (33.2%) and 51 (66.3%) in *H. pylori* infection-positive individuals, and 152 (33%) and 151 (65.6%) in *H. pylori* infection-negative individuals. On balance, no association was found between TLR9 rs34399053 polymorphism with susceptibility to *H. pylori* infections in allelic and genotypic models (Table 3). For TLR9 rs150459369 SNP, CC, CT, and TT genotypes frequency were 77 (100%), 0 (0%) and 0 (0%) in *H. pylori* infection-positive individuals and 220 (95.6%), 10 (4.4%) and 0 (%) in *H. pylori* infection-negative individuals. In addition, C and T alleles frequency were 154 (100%) and 0 (0 %) in *H. pylori* infection-positive individuals and 450 (97.8%) and 10 (2.2%) in *H. pylori* infection-negative individuals. Frequency of variant allele, T, and variant genotypes, CT and TT, were as follows: 0 (0%) and 0 (0%) in *H. pylori* infection-positive individuals, and 10 (2.2%) and 10 (4.4%) in *H. pylori* infection-negative individuals. By and large, no association was found between TLR9 rs150459369 polymorphism with susceptibility to *H. pylori* infections in allelic and genotypic models (Table 3). For TLR9 rs5743836 SNP, TT, TC and CC genotypes frequencies were 77 (65.8%), 40 (34.2%), and 0 (0%) in *H. pylori* infection-positive individuals and 35 (68.6%), 15 (29.5%), and 1 (1.9%) in *H. pylori* infection-negative individuals. Overall, no association was detected between TLR9 rs5743836 polymorphism and susceptibility to *H. pylori* infections in allelic and genotypic models (Table 3).

Association between TLR10 polymorphisms with susceptibility to *H. pylori* infections

The role of two polymorphisms, TLR10 rs10004195 and TLR10 rs4129009, in susceptibility to *H. pylori* infections were determined. For TLR10 rs10004195 SNP, AA, AT and TT genotypes frequency were 581 (33.3%), 740 (42.3%) and 426 (24.4%) in *H. pylori* infection-positive individuals and 339 (27.5%), 627 (51%) and 266 (21.5%) in *H. pylori* infection-negative individuals. Additionally, frequency of variant genotypes, AT and TT, were as follows: 1166 (66.7%) and 893 (72.4%) in *H. pylori* infection-positive and -negative individuals, respectively. All in all, no

association was found between TLR10 rs10004195 polymorphism with susceptibility to *H. pylori* infections in genotypic model (Table 3). Frequency of variant genotypes, TC and CC, were as follows: 990 (66.1%) and 667 (65.5%) in *H. pylori* infection-positive and -negative individuals, respectively. Totally, no association was found between TLR10 rs4129009 polymorphism with susceptibility to *H. pylori* infections in genotypic model (Table 3).

Finally, no study was found in terms of evaluating association between TLR3, 6-8, 11-13 polymorphisms and susceptibility to *H. pylori* infections.

