

Genetic polymorphisms in TLR1, TLR2, TLR4, and TLR10 of *Helicobacter pylori*-associated gastritis: a prospective cross-sectional study in Thailand

Taweesak Tongtawe^{a,g}, Theeraya Bartpho^a, Soraya Kaewpitoon^{e,f}, Natthawut Kaewpitoon^h, Chavaboon Dechsukhum^{b,g}, Wilairat Leraanansaksiri^c, Ryan A. Loyd^{e,g}, Krajang Talabnin^d, Likit Matrakool^{a,g} and Sukij Panpimanmas^{a,g}

The toll-like receptors (TLRs) mediate the recognition of *Helicobacter pylori* and initiate the innate immune response to infection. We hypothesized those genetic polymorphisms in the TLR1, TLR2, TLR4, and TLR10 influence bacterial infection, affecting susceptibility *H. pylori* to disease outcomes. Genomic DNA was extracted and genotypes of *TLR1* (*rs4833095*), *TLR2* (*rs3804099* and *rs3804100*), *TLR4* (*rs10759932*), and *TLR10* (*rs10004195*) polymorphism were detected by the TagMan single-nucleotide epolymorphisms genotyping assay using the real-time PCR hybridization probe method. The *TLR1* (*rs4833095*), C allele and the *TLR10* (*rs10004195*), A allele frequency was significantly increased risk in the *H. pylori* infection group (odds ratio = 1.76, 95% confidence interval = 1.84–2.15, $P = 0.01$ and odds ratio = 1.81, 95% confidence interval = 1.18–3.26, $P = 0.04$, respectively). The *TLR1* (*rs4833095*), C allele and *TLR10* (*rs10004195*), A allele are susceptible TLRs polymorphisms in the Thai population. These findings

suggest that *TLR1 rs4833095* and *TLR10 rs10004195* may play crucial roles in *H. pylori* susceptibility and gastric pathogenesis. *European Journal of Cancer Prevention* 27:118–123 Copyright © 2018 The Author(s). Published by Wolters Kluwer Health, Inc.

European Journal of Cancer Prevention 2018, 27:118–123

Keywords: gastritis, *Helicobacter pylori*, TLR1, TLR10, TLR2, TLR4, toll-like receptor polymorphisms

^aDepartment of Surgery, ^bSchool of Pathology, ^cSchool of Preclinic, ^dMedical Technology and Medical Biochemistry, Institute of Medicine, ^eFamily Medicine and Community Medicine, ^fParasite Research Unit, Suranaree University of Technology, ^gSuranaree University of Technology Hospital and ^hFaculty of Public Health, Vongchavalitkul University, Nakhon Ratchasima, Thailand

Correspondence to Taweesak Tongtawe, MD, FRCST, Department of Surgery, Institute of Medicine, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand

Tel: +66 81 901 0891; e-mail: taweesak.t@sut.ac.th

Received 5 April 2016 Accepted 10 March 2017

Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium that colonizes the stomach. The global prevalence rate of *H. pylori* infection is as high as 90% in some developing countries (Goh *et al.*, 2011) and an estimated 20–30% of infected individuals subsequently develop disease, including peptic ulcers, gastric cancer, or mucosal-associated lymphoid tissue lymphomas (Basso *et al.*, 2010). The risk of different clinical outcome expression of *H. pylori* infection is believed to rely on interactions between host genetic factors and bacterial factors (Bagheri *et al.*, 2014). Toll-like receptors (TLRs) are pathogen-associated molecular pattern receptors, mediators of innate immunity that are essential for the recognition of microbial antigens by the immune cells. The gastric epithelial cell signaling response to *H. pylori* infection. TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10 on the cell surface or *TLR9* in intracellular vesicles interact with *H. pylori* virulence factor causes the

