INTRODUCTION

Helicobacter pylori are a kind of microaerobic gram-negative bacteria, which invade the body and mainly settle in gastric mucosa. About 50% of people in the world are infected with Helicobacter pylori, and the average rate is about 60% in China, with more than 10 million people becoming newly infected every year. The World Health Organization (WHO) identified Helicobacter pylori as a class I carcinogen strongly associated with bacterial infection and gastric cancer. The occurrence and development of some common stomach diseases, such as chronic atrophic gastritis, intestinal metaplasia, peptic ulcer, and gastric cancer, are closely associated with Helicobacter pylori infection. Therefore, the accuracy of risk assessment of Helicobacter pylori infection can be improved and individualized health education can be provided for patients with Helicobacter pylori infection by nurses.
infection rate increases sharply in childhood. According to statistics, about 50% of the world’s population is infected with Helicobacter pylori. Helicobacter pylori infection rate has obvious regional differences, and is related to economic conditions and living habits. In developed countries, the carrier rate of Helicobacter pylori is generally less than 30% in healthy people, while in developing countries; the carrier rate is generally about 50%–70%. Although Helicobacter pylori infection rate is very high, less than 3% of patients eventually develop gastric cancer, indicating that host factors play an important role in H. pylori infection-related diseases. Toll-like receptor (TLR) is a pattern recognition receptor currently discovered, which can specifically recognize bacteria, viruses and other pathogenic microorganisms, and then activates the body’s non-specific immune response to pathogenic microorganisms. At present, more and more evidences show that TLR is closely related to the occurrence and development of Helicobacter pylori infection-related diseases, and the gene polymorphism of TLR can affect the host’s genetic susceptibility to helicobacter pylori infection-related diseases.

TLR4 gene is located at chromosomal locus 9q33.1 and encodes three exons. TLR4 can widely recognize ligands in non-specific immunity, making it one of the most studied molecules. SNP rs4986790 is a non-synonymous SNP located in the third exon of TLR4, which can affect the stability of the extracellular domain of TLR4 and lead to adenine-guanine (A-G) base exchange, thus eventually leading to amino acid replacement, namely glycine replacing aspartic acid. Substitution of amino acids may reduce the binding of TLR4 to ligands and the host’s responsiveness to lipopolysaccharides, thus reducing the transport of TLR4 to the cell surface. Since TLR4 may have a role in Helicobacter pylori, any mutations of this gene influencing the output of TLR4 may be of alternative risk factors for the occurrence of Helicobacter pylori infection. Some studies have indicated the association between TLR4 gene rs4986790 polymorphism and Helicobacter pylori risk. But other studies hold the controversial idea. Furthermore, genome-wide association studies (GWASs) and meta-analysis has not been conducted for this association. Thus, we performed the case-control study and meta-analysis to explore whether TLR4 gene rs4986790 polymorphism confers risk to Helicobacter pylori infection. Combined with individual gene polymorphism, the accuracy of risk assessment of Helicobacter pylori infection can be improved and individualized health education can be provided for patients.

2 | MATERIALS AND METHODS

2.1 | Research subjects

From February 2021 to December 2021, 489 cases of physical examination were performed in physical examination Center of Zhejiang Provincial People’s Hospital. 254 patients were Helicobacter pylori positive. No antibiotics, bismuth or non-steroidal anti-inflammatory drugs were taken 2 weeks before physical examination. Helicobacter pylori infection was confirmed by 13C-urea breath test (UBT). The present study was approved by the Ethics Committee of Zhejiang Provincial People’s Hospital, People’s Hospital of Hangzhou Medical College. The patients and healthy controls all provided informed consent. This investigation was conducted based on the principles of the declaration of Helsinki.

2.2 | Polymorphism genotyping

2 ml of EDTA-K2 anticoagulant venous blood was extracted and stored at ~80°C for future use. Whole blood genome DNA was extracted nucleoid extraction kit using Bioteke magnetic bead method (Thermo Scientific, Waltham, Massachusetts, USA). The TLR4 gene rs4986790 was genotyped by the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method. Table 1 shows the sequences of all primers and the products sizes.