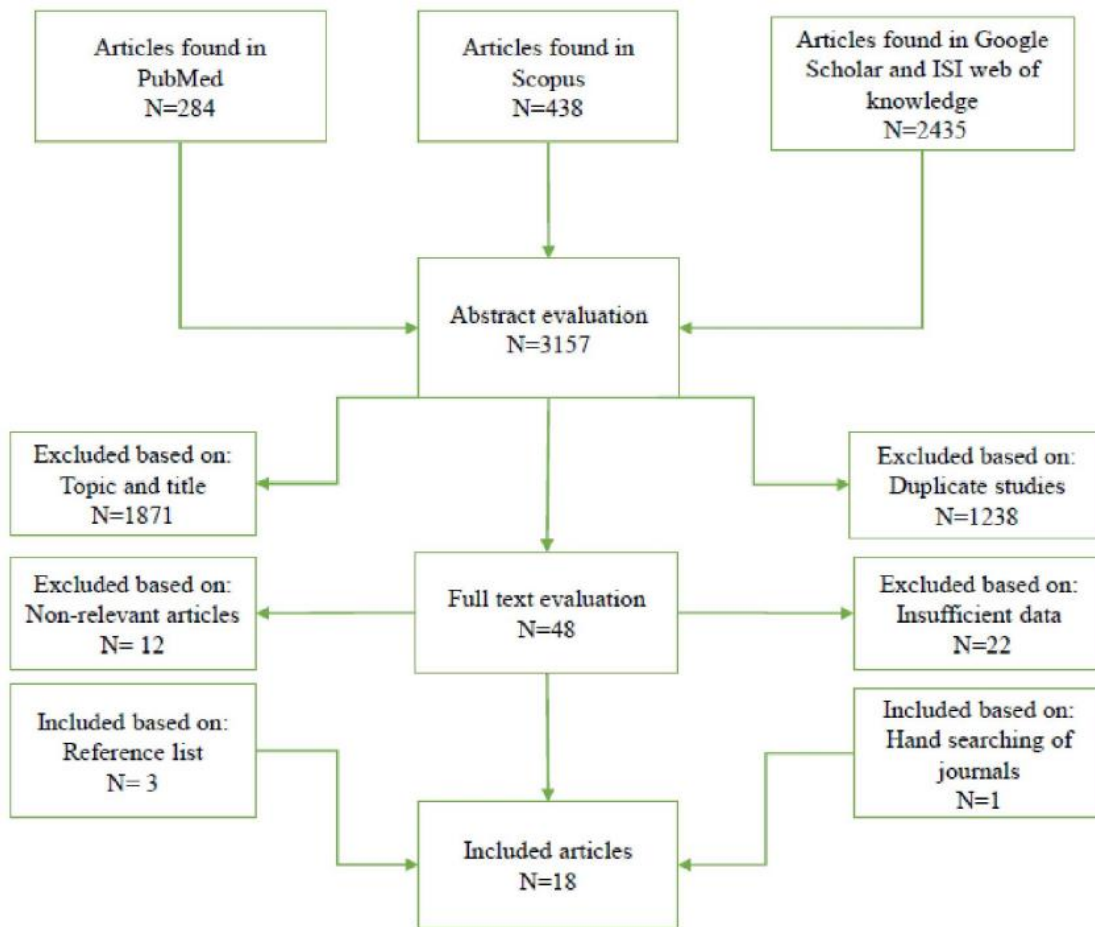


Fig. 1. Flowchart of eligible studies selection process in the meta-analysis

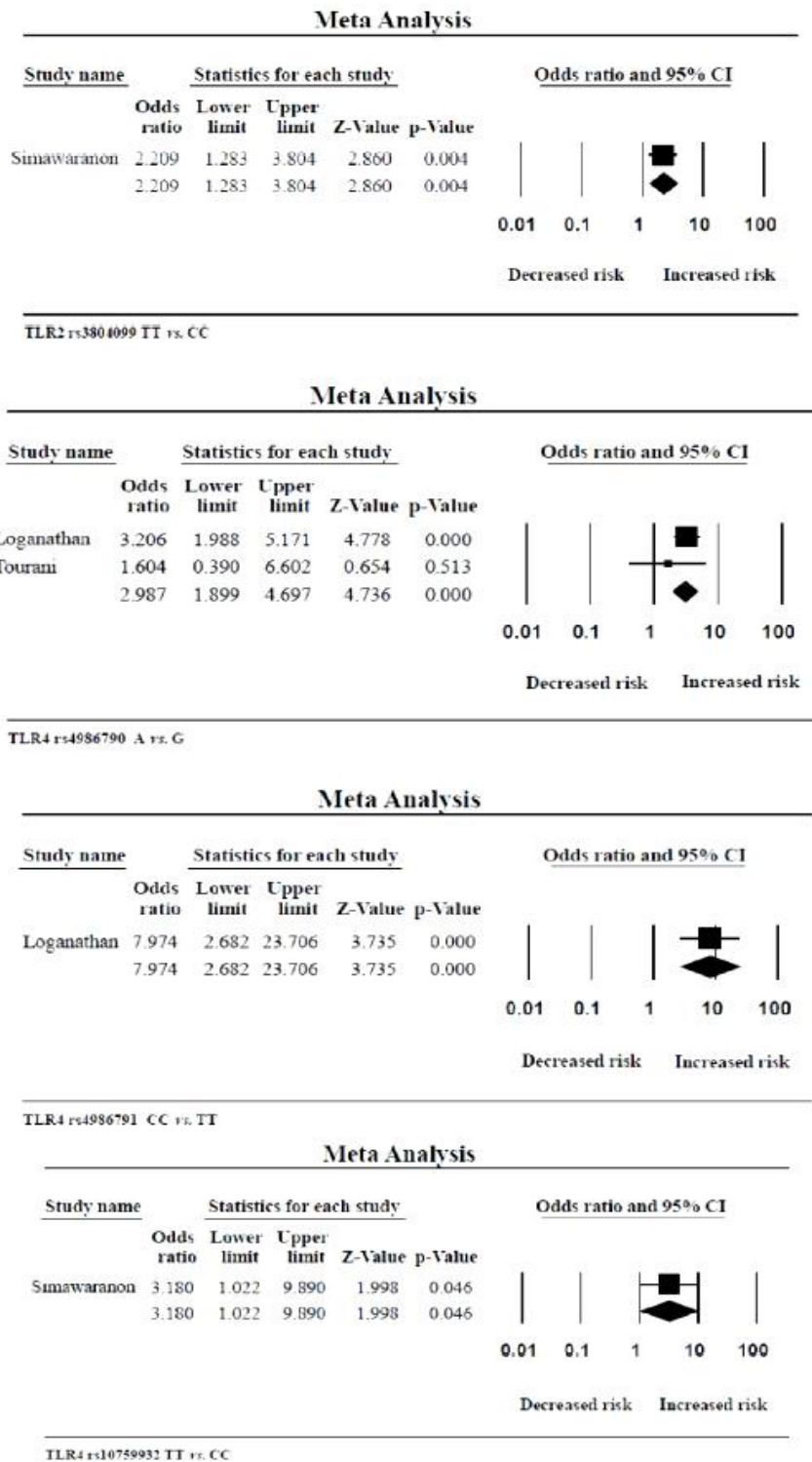


Fig. 2. Forest plot of the meta-analysis on the association between TLR2 rs3804099, TLR4 rs4986790, TLR4 rs4986791, TLR4 rs10759932, TLR4 rs1927914, and TLR9 rs352140 and susceptibility to *H. pylori* infections.

Table 1. The main data of included studies in the meta-analysis

Country	Genotyping method	<i>H. pylori</i> positive (N)	<i>H. pylori</i> negative (N)	Total (N)	Disease type	Polymorphism	<i>H. pylori</i> detection method	Ref.
China	Real-time PCR	1511	1042	2553	GA DYS IM SG	TLR1 rs4833095 TLR10 rs10004195 TLR10 rs4129009	¹³ C-UBT	12
China	Real-time PCR Mass spectrometry	190	94	284	GC	TLR2 -196 to -174 del TLR4 rs11536889	Serum anti- <i>H. pylori</i> IgG antibody	13
Japan	PCR	937	699	1636	GA	TLR2 -196 to -174 del	Serum anti- <i>H. pylori</i> IgG antibody	14