host immune responses and lead to the activation of NF- κ B and subsequent proinflammatory cytokine release (Smith, 2014). The TLRs polymorphisms can confer host susceptibility to *H. pylori* infection and likely influence association with *H. pylori*-related diseases by modulating the release of proinflammatory or anti-inflammatory cytokines, which underlie gastric inflammation and the immune response to *H. pylori*. *TLR1* polymorphisms have been reported to confer a high risk of *H. pylori* infection. The *TLR1 rs4833095* CT genotype or the T allele has been shown to be correlated with a decreased risk of *H. pylori* infection and reduced risks of chronic atrophic gastritis and intestinal metaplasia in Chinese patients (Yang *et al.*, 2013). The polymorphisms of TLR2 are associated with susceptibility to gastric cancer among various ethnic groups such as in Japanese, Chinese, and Caucasian populations. TLR4 are more frequent in relation to gastritis, and gastric cancer has been found worldwide including Europe, Asia, and America (Ishihara S *et al.*, 2004 Huang H *et al.*, 2010; Castano-Rodriguez *et al.*, 2014a, 2014b). Especially in the Chinese population, Castano-Rodriguez *et al.* (2014a, 2014b) reported that six TLR4 polymorphisms (*rs11536889*, *rs10759931*, *rs1927911*, *rs10116253*,

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

rs10759932, and *rs2149356*) were associated significantly with gastric cancer. However, there is no association between TLR4 polymorphisms and *H. pylori* related gastritis diseases in some studied populations such as in an ethnic Kashmiri population (Qadri *et al.*, 2014) or a weak association revealed very rare in Japanese population (Tahara *et al.*, 2007; Hishida *et al.*, 2011). Polymorphisms in *TLR10* have a known direct association with *H. pylori* susceptibility in European and Chinese populations. The *TLR10 rs10004195*, T allele decreased the risk of chronic atrophic gastritis and haplotype TT had a protective effect on *H. pylori* infection or precancerous gastric lesions (Tang *et al.*, 2015). Ravishankar Ram *et al.* (2015) found that individuals with the *rs10004195* T allele were not protected against the development of GC in Malaysian patients whereas haplotype AA of *rs10004195* was associated with an increase in GC irrespective of *H. pylori* infection. Among different populations, the association between the TLR(s) polymorphism and *H. pylori* infection-related diseases is inconsistent, but the impact of TLR polymorphisms on the *H. pylori*-related pathologic process has not yet been established in Thailand patients. The investigation of polymorphisms in Thai populations could provide novel insights into targeted treatment in genetically susceptible individuals and improve the prevention of *H. pylori*-related GC. Therefore, we hypothesized that specific genetic polymorphisms in TLR1, TLR2, TLR4, and TLR10 genes are likely associated with *H. pylori*-related gastric diseases and outcomes by affecting the *H. pylori* susceptibility of the hosts. It can be expected that the association of genetic polymorphisms in the host and clinical outcomes of *H. pylori*-related gastritis will be uncovered with advancing technologies and, in the near future, there is every prospect of defining full genetic risk profiles.

Patients and methods

Patients

Four hundred patients undergoing esophagogastroduodenoscopy for investigation of chronic abdominal pain participated in this study from December 2014 to March 2016. The following exclusion criteria were applied: previous *H. pylori* eradication treatment in the previous 2 months, significant medical illnesses, history of previous gastric surgery, and the use of antimicrobials or gastrointestinal medications such as proton-pump inhibitor or bismuth compounds within the previous 2 months. The study was carried out in accordance with good clinical practice 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, Suranaree University of Technology (EC-58-58 and EC-58-59).

Diagnosis of *H. pylori*-associated gastritis

A diagnosis of *H. Pylori*-associated gastritis was made if *H. pylori* were seen on histopathological examination and the rapid urease test was positive. Finally, we proved bacterial infection by PCR.

Biopsy specimens

The esophagogastroduodenoscopy procedures were performed using an upper GI video endoscope (Olympus EVIS EXERA III, CV-190, Japan). The entire stomach was examined first by conventional endoscopy and then biopsies were performed using the 'Site Specific Biopsy' technique (Tongtawee *et al.*, 2015a, 2015b). Gastric tissue specimens for histological analysis were sent to the pathologist. The hematoxylin and eosin stain, and Giemsa stain were used for the identification of *H. pylori*.

