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Gastrointestinal Tract

Role of toll-like receptor 10 gene polymorphism and gastric mucosal pattern in patients with chronic gastritis

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ABSTRACT

Background/Aims: *Helicobacter pylori* stimulates the host's toll-like receptors (TLRs). Single-nucleotide polymorphism (SNP) of TLRs is related to the manipulation of regulatory cytokines and also implicated in the varied outcomes of the inflammatory response, including the development of precancerous lesions of gastric mucosa and disease progression. We hypothesized that TLR10 *rs10004195* polymorphism is associated with gastric mucosal patterns.

Materials and Methods: TLR10 *rs10004195* polymorphisms were identified in a total of 400 gastritis patients using the TagMan SNP genotyping assay. Gastric mucosal patterns were classified by Conventional Narrow Band Imaging gastroscopy (C-NBI gastroscopy). Logistic regression was used to analyze the association.

Results: The gastritis patients was Type 1, 37.5% of Thai patients. The T/T homozygous genotype was exhibited by the highest percentage (46.5%) of patients, and the A/A homozygous and A/T heterozygous genotypes were exhibited by 20.25% and 33.25%, respectively, of patients. TLR10 rs10004195 was significantly associated with gastric mucosal patterns. After adjusting for confounding factors, patients with the A/A homozygous genotype showed a significantly increased risk of severe inflammation (OR=1.35, 95% CI=0.97-2.13, p=0.028). Patients with the A/T heterozygous and T/T homozygous genotypes showed a significantly increased risk of mild inflammation (OR=1.24, 95% CI=0.78-2.07, p=0.042 and OR=1.78, 95% CI=0.51-3.35, p=0.001, respectively).

Conclusion: Our results indicate that the presence of TLR10 *rs10004195*, A/T heterozygous, and T/T homozygous genotypes is associated with type 1, 2, and 3 whereas that of the A/A homozygous genotype is associated with type 4 and 5 of gastric mucosal patterns. This suggests that the A/A homozygous genotype contributes to severe inflammation in *H. pylori*-associated gastritis in Thai patients.

Keywords: Toll-like receptor 10, genetic polymorphism, *Helicobacter pylori*, gastric mucosal pattern

Original Article

INTRODUCTION

Toll-like receptors (TLRs) are pattern-recognition receptors in immune responses against infection (1). They recognize a variety of pathogen-associated molecular patterns (PAMPs) expressed by pathogens such as *Helicobacter pylori* (2). Recognition of TLRs and PAMPs leads to activation of transcription factors such as NF-κB and the subsequent stimulation of the production of inflammatory cytokines and chemokines (3,4). TLR10 is located on the cell surface; it is an anti-inflammatory pattern-recognition receptor (5). TLR10 is expressed in gastric epithelial cells during gastric mucosa infection. It has been concluded that TLR10 is related to the innate immune response to *H. pylori* infection and signaling of the TLR2/TLR10 heterodimer during recognition of *H. pylori* lipopolysaccharides and also heterodimers of TLR6/TLR10 . These combinations facilitate the multiple distinct bacterial patterns to recognize of diversify innate immune recognition (6-10).

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Genetic variants in TLRs play crucial roles in affecting *H. pylori* susceptibility of the hosts and the process of *H. pylori*-induced gastric carcinogenesis (11,12). Recently, a genome-wide association study (GWAS) found the presence of single-nucleotide polymorphisms (SNPs) in TLR10 to be significantly associated with *H. pylori* susceptibility in the European population (13). Few studies on TLR10 polymorphisms associated with the gastric pathology have been reported. Polymorphisms in TLR10 *rs10004195* and *rs4129009* have been identified. These polymorphisms have a known direct association with *H. pylori* susceptibility in the Chinese population; the *rs10004195* T allele has been shown to decrease the risk of chronic atrophic gastritis, and the haplotype TT of *rs4833095* and *rs10004195* has been shown to have a protective effect on *H. pylori* infection or precancerous gastric lesions (14). The *rs10004195* T allele did not protect against GC development in Malaysian patients, whereas the haplotype AA of *rs10004195* was associated with an increase in the incidence of GC in patients with *H. pylori* infection (15). However, further confirmation with respect to other populations, particularly pertaining to the relationship of these genotypes with the gastric pathology, is needed. The impact of TLR polymorphisms on the *H. pylori*-related pathologic process or inflammation in *H. pylori*-infected gastric mucosa has not yet been established in Thai patients.