Japan	PCR-CTPP	1191	401	1592	GA	TLR4 rs11536889	Serum anti- <i>H. pylori</i> IgG antibody	15
Thailand	Real-time PCR	204	196	400	GC Gastritis	TLR1 rs4833095 TLR2 rs3804099 TLR2 rs3804100 TLR4 rs10759932 TLR10 rs10004195	Histopathological examination Real-time PCR	16
Thailand	Real-time PCR	204	196	400	Gastritis	TLR1 rs4833095	Histopathological examination	17
Malaysia	Real-time PCR	62	33	95	GC NUD PUD	TLR1 rs4833095 TLR10 rs10004195	RUT Culture Histopathological examination	18
India	PCR-RFLP ARMS-PCR	77	230	307	PUD	TLR4 rs1927914 TLR4 rs4986790 TLR4 rs4986791 TLR9 rs352140 TLR9 rs34399053 TLR9 rs150459369	RUT	19
India	PCR-RFLP	104	26	130	GC	TLR4 rs4986790 TLR4 rs4986791	PCR (glmM)	20
Iran	ASPCR PCR-RFLP	55	45	100	PUD	TLR2 -196 to -174 ins/del TLR2 rs121917864 TLR2 rs5743708	RUT Serum anti- <i>H. pylori</i> IgG antibody Histopathological examination PCR (glmM)	21