DNA preparation

Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue of 400 gastric patients using the QIAamp DNA formalin-fixed, paraffin-embedded tissue kit (Qiagen, Duesseldorf, Germany). The DNA extraction was performed according to the manufacturer's instructions. Briefly, deparaffinize the paraffin-embedded tissues in xylene and hydrate in 100% ethanol.

Single-nucleotide polymorphisms selection and genotyping

Four common single-nucleotide polymorphisms (SNPs) in TLR1, TLR2, TLR4, and TLR10 genes have been reported with functional effects or association with gastric cancer. *TLR1 rs4833095* (C>T), *TLR2 rs3804099* (T>C), *rs3804100* (T>C), *TLR4 rs10759932* (T>C), and *TLR10 rs10004195* (T>A) were selected according to the SNP database of the National Center for Biotechnology Information. The genotype of TLR(s) polymorphism was determined by TagMan allelic discrimination using a predesigned Custom TagMan SNP Genotyping Assay by real-time PCR (Roche diagnostics, Neuilly sur Seine, France). Forward and reverse primers were used along with wild-type probe VIC and probes FAM used for the variant allele. Primers and probes were supplied by Applied Biosystems (Foster city, California, USA). The real-time PCR system was used according to the manufacturer's instructions (LightCycler 480 II instrument; Roche Diagnostics, Neuilly sur Seine, France). 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. The success rate of genotyping for each SNP was more than 94%. Negative controls and duplicate samples were used to check the accuracy of genotyping and initially analyzed using LightCycler 480 Software 1.5 (Roche Applied Science, Mannheim, Germany).

Statistical analysis

The patients' demographic data, patients' symptom, endoscopic findings, and TLRs were examined through

univariate analysis. Backward stepwise procedures were used to carry out the multivariate analysis; the final model included only those variables that were found to be statistically significant in the univariate analysis. The associations were expressed as odds ratios (OR) with their confidence intervals (95% CI). The data were analyzed using the SPSS software (IBM SPSS Statistics for Windows, version 20, Armonk, New York, USA). Significance was set at P less than 0.05.

Results

Characteristics of study population

A total of 400 patients, 204 *H. Pylori*-positive cases and 196 *H. Pylori*-negative cases, were matched by sex, age, patient symptoms, and endoscopic findings (Table 1). In the Thai population, the mean age was 46 ± 1.5 years (18–70 years) for the *H. Pylori*-infected patients and 42 ± 2.5 years (19–68 years) for the *H. Pylori*-infected patients. Most of the gastritis was chronic dyspepsia (74.3 or 71.8%). Nonulcer gastritis/duodinitis was found to be the predominant endoscopic finding identified, 140 in *H. Pylori*-positive cases and 128 in *H. Pylori*-negative cases, but there were no statistically significant differences in the distribution of sex, age, patient symptoms, and endoscopic findings between *H. Pylori*-infected patients and uninfected patients.

Association between toll-like receptors polymorphism and *H. pylori* infection

Figure 1 presents the genotype distribution of all SNPs. Among all patients, the distribution of *TLR2 rs3804099*, *rs3804100*, and *TLR4 rs10759932* showed a higher frequency of the TT genotype than the TC/TT genotypes,

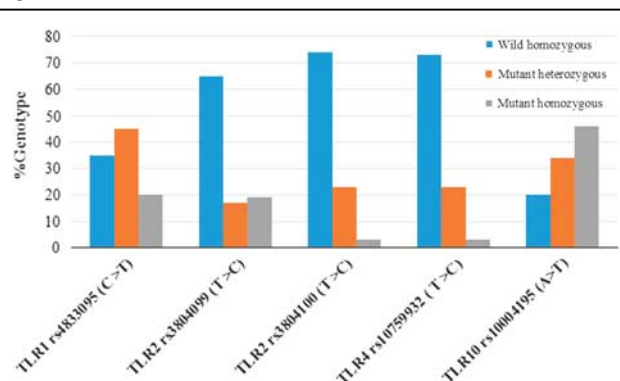
Table 1 Association between demographic data, patients' symptoms, and endoscopic findings *H. pylori* infection (univariate analysis)