2.3 | Data Collection

PubMed, EMBASE, the Cochrane library, Google academic, and CNKI (Chinese national knowledge internet) were independently searched by two authors. Search time begins at database foundation and ends at March 2022. And the searching keywords were as follows: “polymorphism” or “polymorphisms”, “Helicobacter pylori infection” or “Toll-like receptor-4”. Only English language studies and Chinese language studies are enrolled.

2.4 | Inclusion and exclusion criteria

The inclusion criteria are as follows: (a) a case-control study, which evaluates the association between TLR4 gene rs4986790 polymorphism and Helicobacter pylori infection risk. (b) having enough data to count OR and 95%CI. The exclusion criteria are as follows: (a) not a case-control study; (b) not having enough data to count OR and 95%CI. (c) experiment objective was animals.

2.5 | Data extraction and methodological quality assessment

All the data and information were strictly searched and evaluated by the first author and second author according to inclusion and exclusion criteria. The necessary element includes literature name, genotyping methods, control source, Newcastle-Ottawa Scale (NOS) score and HWE. If the first author and second author have different opinions, the corresponding author will join in the discussion and decide the final results. The main criteria consisted of three aspects including choice of enrolled cases and controls (0–4 scores); between-group comparability (0–2 scores); exposure outcomes together with factors (0–3 scores). Furthermore, the ethics approval and patient consent was waived by Ethics
Committee of Zhejiang Provincial People’s Hospital (Affiliated People’s Hospital, Hangzhou Medical College) because neither human beings nor animals were participated in the present meta-analysis.

### 2.6 Statistical analysis

The variables were compared using $\chi^2$ test. Both the OR and 95%CI were used to evaluate this association power. The Q-statistic and $I^2$ statistics were used to evaluate the heterogeneity degree. A total of five genetic models were used to make an assessment on the association between TLR4 gene rs4986790 polymorphism and Helicobacter pylori infection risk as the previous literature reported. The selection of fixed-effects or random-effects model was based on the degree of heterogeneity. Meta-regression was used to find the source of heterogeneity. Sensitive analysis and publication bias were based on previous meta-analysis. The Stata 15.0 was responsible for all statistical work.

### 3 RESULTS

#### 3.1 General information

Figure 1 showed the flow diagram of the present meta-analysis search process. There were seven literatures included in the meta-analysis altogether. Main data and information of all literatures

| TABLE 1 Primer sequences, product size for TLR4 rs4986790 polymorphism |
|-----------------|-----------------|-----------------|
| **polymorphism** | **Primer sequences** | **product size** |
| TLR4 rs4986790  | Forward outer: 5′-TGAACCCTATGAACTTTATCC-3′ | Common product size: 383 bp |
| | Reverse outer: 5′-GTTAACTAATTCTAAATGTTGCCATC-3′ | AA: 147 bp |
| | Forward inner(A allele): 5′-GCATACTTAGACTACCTACCTGATGA-3′ | GG: 287 bp |
| | Reverse inner(G allele): 5′-CAAACAAATAAAGTGCAATAATAC-3′ | AG: 147 bp and 287 bp |

**FIGURE 1** PRISMA 2009 Flow Diagram
were listed in Table 2. In total, seven literatures consisted of four literatures from Asian countries, two literatures from European countries and one literature from Brazil. Different genotyping methods were used such as DNA sequencing, PCR-RFLP. The publication year ranged from 2015 to 2020 and the controls were population-based or hospital-based. All the genotyping frequency of controls was conform to HWE. The results of NOS showed high-quality of all studies and average score was 8.1, which is shown in Table 3.