TOLL-LIKE RECEPTOR GENES AND *H. PYLORI* INFECTIONS

Iran	PCR-CTPP PCR-RFLP	56	44	100	PUD	TLR4 rs11536889 TLR4 rs4986790 TLR4 rs4986791	RUT Histopathological examination Serum anti- <i>H. pylori</i> IgG antibody	22
Iran	PCR-RFLP	195	241	436	Gastritis	TLR4 rs4986790	RUT PCR (16s rRNA and glmM) *Histological examination	23
Scotland	Applied Biosystems 5 nuclease SNP genotyping assay	117	51	168	GA	TLR9 rs5743836	¹⁴ C-UBT Serology RUT Culture Histopathological examination	24
Scotland	PCR-RFLP Real-time PCR	103	46	149	GC	TLR4 rs4986790	¹⁴ C-UBT Serology RUT Culture Histopathological examination	25
Germany	Fluorescence- labeled hybridization FRET probes	229	53	548	GC HRG	TLR1 rs5743618	Serum anti- <i>H. pylori</i> IgG antibody	26
Germany	Real-time PCR	594	358	952	MALT lymphoma	TLR4 rs4986790	RUT and Histopathological examination	27
Germany Lithuania Latvia	PCR-RFLP	203	97		GC GA	TLR4 rs11536889	Serum anti- <i>H. pylori</i> IgG antibody	28
Brazil	PCR-RFLP	232	254	486	Duodenal ulcer Gastritis	TLR2 (+2251) TLR4 rs4986790 TLR5 rs5744168	¹³ C-UBT Culture RUT PCR (urea)	29

MALDI-TOF= Matrix assisted laser desorption ionization time-of-flight; ELISA= Enzyme-linked immunosorbent assay; PCR-RFLP= Polymerase chain reaction-restriction fragment length polymorphism; PCR-CTPP= Polymerase chain reaction with confronting two-pair primers, ASPCR= Allele-specific polymerase chain reaction; HPLC= High-performance liquid chromatography; ¹³C- and ¹⁴C-UBT= ¹³C- and ¹⁴C-urea breath test; RUT= Rapid urease test; GC= Gastric cancer, NUD= Non-ulcer dyspepsia; PUD= Peptic ulcer disease; GA= Gastric atrophy, MALT= Mucosa-associated lymphoid tissue lymphoma; HRG= High-risk gastritis, DYS= Dysplasia; IM= intestinal metaplasia; SG= Superficial gastritis; NA= Not available.

*Histopathological examination: H & E and Giemsa staining.

Table 2. Distribution of genotype and allele frequencies of polymorphisms in the TLRs genes in *H. pylori* positive and negative individuals