Therefore, we hypothesized that genetic polymorphisms in TLR10 *rs10004195* genes are correlated with *H. pylori*-related inflammation in the gastric mucosa pattern using the conventional NBI endoscopy technique.

MATERIALS AND METHODS

Patients

A total of 400 patients undergoing esophagogastroduodenoscopy for the investigation of chronic abdominal pain were enrolled in this study from December 2014 to March 2016. The following exclusion criteria were applied: patients whom *H. pylori* eradication in the past 2 months, significant medical illnesses, history of gastric surgery, and use of antimicrobials or gastrointestinal medications such as PPIs or bismuth compounds within the previous 2 months. The study was performed according to good clinical practices and the guidelines of the Declaration of Helsinki. All patients provided written informed consent, and the study protocol was approved by the Ethics Committee for Research Involving Human Subjects at the Suranaree University of Technology (EC-58-58 and EC-58-59).

Biopsy Specimens

The esophagogastroduodenoscopy procedures were performed using an upper GI video endoscope (Olympus EVIS EXERA III, CV-190, Japan). The whole stomach was examined with conventional endoscopy and biopsies using the site-specific biopsy technique (16). Gastric tissue specimens were sent to the pathologists for histological analysis. The hematoxylin and eosin and Giemsa stains were used for *H. pylori* identification.

Image Evaluation

Gastroscopic examinations were digitally recorded, and still images of the observation sites were captured for the reproducibility study. The images were transferred to a software program without distorting brightness, contrast, or color balance. A total of 400 pictures from 400 patients were selected for the inter- and intraobserver agreement study. All endoscopists were blinded to the results of the *H. pylori* status and histology before reviewing the gastroscopic pictures.

DNA Preparation

Genomic DNA of 400 specimens was extracted from formalin-fixed, paraffin-embedded (FFPE) tissue using the QIAamp DNA FFPE tissue kit (Qiagen, Duesseldorf, Germany). Genomic DNA extraction was performed according to the manufacturer's instructions. Briefly, deparaffinize the paraffin-embedded tissues in xylene and hydrate in 100% ethanol and subsequently digested by lysis buffer and proteinase K. Genomic DNA was purified from the tissue lysate using the QIAamp spin column and eluted and stored at -20°C.

Genotyping Assay

Single-nucleotide polymorphisms located in TLR10 *rs10004195* (A>T) were selected from the SNP database of the National Center for Biotechnology Information. The genotype of TLR polymorphism was determined by TagMan allelic discrimination using the predesigned custom TagMan SNP genotyping assay by real-time PCR according to the manufacturer's instructions (LightCycler® 480 II Instrument, Roche diagnostics, Neuilly sur Seine, France). Forward and reverse primers were used along with the wild-type probe VIC and probe FAM for variant alleles (Applied Biosystems). Briefly, the PCR conditions were as follows: 95°C for 10 min, 55 cycles of 95°C for 15 s and 60°C for 1 min. Negative controls and duplicate samples were checked for the accuracy of genotyping. The genotypes of polymorphisms were analyzed by using the LightCycler® 480 Software 1.5 (Roche diagnostics, Neuilly sur Seine, France). The success rate of genotyping was more than 94%.

Statistical Analysis

Statistical Package for Social Sciences version 20.0 (IBM Corp.; Armonk, NY, USA) used for Windows was used for statistical analysis. The association between genotypes of TLR10 and gastric mucosal patterns was analyzed using logistic regression, with significance set at $p<0.05$. The association was expressed as odds ratios (OR) with their confidence intervals (95% CI). Adjustments for confounding factors were performed by incorporating all the relevant factors, such as age and sex, into the analysis.