Variables	Positive for <i>H. pylori</i> [n (%)]	Negative for <i>H. pylori</i> [n (%)]	P value*
	Total (N=204)	Total (N=196)	
Sex			0.58
Male	71 (35)	65 (33)	
Female	133 (65)	131 (67)	
Age (years)			0.13
Mean \pm SD	46 ± 1.5	42 ± 2.5	
Patients' symptoms			0.42
Chronic dyspepsia	152 (74.3)	141 (71.8)	
Gastrointestinal bleeding	11 (5.4)	13 (6.8)	
Iron-deficiency anemia	25 (12.3)	20 (10.3)	
Recurrent vomiting	7 (3.5)	7 (3.5)	
Chronic diarrhea	9 (4.5)	15 (7.6)	
Endoscopic finding			0.32
Nonulcer gastritis/ duodinitis	140 (68.7)	128 (65.2)	
Peptic ulcer (GU/DU)	31 (15.3)	34 (17.3)	
Gastroesophageal reflux disease	20 (9.8)	24 (12.3)	
Gastric polyp	13 (6.2)	10 (5.2)	

DU, duodenal ulcer; GU, gastric ulcer.

*Significance is set at $P < 0.05$.

Fig. 1



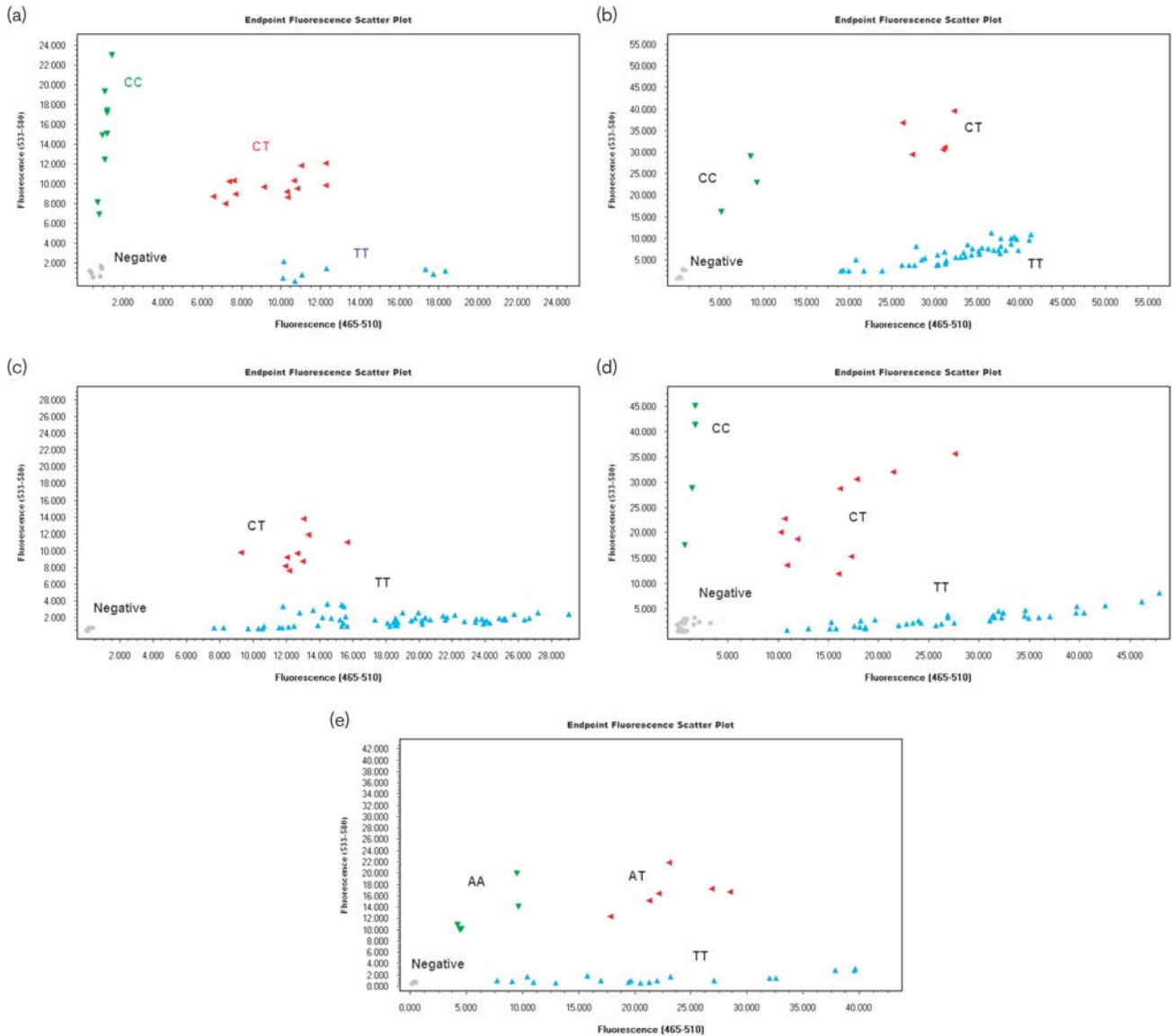
Genotype (heterozygous and homozygous) distributions of Toll-like receptor polymorphisms -*TLR1 rs4833095*, *TLR2 rs3804099* and *rs3804100*, *TLR4 rs10759932* and *TLR10 rs10004195*.