TLR	Variant	SNP number	<i>H. pylori</i> infection positive (N)					<i>H. pylori</i> infection negative (N)					Ref.
			Genotype/Allele					Genotype/Allele					
			CC	CT	TT	C	T	CC	CT	TT	C	T	
TLR1	Ser248Asn (C→T)	rs4833095	629	660	199	NA	NA	379	499	140	NA	NA	12
			122	4	78	NA	NA	14	182	0	NA	NA	16
			23	19	15	NA	NA	9	8	11	NA	NA	18
			122	4	78	NA	NA	14	182	0	NA	NA	17
	Ile602Ser (I→S)	rs5743618	II	IS	SS	I	S	II	IS	SS	I	S	26
TLR2	-196 to -174 (ins → del)		ins/ins	ins/del	del/del	ins	del	ins/ins	ins/del	del/del	ins	del	
			418	414	105	NA	NA	304	316	79	NA	NA	14
			81	90	19	252	128	46	39	6	131	51	13
			42	NA	NA	93	17	26	NA	NA	67	23	21
	T→C	rs3804099	TT	TC	CC	T	C	TT	TC	CC	T	C	
			126	27	51	NA	NA	131	41	24	NA	NA	16
	T→C	rs3804100	TT	TC	CC	T	C	TT	TC	CC	T	C	
			147	49	8	NA	NA	149	43	4	NA	NA	16
	+2251 (G→A)		GG	GA	AA	G	A	GG	GA	AA	G	A	
			225	2	0	NA	NA	252	4	0	NA	NA	29
Arg677Trp (C→T)	rs121917864	CC	CT	TT	C	T	CC	CT	TT	C	T		
		4	NA	NA	58	52	3	NA	NA	47	43	21	
Arg753Gln (G→A)	rs5743708	GG	GA	AA	G	A	GG	GA	AA	G	A		
		50	NA	NA	102	8	44	NA	NA	89	1	21	
TLR4	Asp299Gly +896 (A→G)	rs4986790	AA	AG	GG	A	G	AA	AG	GG	A	G	
			155	40	0	NA	NA	194	37	2	NA	NA	23
			86	18	0	NA	NA	21	5	0	NA	NA	20
			88	15	0	NA	NA	42	4	0	NA	NA	25
			524	69	1	NA	NA	313	45	0	NA	NA	27
			206	25	1	NA	NA	222	28	4	NA	NA	29
			45	25	7	115	39	189	38	3	416	44	19
	Thr399Ile 1196 (C→T)	rs4986791	CC	CT	TT	C	T	CC	CT	TT	C	T	
			93	11	0	NA	NA	21	5	0	NA	NA	20
			38	30	9	106	48	202	22	6	426	34	19
			49	NA	NA	103	9	42	NA	NA	86	2	22
	+3725 (G→C)	rs11536889	GG	GC	CC	G	C	GG	GC	CC	G	C	
			627	474	90	NA	NA	199	162	40	NA	NA	15
279			51	9	609	69	182	44	4	408	52	28	
			110	62	10	282	82	50	36	3	136	42	13

TOLL-LIKE RECEPTOR GENES AND *H. PYLORI* INFECTIONS

			37	NA	NA	93	19	26	NA	NA	70	18	22
	T→C	rs10759932	TT	TC	CC	T	C	TT	TC	CC	T	C	
			153	37	14	NA	NA	139	53	4	NA	NA	16
	C→T	rs1927914	CC	CT	TT	C	T	CC	CT	TT	C	T	
			0	8	69	8	146	0	150	80	150	310	19
TLR5	+1174	rs5744168	CC	CT	TT	C	T	CC	CT	TT	C	T	
	(C→T)		206	19	0	NA	NA	239	16	0	NA	NA	29
			CC	CT	TT	C	T	CC	CT	TT	C	T	
	C→T	rs352140	25	46	6	96	58	100	98	32	298	162	19
			CC	CT	TT	C	T	CC	CT	TT	C	T	
TLR9	C→T	rs34399053	26	51	0	103	51	79	150	1	308	152	19
			CC	CT	TT	C	T	CC	CT	TT	C	T	
	C→T	rs150459369	77	0	0	154	0	220	10	0	450	10	19
			TT	TC	CC	T	C	TT	TC	CC	T	C	
	-1237	rs5743836	77	40	0	NA	NA	35	15	1	NA	NA	24
	(T→C)		CC	CT	TT	C	T	CC	CT	TT	C	T	
			77	0	0	154	0	220	10	0	450	10	19
			AA	AT	TT	A	T	AA	AT	TT	A	T	
			498	712	276	NA	NA	308	493	207	NA	NA	12
TLR10	C→T	rs10004195	59	10	135	NA	NA	22	123	51	NA	NA	16
			24	18	15	NA	NA	9	11	8	NA	NA	18
			TT	TC	CC	T	C	TT	TC	CC	T	C	
	Ile775Val	rs4129009	507	715	275	NA	NA	351	490	177	NA	NA	12