### 3.2 | Allele and genotype-wide meta-analysis

The positive findings between TLR4 gene rs4986790 polymorphism and Helicobacter pylori infection risk in Asian population by allele contrast (G vs. A: OR = 2.07, 95%CI = 1.23–3.49, \( p = 0.006 \), Table 4 and Figure 2), homozygote comparison (GG vs AA: OR = 3.94, 95% CI = 1.48–10.50, \( p = 0.006 \), Table 4 and Figure 3) and recessive genetic model (GG vs. GA/AA: OR = 3.97, 95% CI = 1.72–9.19, \( p = 0.001 \), Table 4 and Figure 4). Main results between TLR4 gene rs4986790 polymorphism and Helicobacter pylori infection risk were shown in Table 4.

### 3.3 | Between-study heterogeneity, sensitivity analysis and publication bias

There was no significant heterogeneity in any genetic model. Meta-regression demonstrated that ethnicity was the important factor, which takes up great heterogeneity to current results. No matter which literature was excluded, the result of our meta-analysis was unchanged, indicating the consistence and stability of the present meta-analysis. The result of sensitive analysis showed that the current results were stable. No obvious asymmetrical was found by Egger’s test (\( p = 0.560 \)).

### 3.4 | Research subjects and genotype frequency

No significant difference was found among the Helicobacter pylori infection (+) group and Helicobacter pylori infection (−) group concerning gender or age. TLR4 gene rs4986790 polymorphism is associated with Helicobacter pylori infection risk and GG genotype contributes increased risk to the population of Zhejiang region (\( p = 0.019 \)) (Table 5).

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**Table 2** Main characteristics of all case-control studies included in meta-analysis

| Literature     | Ethnics (Country) | Genotyping methods | Source of control | Sample size | HWE conformity | NOS | Genotype frequency (Case) | Genotype frequency (Control) | Year |
|----------------|-------------------|--------------------|-------------------|-------------|----------------|-----|---------------------------|-------------------------------|------|
| Moura et al.   | Brazilian (Brazil) | PCR-RFLP           | PB                | 232/104     | Yes            | 8   | 206 25 1                  | 222 28 4                      | 2008 |
| Loganathan et al. | Asian (India)     | ARMS-PCR           | PB                | 77/230      | Yes            | 8   | 45 25 7                   | 189 38 3                      | 2016 |
| Wu et al.      | Asian (China)     | allele specific PCR | HB                | 204/210     | Yes            | 9   | 204 0 0                   | 210 0 0                       | 2006 |
| Tseng et al.   | Caucasian (United states) | ARMS-PCR | HB | 36/148 | Yes | 8 | 30 6 0 | 126 21 1 | 2006 |
| Eed et al.     | Asian (Arabia)    | TaqMan             | PB                | 210/80      | Yes            | 8   | 141 39 30                | 56 21 3                        | 2021 |
| Meliţ et al.   | Caucasian (Romania) | PCR-RFLP          | PB                | 225/250     | Yes            | 9   | 180 33 12                | 198 40 12                      | 2019 |
| Tourani et al. | Asian (Iran)      | PCR-RFLP          | PB                | 56/44       | Yes            | 7   | 50 6 0                   | 41 3 0                        | 2017 |

**Table 3** Quality assessment of the seven case-control studies according to the Newcastle-Ottawa Scale

| Literature     | selection of enrolled study subjects | between-group comparability | exposure outcomes and factors | Total |
|----------------|--------------------------------------|-------------------------------|-------------------------------|-------|
| Moura et al.   | 3                                    | 2                             | 3                             | 8     |
| Loganathan et al. | 3                                 | 2                             | 3                             | 8     |
| Wu et al.      | 4                                    | 3                             | 2                             | 9     |
| Tseng et al.   | 3                                    | 2                             | 3                             | 8     |
| Eed et al.     | 3                                    | 2                             | 3                             | 8     |
| Meliţ et al.   | 4                                    | 3                             | 2                             | 9     |
| Tourani et al. | 2                                    | 2                             | 3                             | 7     |
| Average        | 3.1                                  | 2.3                           | 2.7                           | 8.1   |
**TABLE 4** Meta-analysis of the TLR4 rs4986790 polymorphism and Helicobacter pylori infection risk

