Toll-like receptor 2 and 4 polymorphisms associated with Helicobacter pylori *susceptibility and gastric cancer*

Taweesak Tongtawee[1], Theeraya Simawaranon[1], Wareeporn Wattanawongdon[1], Chavaboon Dechsukhum[2], Wilairat Leeanansaksiri[3]

[1]Department of Surgery, Institute of Medicine, Suranaree University of Technology, Nakhon Ratchasima, Thailand
[2]School of Pathology, Institute of Medicine, Suranaree University of Technology, Nakhon Ratchasima, Thailand
[3]School of Preclinic, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand

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ABSTRACT

Background/Aims: *Genetic polymorphisms in Toll-like receptors (TLRs) are important influence on gastric lesion development and* Helicobacter *pylori susceptibility.*

Materials and Methods: *TLR2 rs3804099 and rs3804100 and TLR4 rs10759932 were determined in a total of 400 patients. The association among genotypes and the risk of gastric lesion development and* H. pylori *susceptibility were evaluated by the odds ratios (ORs) and 95% confidence intervals (95% CIs) from logistic regression analyses.*

Results: *TLR4 rs10759932, C/C homozygous genotype was associated with an increased risk of premalignant/malignant (OR=2.48, 95% CI=1.96-4.62, p=0.015). The recessive model of TLR4 rs10759932 showed a decreased risk of* H. pylori *susceptibility (adjusted OR=0.52, 95% CI=0.38-0.82, p=0.046). Meanwhile, the recessive model was associated with an increased risk of non-malignant (OR=3.46, 95% CI=2.25-5.67, p=0.001). In subjects with* H. pylori *infection, the recessive model was associated with an increased risk of non-malignant (OR=2.28, 95% CI=1.24-3.57, p=0.001) and premalignant/malignant (OR=1.83, 95% CI=1.16-2.84, p=0.027).*

Conclusion: *TLR4 rs10759932, but not TLR2 rs3804099 and rs3804100, was associated with risk of premalignant and/or malignant and H. pylori susceptibility.* H. pylori *infection seems to contribute to chronic gastritis, and premalignant/malignant supported the development of the premalignant/malignant lesions involved in* H. pylori *infection that is critical to gastric cancer in Thai patients.*

Keywords: *Gastric cancer, Toll-like receptor 2, Toll-like receptor 4, Genetic polymorphisms,* Helicobacter pylori

INTRODUCTION

Colonization of *Helicobacter pylori* in a human stomach causes chronic infection. Inflammation in the gastric mucosa induces the development of peptic ulcer. Patients with chronic active gastritis progress to atrophy gastritis and intestinal metaplasia due to inflammation in the gastric mucosa that can trigger gastric cancer (GC) (1,2). However, 15%-30% of patients with *H. pylori*-infected chronic gastritis develop peptic ulcerations, distal gastric adenocarcinoma, or gastric lymphoma (3,4).

With genetic heterogeneity, host factors are important in gastric carcinogenesis due to *H. pylori* infection, Toll-like receptors (TLRs), or host molecule response to pathogen-associated molecular patterns that bind to the spectrum of ligands (5,6). TLR recognition plays a crucial role in the defense against infection and immune system regulation. Thus, polymorphisms in the TLR genes affect host susceptibility to infection (7). TLR polymorphism might cause an imbalance of pro- and anti-inflammatory cytokine responses and modulate immune pathogenesis and cancer. TLRs detect endogenous ligands released of damaged tissues, necrotic cells, or cancer cells (6). Today, genetic polymorphisms in TLRs are associated with *H. pylori*-related diseases, *H. pylori* susceptibility, and inflammation (8).

TLR2 and TLR4 are implicated in the recognition of various bacterial cell wall components, such as lipopolysaccharide, peptidoglycans, and lipoproteins. Numerous studies of TLR2 and TLR4 polymorphisms are associated with *H. pylori* infection and GC. They report that impaired TLR function and TLR signaling pathways may result in an elevated risk of infection including occurrence of various pathologies and cancer (9-12). Additionally, TLR4 polymorphisms are associated with modified immune responses of the gastric mucosa (13-15) and substantially contribute to GC (16,17). However, in Thailand, no study investigating the role of the TLR2 and TLR4 polymorphisms on the *H. pylori*-related pathological process and the impact of TLR polymorphisms on the *H. pylori*-related gastric lesions including non-malignant, premalignant,

Corresponding Author: ***Taweesak Tongtawee; taweesak.t@sut.ac.th***
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and malignant has yet been established in Thai patients. The investigation of polymorphisms in the Thai population could provide novel insights into targeted treatment in genetically susceptible individuals and improve the prevention of *H. pylori*-related GC. Therefore, the study was aimed to evaluate the association of TLR2 *rs3804099* and *rs3804100* and TLR4 *rs10759932* polymorphisms and the risk of *H. pylori* susceptibility as well as gastric mucosal pathology by affecting the variant genotype. The results could provide details of the association of genetic polymorphisms and *H. pylori* infection and gastric mucosal pathology in Thai patients.