TLR= Toll-like receptor; SNP= Single nucleotide polymorphism; ins= Insertion, del= Deletion; NA= Not available

Table 3. Association between polymorphisms in the TLRs genes, genotypes/alleles, and *H. pylori* infection

Polymorphism	Genotype/Allele	Odds Ratio	95% CI	P-value	I ² (%)	Effect model
TLR1 rs4833095	C vs. T	NA	NA	-	-	-
	CC vs. TT	1.605	(0.485-5.309)	0.439	69.2	R
	CC vs. CT	0.048	(0.002-1.247)	0.068	98.4	R
TLR1 rs5743618	I vs. S	NA	NA	-	-	-
	II vs. SS	1.277	(0.394-4.136)	0.684	0.00	F
	II vs. IS	1.210	(0.354-4.142)	0.304	0.00	F
TLR2 -196 to -174 ins>del	ins vs. del	0.876	(0.366-2.097)	0.766	79	R
	ins/ins vs. del/del	1.028	(0.754-1.402)	0.862	27	F
	ins/ins vs. ins/del	0.995	(0.821-1.208)	0.963	19.2	F
TLR2 rs3804099	T vs. C	NA	NA	-	-	-
	TT vs. CC	2.209	(1.283-3.804)	0.004	0.00	F
	TT vs. TC	0.685	(0.397-1.179)	0.172	0.00	F
TLR2 rs3804100	T vs. C	NA	NA	-	-	-
	TT vs. CC	2.027	(0.597-6.878)	0.257	0.00	F
	TT vs. TC	1.155	(0.723-1.846)	0.547	0.00	F
TLR2 (+2251)	G vs. A	NA	NA	-	-	-
	GG vs. AA	-	-	-	-	-
	GG vs. GA	0.560	(0.102-3.087)	0.506	0.00	F

TLR2 rs121917864	C vs. T	0.980	(0.561-1.712)	0.943	0.00	F
	CC vs. TT	NA	NA	-	-	-
	CC vs. CT	-	-	-	-	-
TLR2 rs5743708	G vs. A	6.980	(0.856-56.901)	0.070	0.00	F
	GG vs. AA	NA	NA	-	-	-
	GG vs. GA	NA	NA	-	-	-
TLR4 rs4986790	A vs. G	2.987	(1.899-4.697)	0.000	0.00	F
	AA vs. GG	1.234	(0.148-10.261)	0.846	69.6	R
	AA vs. AG	1.293	(0.887-1.884)	0.182	53.1	R
TLR4 rs4986791	C vs. T	5.469	(3.432-8.713)	0.000	0.00	F
	CC vs. TT	7.974	(2.682-23.706)	0.000	0.00	F
	CC vs. CT	1.984	(0.144-27.401)	0.609	93.6	R
TLR4 rs11536889	G vs. C	0.895	(0.688-1.165)	0.410	0.00	F
	GG vs. CC	0.813	(0.562-1.175)	0.271	12.3	F
	GG vs. GC	0.872	(0.717-1.060)	0.169	0.00	F
TLR4 rs10759932	T vs. C	NA	NA	-	-	-
	TT vs. CC	3.180	(1.022-9.890)	0.040	0.00	F
	TT vs. TC	0.634	(0.393-1.024)	0.062	0.00	F
TLR4 rs1927914	C vs. T	8.831	(4.222-18.470)	0.000	0.00	F
	CC vs. TT	-	-	-	-	-
	CC vs. CT	-	-	-	-	-
TLR5 rs5744168	C vs. T	NA	NA	-	-	-
	CC vs. TT	-	-	-	-	-
	CC vs. CT	1.378	(0.691-2.749)	0.363	0.00	F
TLR9 rs352140	C vs. T	1.111	(0.762-1.622)	0.584	0.00	F
	CC vs. TT	0.750	(0.283-1.990)	0.563	0.00	F
	CC vs. CT	1.878	(1.071-3.290)	0.028	0.00	F
TLR9 rs34399053	C vs. T	1.003	(0.681-1.479)	0.987	0.00	F
	CC vs. TT	1.000	(0.040-25.296)	1.000	0.00	F
	CC vs. CT	1.033	(0.599-1.782)	0.907	0.00	F
TLR9 rs150459369	C vs. T	0.139	(0.008-2.383)	0.173	0.00	F
	CC vs. TT	-	-	-	-	-
	CC vs. CT	0.135	(0.008-2.339)	0.169	0.00	F
TLR9 rs5743836	T vs. C	NA	NA	-	-	-
	TT vs. CC	0.153	(0.006-3.841)	0.253	0.00	F
	TT vs. TC	1.212	(0.593-2.479)	0.598	0.00	F
TLR10 rs10004195	A vs. T	NA	NA	-	-	-
	AA vs. TT	0.839	(0.680-1.036)	0.102	0.00	F
	AA vs. AT	0.258	(0.029-2.288)	0.224	96.8	R
TLR10 rs4129009	T vs. C	NA	NA	-	-	-
	TT vs. CC	1.076	(0.852-1.358)	0.539	0.00	F
	TT vs. TC	1.010	(0.845-1.207)	0.911	0.00	F