RESULTS

In this study, we examined the possible association of polymorphisms of genes involved in *H. pylori*-induced inflammation with gastric mucosal patterns. We studied the polymorphism in the TLR10 *rs10004195* gene. A total of 400 patients comprising 136

males and 264 females aged between 17 and 80 years (mean±SD: 44.6±15.9) were included. TLR10 *rs10004195* was genotyped, and its genotypic frequencies are summarized in Table 1. The T/T homozygous genotype was exhibited by the highest percentage of Thai patients. It was 46.5% of T/T homozygous while 20.25 and 33.25% of A/A homozygous and A/T heterozygous, respectively**.** TLR10 *rs10004195* is shown in Figure 1.

Table 1. Demographic data, TLR10 SNP *rs10004195*, and gastric mucosal patterns of gastritis patients

Characteristics	N=400
Male/female (n)	136/264
Mean age±SD (years)	44.6±15.9
TLR10 AA genotype	81 (20.25%)
AT genotype	133 (33.25%)
TT genotype	186 (46.5%)
Gastric mucosal patterns	
Type 1	150 (37.5%)
Type 2	94 (23.5%)
Type 3	98 (24.5%)
Type 4	31 (7.75%)
Type 5	27 (6.75%)

N: number of patients; SD: standard deviation

The gastric mucosal patterns were classified according to inflammation grading designed as follows: type 1, regular arrangement of collecting venules; type 2, cone-shaped gastric pits; type 3, rod-shaped gastric pits with prominent sulci; type 4, ground glass-like morphology; and type 5, dark brown patches with a bluish margin and irregular border (16). Our results showed that gastritis patients was Type 1 gastric mucosal pattern, 37.5% of Thai patients. Type 2 and 3 was 23.5% and 24.5% while type 4 and 5 as tended to be severe inflammation was 7.05% and 6.75%, respectively (Table 1). Genotypes frequencies of patients among gastric mucosal patterns showed that the highest frequencies, 71% of type 4 and 63% of type 5 belong to A/A homozygous genotype. The T/T homozygous genotype was highly exhibited by 59%, 49%, and 42% belong to type 1, 2, and 3, respectively (Table 2). However, distribution of A/T heterozygous genotype was present in 36%, 46%, and 28% of patients with type 1, 2, and 3 (Figure 2).

Table 2. Association between TLR10 SNP *rs10004195* and gastric mucosal patterns using C-NBI endoscopy

	Gastric mucosal patterns, N(%)							
TLR10 genotype	**Type 1 (N=150)**	**Type 2 (N=94)**	**Type 3 (N=98)**	**Type 4 (N=31)**	**Type 5 (N=27)**	**OR**	**95% CI**	**p**
AA homozygous	7 (5)	5 (5)	30 (31)	22 (71)	17 (63)	1.35	0.97-2.13	0.028
AT heterozygous	54 (36)	43 (46)	27 (28)	5 (16)	4 (15)	1.24	0.78-2.07	0.042
TT homozygous	89 (59)	46 (49)	41 (42)	4 (13)	6 (22)	1.76	0.51-3.53	0.001

N: number of patients; OR: odds ratios; CI: confidence intervals; C-NBI: Conventional Narrow Band Imaging