| Comparison          | Population | N | Test of association | Test of heterogeneity |
|---------------------|------------|---|---------------------|-----------------------|
|                     |            |   | OR  | 95%CI | P  | Mode  | χ²  | P  | I²  |
| G versus A          | Overall    | 7 | 1.36 | 0.87-2.12 | 0.179 | Random | 19.47 | 0.003 | 69.2 |
|                     | Asian      | 4 | 2.07 | 1.23-3.49 | 0.006 | fixed | 5.21 | 0.157 | 42.4 |
|                     | Caucasian  | 2 | 1.00 | 0.70-1.43 | 0.998 | fixed | 0.03 | 0.865 | 0   |
|                     | Brazilian  | 1 | 0.81 | 0.48-1.36 | 0.423 | /     | /    | /    | /   |
| GG versus AA        | Overall    | 7 | 1.98 | 0.78-4.98 | 0.149 | Random | 12.28 | 0.056 | 51.2 |
|                     | Asian      | 4 | 3.94 | 1.48-10.50 | 0.006 | fixed | 3.72 | 0.293 | 19.4 |
|                     | Caucasian  | 2 | 1.22 | 0.55-2.70 | 0.616 | fixed | 0.81 | 0.368 | 0   |
|                     | Brazilian  | 1 | 0.27 | 0.03-2.43 | 0.243 | /     | /    | /    | /   |
| GA versus AA        | Overall    | 7 | 1.18 | 0.78-1.77 | 0.437 | fixed | 11.67 | 0.070 | 48.6 |
|                     | Asian      | 4 | 1.43 | 0.60-3.42 | 0.419 | Random | 9.17  | 0.027 | 67.3 |
|                     | Caucasian  | 2 | 0.96 | 0.61-1.51 | 0.862 | fixed | 0.24 | 0.622 | 0   |
|                     | Brazilian  | 1 | 0.96 | 0.54-1.70 | 0.895 | /     | /    | /    | /   |
| GG versus GA/AA     | Overall    | 7 | 1.93 | 0.81-4.62 | 0.137 | fixed | 11.05 | 0.087 | 45.7 |
|                     | Asian      | 4 | 3.97 | 1.72-9.19 | 0.001 | fixed | 3.05  | 0.384 | 1.6  |
|                     | Caucasian  | 2 | 1.24 | 0.56-2.72 | 0.595 | fixed | 0.76 | 0.383 | 0   |
|                     | Brazilian  | 1 | 0.27 | 0.03-2.44 | 0.244 | /     | /    | /    | /   |
| GG/GA versus AA     | Overall    | 7 | 1.30 | 0.85-2.01 | 0.230 | Random | 14.48 | 0.025 | 58.6 |
|                     | Asian      | 4 | 1.81 | 0.89-3.70 | 0.102 | Random | 7.00  | 0.072 | 57.1 |
|                     | Caucasian  | 2 | 0.98 | 0.65-1.48 | 0.932 | fixed | 0.11 | 0.738 | 0   |
|                     | Brazilian  | 1 | 0.637 | /     | /   | /    | /    | /    | /   |

Abbreviations: CI, confidence interval; OR, odds ratio.
Results with significant correlation (p < 0.05) are emphasized by the bold values.

**FIGURE 2** Forest plot for the associations between TLR4 gene rs4986790 polymorphism and Helicobacter pylori infection risk through allele contrast (G vs A). OR, odds ratio; CI, confidence interval
DISCUSSION

The basic research on Helicobacter pylori infection has yielded remarkable results in recent years; it has also risen to the search for genes. However, Helicobacter pylori infection is a complex inflammatory response in the body, and it is difficult to explain the impact of Helicobacter pylori infection based on a single cytokine alone. At present, most studies have been exploring the association between genetic polymorphism and diseases susceptibility, so as to further clarify the factors related to Helicobacter pylori infection and have a deeper understanding of Helicobacter pylori infection from people's individual differences. Helicobacter pylori infection is associated with the occurrence and development of chronic atrophic gastritis, intestinal metaplasia, peptic ulcer and gastric cancer. Studies have shown that TLR gene polymorphism plays an important role in Helicobacter pylori infection and clinical outcome after infection8, which can affect the host's genetic susceptibility to helicobacter pylori infection and related diseases to a certain extent.