Bold= Statistically significant result; R= Random-effect model; F= Fixed-effect model; CI= Confidence interval; NA= Not available

Discussion

Genetic polymorphisms in TLRs, as key members of the innate immune system, mediate recognition of *H. pylori* and are assumed to play an important role in susceptibility to *H. pylori* infections [30]. However, there have been many conflicting reports concerning TLRs polymorphisms and their possible role in susceptibility to *H. pylori* infections. The current study is the first comprehensive systematic review and meta-analysis on the evaluation of association between TLR1, 2, 4, 5, 9 and 10 polymorphisms with of *H. pylori* infection risk. TLR1 is a PRR which forms heterodimer with TLR2 and recognizes lipoprotein/lipopeptides of *H. pylori* [31]. Recently, several studies have shown that individuals carrying TLR1 rs4833095 CT and TT genotypes and T allele as well as TLR1 rs5743618 SS genotype significantly decrease the risks of *H. pylori* infection and *H. pylori*-related diseases [12, 26]. In the same manner, individuals carrying CC or TT homozygous genotypes are at increased risks [16-18]. However, in this study, the genotypic model failed to show any significant association between TLR1 rs4833095 and rs5743618 polymorphisms and the susceptibility to *H. pylori* infections (Table 3). This difference may be due to the differences in ethnicities, assessment methods, population size and age.

Another member of the PRRs is TLR2 which is implicated in the identification of Gram-positive bacteria, mycobacteria, spirochetes, viruses, hepatitis C and B viruses, herpes simplex and cytomegalovirus, and fungi [31]. In

the case of *H. pylori*, SNPs in genes that encode TLR2 are associated with *H. pylori* infection and *H. pylori*-related diseases.

Our systematic review of included articles revealed that TLR2 -196 to 174del polymorphism is not significantly associated with *H. pylori* infection in the Japanese population [14]. However, increased risk of *H. pylori*-related diseases in *H. pylori*-infected individuals was observed in Chinese and Iranian populations carrying the above-mentioned polymorphism [13, 21]. In this meta-analysis, we could not detect any association between TLR2 -196 to 174del polymorphism and susceptibility to *H. pylori* infections (Table 3). This difference may arise from variations in ethnicities. In the studies conducted in the Thai population, no significant association was discerned between TLR2 rs3804099 and rs3804100 polymorphisms and susceptibility to *H. pylori* infections [16]. However, we observed that individuals with the CC mutant homozygote genotype for TLR2 rs3804099 bear a significantly increased risk of *H. pylori* infection (odds ratio = 2.209, 95% CI: 1.283-3.804) (Table 3). In the Brazilian population, TLR2 (+2251) failed to project a significant association with susceptibility to *H. pylori* infections while in the Iranian population TLR2 rs121917864 and rs5743708 proved significant associations with *H. pylori* infection [21, 29]. Our findings suggest that the TLR2 rs5743708 A allele mutant was significantly associated and susceptibility to *H. pylori* infections (odds ratio = 6.980, 95% CI: 0.856-56.901) (Table 3).

