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Toll-like receptor 1 and *10* polymorphisms, *Helicobacter pylori* susceptibility and risk of gastric lesions in a high-risk Chinese population

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ABSTRACT

Genetic polymorphisms of Toll-like receptor (TLR) 1 and 10 may influence *Helicobacter pylori* (*H. pylori*) susceptibility. To evaluate associations between *TLR1* and *10* polymorphisms, *H. pylori* infection, and precancerous gastric lesions, a population-based study was conducted in a high-risk Chinese population.

Three single-nucleotide polymorphisms, *TLR1* rs4833095, *TLR10* rs10004195, and *TLR10* rs4129009 were genotyped by TaqMan SNP genotyping assay in 2553 participants with diverse gastric lesions. The status of *H. pylori* infection was determined by ^{13}C-urea breath test.

TLR1 rs4833095 T and *TLR10* rs10004195 T alleles were the minor alleles and showed in linkage disequilibrium (D' = 0.98, r^2 = 0.73) in the Chinese population. A decreased risk of *H. pylori* infection was observed in subjects with *TLR1* rs4833095 CT genotype [adjusted odds ratio (OR) = 0.80; 95% confidence interval (CI): 0.66–0.96] or T allele (OR = 0.82; 95%CI: 0.69–0.99). Moreover, subjects carrying *TLR1* rs4833095 TT genotype were associated with reduced risks of chronic atrophic gastritis (CAG, OR = 0.66; 95%CI: 0.45–0.97) and intestinal metaplasia (IM, OR = 0.57; 95%CI: 0.36–0.90). The risk of CAG was also decreased in subjects carrying *TLR10* rs10004195 T allele (OR = 0.75; 95%CI: 0.57–0.99). Furthermore, haplotype analysis indicated that haplotype TT of rs4833095 and rs10004195 had a protective effect on *H. pylori* infection (OR = 0.83; 95%CI: 0.72–0.96) or precancerous gastric lesions (OR = 0.78; 95%CI: 0.64–0.96 for CAG, and OR = 0.74; 95%CI: 0.57–0.96 for IM).

These findings suggest that *TLR1* rs4833095 and *TLR10* rs10004195 may play crucial roles in *H. pylori* susceptibility and gastric pathogenesis.

1. Introduction

Helicobacter pylori (*H. pylori*), a Gram-negative bacterium, has been established as the most important risk factor for gastric cancer (GC) (Kusters et al., 2006; Suerbaum and Michetti, 2002). Persistent *H. pylori* infection induces chronic inflammation in gastric mucosa that plays a fundamental role in evolution of superficial gastritis (SG), chronic atrophic gastritis (CAG), intestinal metaplasia (IM) and dysplasia (DYS) (Correa, 1988; Peek and Blaser, 2002). Interestingly, a small proportion of individuals are never infected with *H. pylori* (Bardhan, 1997), suggesting that host genetic variants may influence *H. pylori* susceptibility and gastric pathogenesis.

Toll-like receptors (TLRs) constitute a family of pattern recognition receptors (PRRs) and participate in immune responses against infection (Akira et al., 2006). As the type I transmembrane proteins, TLRs can recognize a wide variety of conserved pathogen-associated molecular patterns expressed by pathogens including *H. pylori* (Kutikhin, 2011). Recognition of specific ligands by TLRs leads to activation of transcription factors such as nuclear factor kappa B (NF-κB), and subsequently stimulates the production of various inflammatory related cytokines and chemokines (Gay et al., 2014; Kawai and Akira, 2011). Currently, 10 members of TLRs family (TLR1 to TLR10) have been identified in humans (Takeuchi and Akira, 2010).

Abbreviations: CAG, chronic atrophic gastritis; CI, confidence interval; ^{13}C-UBT, ^{13}C-urea breath test; DYS, dysplasia; GC, gastric cancer; *H. pylori*, *Helicobacter pylori*; IM, intestinal metaplasia; Ind DYS, indefinite DYS; LD, linkage disequilibrium; MAF, minor allele frequency; OR, odds ratio; SG, superficial gastritis; SNP, single-nucleotide polymorphism; TLR, Toll-like receptor.

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Genetic variants in TLRs are proposed to play crucial roles in susceptibility to *H. pylori* infection and process of *H. pylori*-induced gastric carcinogenesis (Netea et al., 2012; Schroder and Schumann, 2005). Indeed, accumulated evidences have suggested that genetic polymorphisms and consequently gene expression of TLR2, 4, 5, 9 can influence *H. pylori*-related gastric lesions (Castaño-Rodriguez et al., 2014). Our previous study has also shown that *TLR2* and *5* polymorphisms may play important roles in *H. pylori*-associated GC and its precursors (Zeng et al., 2011).

A limited number of studies have reported that genetic variants of *TLR1* were associated with *H. pylori*-associated gastric lesions (Cabrera-Andrade et al., 2014; Yang et al., 2013), much less study on *TLR10* polymorphisms in association with gastric lesions. Recently, a genome-wide association study (GWAS) identified single-nucleotide polymorphisms (SNPs) in *TLR1* and *10* significantly associated with *H. pylori* susceptibility in European population (Mayerle et al., 2013). However, these findings need to be further confirmed and generalized to other ethnic population, particularly in their relationships with gastric pathogenesis.