TLR4 gene rs4986790 polymorphism confers different susceptibility on various diseases. A number of studies have shown that TLR4 rs4986790 polymorphism is associated with risk of multiple diseases such as pneumonia, open angle glaucoma, cancer, Type 2 diabetes and Inflammatory Bowel Disease21-24. And TLR4 rs4986790 polymorphism is not associated with risk of asthma, coronary artery disease, atherosclerosis or preeclampsia25-28. The present meta-analysis shows that TLR4 gene rs4986790 polymorphism is associated with Helicobacter pylori infection risk in Asian population. We conducted a case-control study to investigate whether TLR4 gene rs4986790 polymorphism confers risk to Helicobacter pylori infection for the population of Zhejiang region. In the present study, TLR4 gene rs4986790 polymorphism is associated with Helicobacter pylori infection risk and GG genotype contributes increased risk to the population of Zhejiang region. As far as we know, the present study is the first to investigate the TLR4 gene rs4986790 polymorphism with Helicobacter pylori infection risk in Chinese mainland. Our results are conforming to the results of Asian population because Zhejiang is a part of China and China is a
typical Asian country. Surprisingly, the literature published by Wu et al suggested that TLR4 gene rs4986790 polymorphism was not associated with Helicobacter pylori infection for the population of Taiwan region. We consider the difference may derive from regional difference or areal variation. China is a vast country with 56 ethnic groups. Different nationalities, different regions may bring different polymorphisms.

Gene, race, age and sex are important factors that determine susceptibility to Helicobacter pylori infection. With the development of global integration, the population of different countries has become more and more frequent, and the ethnic types of resident population in the region have become more and more diverse. Therefore, nurses should pay more attention to the evaluation of ethnic differences in Helicobacter pylori infection risk assessment.
For example, the GG genotype of TLR4 rs4986790 polymorphism is related to the incidence of Helicobacter pylori infection in Chinese Han population. Nurses can take these factors into consideration in risk assessment to judge the risk of Helicobacter pylori infection in Chinese with GG genotype, thus improving the accuracy of risk assessment. Nurses are the largest health care practitioners and through their care activities can apply the results of genetic laboratory research to clinical applications. Health education is an important way. Change bad living habits, such as smoking, drinking, high-salt and high-fat diet, lack of exercise, etc., and cultivate scientific harmony and rational life style is an important content of hypertension health education. Combining with gene polymorphism, health education can be personalized and improved to learn. Patients with GG genotype are more suffered from Helicobacter pylori infection. Therefore, nurses can focus on health education according to individual genotype differences, and improve the scientific nature of health education through the interpretation of gene polymorphism, so as to improve patient compliance and self-risk management ability.

There were several potential limitations we should acknowledge. Firstly, all PNS patients with Helicobacter pylori infection were from one hospital and our results, conclusions may not be representative for other regions. Secondly, small sample size may bring some bias to our results.

5 | CONCLUSIONS

TLR4 gene rs4986790 polymorphism is associated with Helicobacter pylori infection risk for population of Zhejiang, China. Combined with individual gene polymorphism, the accuracy of risk assessment of Helicobacter pylori infection can be improved and individualized health education can be provided for patients with Helicobacter pylori infection by nurses.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Meina Jiang conceived study design and content concept; Wenjuan He performed the data collection, extraction and analyzed the data. Meina Jiang interpreted and reviewed the data and drafts. Meina Jiang reviewed the final draft. All authors were involved in literature search, writing the paper and had final approval of the submitted and published versions.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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