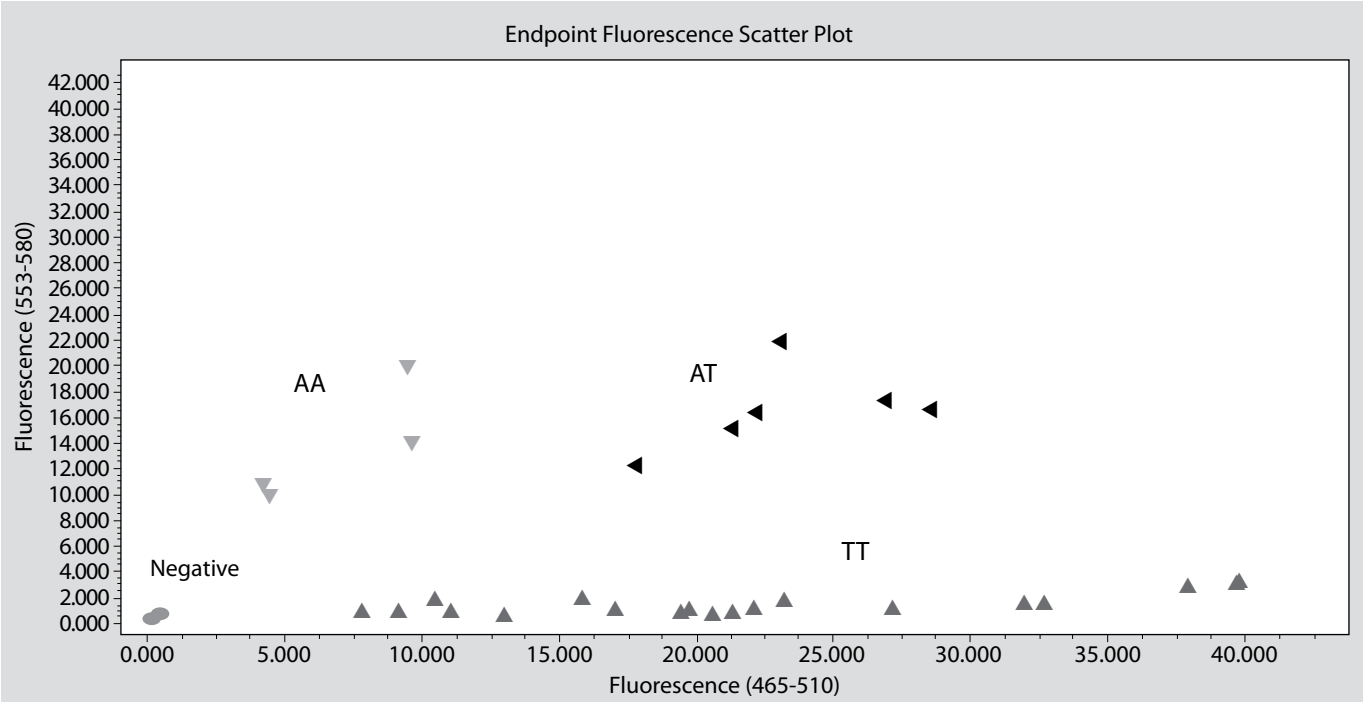

Figure 1. The TLR10 SNP *rs10004195* AA, AT, and TT
SNP: single-nucleotide polymorphism

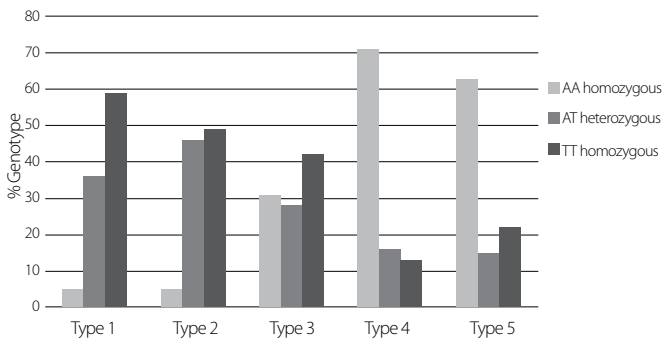

Figure 2. Genotype distribution and percentage of patients belong to each type of gastric mucosal patterns. A/A homozygous genotype showed high frequencies of patients with type 4 and type 5. The T/T homozygous genotype was highly exhibited type 1, 2, and 3. The A/T heterozygous genotype was present in patients with type 1, 2, and 3

In logistic regression analysis, TLR10 *rs10004195* was found to be significantly associated with gastric mucosal patterns. After adjusting for confounding factors, patients with the A/A homozygous genotype showed a significantly increased risk of severe inflammation (OR=1.35, 95% CI=0.97-2.13, p=0.028). Patients with the A/T heterozygous and T/T homozygous genotypes showed a significantly increased risk of mild inflammation (OR=1.24, 95% CI=0.78–2.07, p=0.042 and OR=1.78, 95% CI=0.51-3.35, p=0.001, respectively) (Table 2).