TLR4 is located on the surface of immune cells such as monocytes, mast cells and neutrophils and, similar to TLR2, recognizes a wide number of endogenous ligands released during cellular stress and necrosis, and preserved microbial structures of Gram-negative bacteria [31]. TLR4 expression increases during *H. pylori* infection on gastric epithelial cells and is the LPS receptor [31]. In the current meta-analysis, five SNPs were found in the human TLR4 gene in *H. pylori* positive subjects.

Whilst several studies with different population have reported that the TLR4 polymorphisms *rs4986790*, *rs4986791*, *rs11536889*, *rs10759932* and *rs1927914* are either associated or not associated with the risk of *H. pylori* infection (Table 2), our study offers a significant association only with the TLR4 *rs4986790* G mutant allele (odds ratio = 2.987, 95% CI: 1.899-4.697), TLR4 *rs4986791* TT mutant homozygote genotype (odds ratio = 7.974, 95% CI: 2.682-23.706) and T mutant allele (odds ratio = 5.469, 95% CI: 13.432-8.713), TLR4 *rs10759932* CC mutant genotype (odds ratio = 3.180, 95% CI: 1.022-9.890) and TLR4 *rs1927914* T mutant allele (odds ratio = 8.831, 95% CI: 4.222-18.470) and susceptibility to *H. pylori* infections (Table 3 and Fig. 2).

Monomeric bacterial flagellin has the ability to stimulate innate immune responses mediated by TLR5 which is located on surface epithelial, and membrane of immune cells such as NK cells, monocytes and myeloid dendritic cells [31]. There are controversial studies on recognizing *H. pylori* flagellin by TLR5 and the association of TLR5 polymorphism and susceptibility to *H. pylori* infection [29]. In the

current meta-analysis, there was no evidence linking TLR5 polymorphism and susceptibility to *H. pylori* infection.

The plasmacytoid dendritic cells, B cells and NK cells express TLR9 which is involved in stimulating innate immune responses via detection of bacterial and viral unmethylated CpG DNA [31]. It has also been suggested that TLR9 *rs352140* and *s5743836* polymorphisms play a role in the susceptibility to *H. pylori* infection [19, 24]. Our results also demonstrated that TLR9 *rs352140* CT mutant genotype conferred a significantly increased risk of *H. pylori* infections (odds ratio = 1.878, 95% CI: 1.071-3.290) (Table 3 and Fig. 2).

TLR10 is an anti-inflammatory PRR which is expressed in humans [12, 31]. Tang et al. [10], Simawaranon et al. [16], and Ram et al. [18] reported that TLR10 *rs10004195* is associated with susceptibility to *H. pylori* infection in the Chinese, Thai and Malaysian population. However, no association was found between TLR10 polymorphisms and susceptibility to *H. pylori* infections in our study (Table 3).

TLR11, TLR12 and TLR13 are expressed in mice but we could not find any study on the association of TLR3, 6-8 and 11-13 polymorphisms and susceptibility to *H. pylori* infections.

Conclusion

This meta-analysis indicated that TLR2 *rs3804099*, TLR4 *rs4986790*, TLR4 *rs4986791*, TLR4 *rs10759932*, TLR4 *rs1927914* and TLR9 *rs352140* are associated and increased susceptibility to *H. pylori* infections. Possible reasons for discrepant findings in different

studies may be variances in population size, age and sex, ethnicity and race, methods of diagnosis of infection and genotyping, and differences in the prevalence of *H. pylori* infection. The results of this meta-analysis led to the widely accepted conclusion that *H. pylori* infections are associated with TLRs genetic variations. In conclusion, evidences support the important role of TLRs in *H. pylori* infection as these receptors of the innate immune system have been shown to recognize diverse components of *H. pylori*, the major risk factor for gastric cancer. Given that host genetic

variability in the TLRs are known to be associated with an increased risk of *H. pylori* infection, this knowledge has the potential to allow better prevention of *H. pylori* infection and subsequently gastric cancer through selective treatment and surveillance of individuals harboring high risk genetic profiles.

Conflic of Interest

None.

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