whereas *TLR1 rs4833095* showed a lower frequency of the CC genotype than the CT/TT genotypes. Similarly, the *TLR10 rs10004195* AA genotype showed a lower frequency than the CT/TT genotypes. Genotypes of the *TLR1*, *TLR2*, *TLR4*, and *TLR10* polymorphisms were differentiated using the TagMan SNP genotyping assay by real-time PCR (Fig. 2). We evaluated the association between *TLR1*, *TLR2*, *TLR4*, and *TLR10* polymorphisms and the risk of *H. pylori* infection. Interestingly, *TLR1 rs4833095* showed a significant association with *H. pylori* infection ($P=0.01$); frequencies of CC, CT, and TT genotypes were 60, 2, and 38% in *H. pylori*-positive patients in contrast to 7, 93, and 0% in *H. pylori*-negative patients, respectively. *TLR10 rs10004195* also showed a significant association with *H. pylori* infection ($P=0.04$); frequencies of AA, AT, and TT genotypes were 29, 5, and 66% in *H. pylori*-positive patients and 1, 3, and 26% in *H. pylori*-negative patients, respectively (Table 2). In the multivariate analysis model, we detected a significantly increased risk of *TLR1 rs4833095* for *H. pylori* infection with the homozygous CC (OR = 1.76, 95% CI = 1.84–2.15, $P=0.01$). In addition, compared with *TLR10 rs1004195*, we found that the AA genotype led to a significantly increased risk for *H. pylori*-related infection (OR = 1.81, 95% CI = 1.18–3.26, $P=0.02$) as shown in Table 3.

Discussion

This study indicated that *TLR1 rs4833095* and *TLR10 rs10004195* were associated with *H. pylori* susceptibility in the Thai population. The *TLR1 rs4803395*, C allele was associated with a significantly increased risk for *H. pylori* infection and the *TLR10 rs10004195*, A allele conferred a high risk of *H. pylori* infection. Furthermore, the CT genotype or the T allele of *TLR1 rs4803395* conferred protection against *H. pylori* infection, whereas the AT genotype or the T allele of *TLR10 rs10004195* exerted a protective effect on *H. pylori* infection; however, this was not statistically significant (Table 2).

Fig. 2



TaqMan SNP Genotyping Assay resulting in single indistinguishable cluster in scatter plot. *TLR1* rs4833095 (a), *TLR2* rs4804099 (b), *TLR2* rs4804100 (c), *TLR4* rs1075932 (d) and *TLR10* rs10004195 (E).