DISCUSSION

TLR10, an anti-inflammatory pattern-recognition receptor, is known to be expressed in various cells of the stomach as a result of *H. pylori* infection (5). TLR10 polymorphisms identified using GWAS exhibited a significant association with *H. pylori* susceptibility in the European population (13). Polymorphisms in TLR10 *rs10004195* are known to have a direct association with *H. pylori* susceptibility and the gastric pathology in the Chinese population (14).

In this study, we examined the possible association of polymorphisms in the TLR10 gene involved in *H. pylori*-induced inflammation with gastric mucosal patterns. The polymorphisms in TLR10 r*s10004195* showed that T/T homozygous was a common genotype exhibited by Thai patients. The following SNPs showed an association between the genotype and gastric mucosal patterns: A/T heterozygous and T/T homozygous were associated with types 1, 2, and 3, whereas A/A homozygous was associated with types 4 and 5. This suggested that the A/A homozygous genotype contributes to severe inflammation in *H. pylori*-associated gastritis in Thai patients. The A/T heterozygous and T/T homozygous genotypes may contribute to protection against the inflammation process. The patients with T/T genotype in this study is to provide probable support for recent conclusion that the TLR10 *rs10004195*, T allele decreased risk of chronic atrophic gastritis and haplotype TT had a protective effect on *H. pylori* infection or precancerous gastric lesions (14). Ravishankar Ram et al. (15) found that individuals with the *rs10004195* T allele were not protected against GC development in Malaysian patients and the haplotype AA of *rs10004195* was associated with an increase in GC irrespective of *H. pylori* infection. However, less number of Thai patients belong to type 4 and type 5 than of the other types. These patients are supposed to provide a chance for developing severe inflammation for GC. This may explain the low incidence of GC in Thailand. Therefore, TLR10 *rs10004195* in Thai population is suggested to support the "Thailand enigma" phenomenon. However, in the Chinese and European population, the T and A alleles of *rs10004195* have been reported to be associated with *H. pylori* susceptibility (13,14).

In addition, SNP in TLR10, *rs10004195*, may influence TLR1/2-mediated immune responses (17). Thus, these variants may alter the recognition of *H. pylori* by TLR1/2, resisting *H. pylori* colonization in the gastric mucosa, and subsequently reduce the severity of gastric inflammation. Further studies on the TLR10 expression of these polymorphisms on gastric epithelial cells and the colonization of *H. pylori* are needed.

Our study provided evidence that TLR10 *rs10004195* was associated with gastric mucosal patterns of *H. pylori*-induced gastric diseases. However, further evaluation of additional SNPs of TLR10 polymorphisms associated with gastric mucosal patterns could provide a better understanding. These findings may explain some variations in the TLR10 gene that result in individual susceptibility to *H. pylori*-related diseases and also play important roles in gastric pathogenesis related to clinical outcomes of *H. pylori* infection.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Suranaree University of Technology (Decision Date: 01.12.2015/Decision No: EC-58-59).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - T.T., C.D.; Design - T.T., C.D.; Supervision - T.T., T.B., C.D., W.L.; Resource - T.T., L.M., S.P.; Materials - T.T., T.B.; Data Collection and/or Processing - T.T., T.B.; Analysis and/or Interpretation - T.T., T.B.; Literature Search - T.T., T.B., W.W.; Writing - T.T., T.B., W.W.; Critical Reviews - T.T., T.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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