In our current study, we focused on functionally relevant SNPs mapped to TLR1/TLR10 gene cluster on chromosome 4p14 with minor allele frequency (MAF) over 0.05 in the Chinese population. *TLR1* rs4833095 (Ser248Asn), *TLR10* rs10004195 on promoter region, and *TLR10* rs4129009 (Ile775Val) were finally selected. We were interested in the hypothesis that these polymorphisms are associated with *H. pylori* susceptibility and precancerous gastric lesions, and testified it in a Chinese population from Linqu County, Shandong Province of China, a rural area with high risk of GC (Ma et al., 2012; Wong et al., 2012; You et al., 2006).

2. Materials and methods

2.1. Study subjects and design

In 2002, we launched an intervention trial in Linqu (Wong et al., 2012). A total of 2813 subjects representing 89% eligible residents aged 35 to 64 years from 12 villages were selected at random and given upper endoscopic as well as *H. pylori* infection examinations at baseline. Information on age, gender, cigarette smoking history and use of antibiotics for each participant was obtained by a standard structured questionnaire. For the current study, a total of 2553 cancer-free participants who had complete baseline data in diagnoses for *H. pylori* infection and gastric histopathology were included. The study was approved by the Institutional Review Board of Peking University School of Oncology and University of Hong Kong. Written informed consent was obtained from each participant.

2.2. Gastric histopathology

Details on gastroscopic procedures and histopathologic criteria have been described elsewhere (Wong et al., 2012). Briefly, for each participant, five biopsies were taken from standard sites of stomach following the Updated Sydney System (Dixon et al., 1996). Histopathologic diagnosis for each biopsy specimen was classified as normal, SG, CAG, IM, indefinite dysplasia (Ind DYS), DYS or GC by three senior pathologists independently according to the Padova International Classification (Rugge et al., 2000). A global diagnosis based on the most severe diagnosis among five biopsy specimens was assigned to each participant.

2.3. ^{13}C-urea breath test

Method of ^{13}C-urea breath test (^{13}C-UBT) was described in previous publications (Klein et al., 1996; You et al., 1998). In brief, baseline samples of exhaled CO_2 were collected after participants fasted overnight. Each participant was requested to swallow a pill of 80 mg ^{13}C-urea (Min. 99 at.% ^{13}C). The samples of exhaled CO_2 were taken after 30 min. $^{13}CO_2$ values were measured by a gas isotopic ratio mass spectrometer, and any concentration of $^{13}CO_2$ at 30 min that exceeded the baseline concentration by more than four parts per 1000 (>4.0‰) was regarded as a positive result.

2.4. DNA preparation and SNPs genotyping

A 5 ml blood sample collected at baseline from each subject was allowed to clot for 30–40 min at room temperature and then centrifuged at 965*g* for 15 min. The resulting serum was separated into vials. The clot and serum were frozen immediately at −20 °C and stored in a −70 °C freezer. Genomic DNA was isolated from the blood sample by standard proteinase K digestion and phenol–chloroform extraction protocol (You et al., 2005). Genotyping for rs4833095, rs10004195, and rs4129009 were performed by TaqMan predesigned SNP genotyping assays in an ABI 7000 real-time polymerase chain reaction (PCR) system, according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). Briefly, primers and probes were supplied by Applied Biosystems, and the PCR conditions were as follows: 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The success rate of genotyping for each SNP was more than 98%. Negative controls and duplicate samples were used to check the accuracy of genotyping.

2.5. Statistical analysis

Hardy–Weinberg equilibrium of genotypes distribution was tested by the goodness-of-fit χ^2 test. Smoking and use of antibiotics were regarded as the dichotomous variable, respectively. Wilcoxon rank-sum test and Pearson's χ^2 were used to examine the differences for age, gender, smoking, use of antibiotic and gastric lesions in the status of *H. pylori* infection. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression for candidate SNPs in associations with *H. pylori* infection and precancerous gastric lesions. For SNP-*H. pylori* joint effect analysis, we generated a composite variable by assembling levels of the two combined factors, and ORs with 95%CI were estimated by adding the composite term into the unconditional logistic model. The multiplicative interactions of SNP-*H. pylori* were evaluated by adding their cross-product terms into the model, and additive interactions were assessed using the algorithm of Andersson (Andersson et al., 2005). All statistical tests were two-sided and *P* value <0.05 was considered statistically significant. Statistical analyses were conducted by STATA software (version 12.0; StataCorp LP, College Station, TX, USA). Linkage disequilibrium (LD) and haplotype analyses were performed by Haploview software (version 4.2, Broad Institute, Cambridge, MA, USA).

3. Results

A total of 2553 subjects were enrolled, including 1511 (59.2%) with *H. pylori*-positive and 1042 (40.8%) with *H. pylori*-negative. Significant differences in age, use of antibiotic and gastric lesions were observed in different status of *H. pylori* infection. Because of a few subjects with normal gastric mucosa, we combined it with SG as one group. Similarly, the subjects with Ind DYS were combined with DYS. The proportions of gastric lesions were 15.6% for SG, 33.3% for CAG, 16.4% for IM, and 34.7% for DYS. Baseline characteristics of the subjects were presented in Table 1 and Supplementary Table 1.