This finding further supports that TLR1 and TLR10 genetic polymorphisms have an impact on the susceptibility to *H. pylori* infection by the modulation of the signaling pathway in the inflammatory response initiated by interactions between the host receptor and bacterial antigens such as lipopolysaccharides. The different outcomes of the inflammatory response, including the development of precancerous lesions of gastric mucosa, are likely mediated by the downstream expression of the key inflammatory cytokines, especially interleukin-1 β (IL-1 β), IL-1 α , IL-6, IL-8, IL-10, tumor necrosis factor- α , and interferons, which have been shown to be varied among different genetic polymorphisms of TLR genes. In line with this hypothesis, some SNPs of these genes were

reported to be closely associated with susceptibility to gastric cancer in the Chinese population (Tang *et al.*, 2015). *H. pylori* infection plays an important role in gastric cancer, but there is a low incidence of gastric cancer in the Thai population (Uchida *et al.*, 2015). In this study, the TLR1 and TLR10 genotype distributions were identified and variant genotypes (heterozygous and homozygous) were predominantly found in Thai patients. The percentage of CC alleles was 65% in *TLR1* rs4833095 and the frequency of the A allele was 80% in *TLR10* rs10004195 (Fig. 1). Variant genotypes of *TLR1* rs4833095 and *TLR10* rs10004195 in Thai population may suggest that these genotypes of *TLR1* rs4833095 and *TLR10* rs10004195 in Thai population can impair

Table 2 Association between toll-like receptors genotypes and *H. pylori* infection (univariate analysis)

Variables	Positive for <i>H. pylori</i> [n (%)]	Negative for <i>H. pylori</i> [n (%)]	P value*
	Total (N=204)	Total (N=196)	
<i>TLR1</i> (rs4833095)			0.01*
CC	122 (60)	14 (7)	
CT	4 (2)	182 (93)	
TT	78 (38)	0	
<i>TLR2</i> (rs3804099)			0.97
TT	126 (62)	131 (67)	
TC	27 (13)	41 (21)	
CC	51 (25)	24 (12)	
<i>TLR2</i> (rs3804100)			0.61
TT	147 (72)	149 (76)	
TC	49 (24)	43 (22)	
CC	8 (4)	4 (2)	
<i>TLR4</i> (rs10759932)			0.38
TT	153 (75)	139 (71)	
TC	37 (18)	53 (27)	
CC	14 (7)	4 (2)	
<i>TLR10</i> (rs10004195)			0.04*
AA	59 (29)	22 (11)	
AT	10 (5)	123 (63)	
TT	135 (66)	51 (26)	

*Significance is set at $P < 0.05$.

Table 3 Predictive value of toll-like receptors for *H. pylori* infection (multivariate analysis)

TLR polymorphism	Genotype/allele	OR (95% confidence)	P value
<i>TLR1</i> (rs4833095)	CC	1.76 (1.84–2.15)	0.01*
	CT	0.79 (1.02–1.15)	0.98
	TT	0.82 (1.20–2.12)	0.89
	CT/TT	0.79 (1.91–2.03)	0.92
<i>TLR10</i> (rs10004195)	AA	1.81 (1.18–3.26)	0.02*
	AT	0.40 (1.13–2.23)	0.1
	TT	0.94 (2.30–3.80)	0.93
	AT/TT	0.81 (1.08–1.43)	0.18

OR, odds ratio; TLR, toll-like receptor.

*Significance is set at $P < 0.05$.

signaling to promote the *H. pylori* infection through mediating host–cell interactions. Therefore, some SNPs of *TLR1* and *TLR10* were proposed to play a role in modifying a risk for the development of gastric cancer or confer a protective effect for certain human cancers including gastric cancer in Thai patients, resulting in the so-called phenomenon ‘Thailand enigma’. This study also allows the detection of the SNPs of *TLR2* rs3804099, rs3804100, and *TLR4* rs10759932 and shows a pattern allelic distribution of these SNPs in Thai patients. Although the polymorphism of *TLR2* rs3804099 was shown to be associated with susceptibility to gastric cancer in the Chinese population (Zeng *et al.*, 2011) and *TLR2* rs3804100 was reported to be commonly associated with an increased risk of *H. pylori* infection-related gastric cancer (Castano-Rodriguez *et al.*, 2014a, 2014b), the actual distribution of each genotype-related disease is unclear. The *TLR4* rs10759932, C allele was shown to be associated with a significantly decreased risk of *H. pylori* infection (Castano-Rodriguez *et al.*, 2014a, 2014b), but there was no association between *TLR4* rs10759932 and

H. pylori infection in Thai patients. *TLR4* rs10759932 might not play a direct role in the susceptibility to *H. pylori* infection or there are some other alternative SNPs located in the *TLR4* gene that contribute toward *H. pylori*-related gastric disease as an analogous list for the *TLR4* gene polymorphisms with the potential for onco-genomic studies (Kutikhin, 2011a, 2011b). Similarly, it was found that *TLR2* and *TLR5*, but not *TLR4*, are required for *H. pylori*-induced NF- κ B activation and chemokine expression by epithelial cells (Smith *et al.*, 2003). In addition, our recent studies in genetic polymorphism in another pathway such as *MDM2* in the Thai population showed an association with *H. pylori* infection (Tongtawee *et al.*, 2015a, 2015b) and the association of *MDM2* with more severe inflammation in *H. pylori*-associated gastritis (Tongtawee *et al.*, 2015a, 2015b). These could provide the supportive evidences that the outcome of the infection mainly depends on the immune response of the host.

Conclusion

Through *TLR1*, *TLR2*, *TLR4*, and *TLR10* polymorphism analysis by a prospective cross-sectional study in Thailand, it was found that the *TLR1* (rs4833095)-C allele and the *TLR10* (rs10004195)-A allele are *H. pylori* infection-susceptible TLRs polymorphisms in the Thai population. The different genotypes of the TLRs polymorphism indicated that the outcome of *H. pylori* infection depends on the immune status of the host. However, larger multicenter studies are needed with higher *H. pylori*-positive cases to test this hypothesis. Further evaluation of TLR polymorphisms associated with gastric cancer could provide an understanding of the underlying factors attributable to the clinical consequences of *H. pylori* infection.

Acknowledgements

The authors would like to acknowledge a grant from SUT Research and Development Fund.

Conflicts of interest

There are no conflicts of interest.

References

- Bagheri N, Azadegan-Dehkordi F, Sanei H, Taghikhani A, Rahimian G, Salimzadeh L, *et al.* (2014). Associations of a *TLR4* single-nucleotide polymorphism with *H. pylori* associated gastric diseases in Iranian patients. *Clin Res Hepatol Gastroenterol* **38**:366–371.
- Basso D, Plebani M, Kusters JG (2010). Pathogenesis of *Helicobacter pylori* infection. *Helicobacter* **15**:14–20.
- Castano-Rodriguez N, Kaakoush NO, Pardo AL, Goh KL, Fock KM, Mitchell HM (2014a). Genetic polymorphisms in the Toll-like receptor signalling pathway in *Helicobacter pylori* infection and related gastric cancer. *Hum Immunol* **75**:808–815.
- Castano-Rodriguez N, Kaakoush NO, Mitchell HM (2014b). Pattern-recognition receptors and gastric cancer. *Front Immunol* **5**:336.
- Figura N, Marano L, Moretti E, Ponzetto A (2016). *Helicobacter pylori* infection and gastric carcinoma: not all the strains and patients are alike. *World J Gastrointest Oncol* **8**:40–54.
- Goh KL, Chan WK, Shiota S, Yamaoka Y (2011). Epidemiology of *Helicobacter pylori* infection and public health implications. *Helicobacter* **16** (Suppl 1):1–9.

- Hishida A, Matsuo K, Goto Y, Naito M, Wakai K, Tajima K, *et al.* (2011). No associations of Toll-like receptor 2 (TLR2) –196 to –174del polymorphism with the risk of *Helicobacter pylori* seropositivity, gastric atrophy, and gastric cancer in Japanese. *Gastric Cancer* **13**:251–257.
- Huang H, Wu J, Jin G, Zhang H, Ding Y, Hua Z, *et al.* (2010). A 5'-flanking region polymorphism in toll-like receptor 4 is associated with gastric cancer in a Chinese population. *J Biomed Res* **24**:100–106.
- Ishihara S, Rumi MA, Kadowaki Y, Ortega-Cava CF, Yuki T, Yoshino N, *et al.* (2004). Essential role of MD-2 in TLR4-dependent signaling during *Helicobacter pylori*-associated gastritis. *J Immunol* **173**:1406–1416.
- Kutikhin AG (2011a). Impact of Toll-like receptor 4 polymorphisms on risk of cancer. *Hum Immunol* **72**:193–206.
- Kutikhin AG (2011b). Association of polymorphisms in TLR genes and in genes of the Toll-like receptor signaling pathway with cancer risk. *Hum Immunol* **72**:1095–1116.
- Qadri Q, Rasool R, Afroze D, Naqash S, Gulzar GM, Yousuf A, *et al.* (2014). Study of TLR4 and IL-8 gene polymorphisms in *H. pylori*-induced inflammation in gastric cancer in an ethnic Kashmiri population. *Immunol Invest* **43**:324–336.
- Ravishankar Ram M, Goh KL, Leow AH, Poh BH, Loke MF, Harrison R, *et al.* (2015). Polymorphisms at locus 4p14 of Toll-like receptors TLR-1 and TLR-10 confer susceptibility to gastric carcinoma in *Helicobacter pylori* infection. *PLoS One* **10**:e0141865.
- Smith SM (2014). Role of Toll-like receptors in *Helicobacter pylori* infection and immunity. *World J Gastrointest Pathophysiol* **5**:133–146.
- Smith MF Jr, Mitchell A, Li G, Ding S, Fitzmaurice AM, Ryan K, *et al.* (2003). Toll-like receptor (TLR) 2 and TLR5, but not TLR4, are required for *Helicobacter pylori*-induced NF-kappa B activation and chemokine expression by epithelial cells. *J Biol Chem* **278**:32552–32560.
- Tahara T, Arisawa T, Shibata T, Hirata I, Nakano H (2007). Absence of common polymorphisms of Toll like receptor 4 (TLR4): Asp299Gly, Thr399Ile in patients with gastroduodenal diseases in Japan. *J Clin Biochem Nutr* **40**:62–65.
- Tang FB, Li ZX, Wang YM, Zhang L, Ma JL, Zhou T, *et al.* (2015). Toll-like receptor 1 and 10 polymorphisms, *Helicobacter pylori* susceptibility and risk of gastric lesions in a high-risk Chinese population. *Infect Genet Evol* **31**:263–269.
- Tongtawee T, Dechsukhum C, Leeansaksiri W, Kaewpitoon S, Kaewpitoon N, Loyd RA, *et al.* (2015a). Genetic polymorphism of MDM2 SNP309 in patients with *Helicobacter Pylori*-associated gastritis. *Asian Pac J Cancer Prev* **16**:7049–7052.
- Tongtawee T, Dechsukhum C, Talabnin K, Leeansaksiri W, Kaewpitoon S, Kaewpitoon N, *et al.* (2015b). Correlation between patterns of Mdm2 SNP 309 and histopathological severity of *Helicobacter pylori* associated gastritis in Thailand. *Asian Pac J Cancer Prev* **16**:7781–7784.
- Uchida T, Miftahussurur M, Pittayanon R, Vilaichone RK, Wisedopas N, Ratanachu-Ek T, *et al.* (2015). *Helicobacter pylori* infection in thailand: a nationwide study of the CagA phenotype. *PLoS One* **10**:e0136775.
- Yang CA, Scheibenbogen C, Bauer S, Kleinle C, Wex T, Bornschein J, *et al.* (2013). A frequent Toll-like receptor 1 gene polymorphism affects NK- and T-cell IFN-gamma production and is associated with *Helicobacter pylori*-induced gastric disease. *Helicobacter* **18**:13–21.
- Zeng HM, Pan KF, Zhang Y, Zhang L, Ma JL, Zhou T, *et al.* (2011). The correlation between polymorphisms of Toll-like receptor 2 and Toll-like receptor 9 and susceptibility to gastric cancer. *Zhonghua Yu Fang Yi Xue Za Zhi* **45**:588–592.