MATERIALS AND METHODS

Patients

A total of 400 patients who received esophagogastroduodenoscopy (EGD) to investigate chronic abdominal pain participated in the study conducted from December 2014 to March 2016. Patients with significant medical illnesses, history of gastric surgery, and *H. pylori* eradication or use of antimicrobials or gastrointestinal medications, such as proton pump inhibitors or bismuth compounds in the past 2 months, were excluded from the study. The study was performed according to the good clinical practices and the Declaration of Helsinki guidelines. The study protocol was approved by the Ethics Committee for Research Involving Human Subjects (EC-58-58). Written informed consent was obtained from the participants.

Gastric tissue specimens

The EGD procedures were performed using an upper gastrointestinal video endoscope (Olympus EVIS EXERA III, CV-190, Japan). The whole stomach was examined and biopsied using a site-specific biopsy technique to analyze these specimens for histopathology (18).

Diagnosis of H. pylori infection

H. pylori-associated gastritis was observed during histopathological examination. Biopsy samples were obtained directly from the observation area, and *H. pylori* was detected using a rapid urease test on site (ProntodyleR, GASTREX, France). *H. pylori* infection was proven by polymerase chain reaction (PCR).

DNA preparation

A total of 400 gastric formalin-fixed paraffin-embedded (FFPE) tissues were used for genomic DNA extraction using the QIAamp DNA FFPE Tissue Kit (Qiagen, Dusseldorf, Germany). The process of genomic DNA extraction was performed according to the manufacturer's instructions. Briefly, the paraffin-embedded tissues were deparaffinized in xylene using three changes for 5 min each and hydrated in 100% ethanol and 95% ethanol. Samples were then subsequently extracted for genomic DNA by digested lysis buffer and proteinase K. The tissue lysate was purified using the QIAamp spin column and eluted and stored at -20 °C.

Genotyping of TLR gene polymorphisms

Three single-nucleotide polymorphisms (SNPs) were selected according to the National Center for Biotechnology Information SNP database. TLR2 SNP *rs3804099* (T>C) and *rs3804100* (T>C) and TLR4 SNP *rs10759932* (T>C) were used in the present study. TLR(s) polymorphism was determined by TaqMan allelic discrimination with a TaqMan SNP Genotyping Assay using a real-time PCR. Forward and reverse primers were designed by the wild-type probe VIC and the variant probe FAM. Real-time PCR was performed 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 genotypes of polymorphisms were analyzed by the LightCycler® 480 software 1.5 (Roche Diagnostics, Neuilly-sur-Seine, France). The genotyping success rate for each SNP was >94%.

Statistical analysis

All statistical analyses were performed by the Statistical Package for the Social Sciences (SPSS) for Windows, version 20.0 (IBM Corp.; Armonk, NY, USA). The association among genotypes and the risk of gastric lesion development and *H. pylori* susceptibility were evaluated by the odds ratios (ORs) and 95% confidence intervals (95% CIs) from logistic regression analyses. A $p<0.05$ was considered as statistically significant.

RESULTS

Patient characteristics and TLR genotyping in clinical samples

A total of 400 patients (136 men and 264 women) with gastritis participated in the study. The median age of the patients was 44.6±15.9 years. Of these patients, 204 cases were infected with *H. pylori*, and 196 were *H. pylori* negative. Gastric lesions included chronic gastritis (n=312), gastric atrophy (GA) (n=26), internal metaplasia (IM) (n=38), and GC (n=24). SNPs of TLR2 *rs3804099* and *rs3804100* and TLR4 *rs10759932* were genotyped in all patients. The proportions of T/T, T/C, and C/C genotypes were 64%, 17.25%, and 18.75% for TLR2 *rs3804099*;

74%, 23%, and 3% for TLR2 *rs3804100*; and 73%, 22.5%, and 4.5% for TLR4 *rs10759932*, respectively. Table 1 shows the baseline characteristics of the subjects.

Table 1. Summary of subject characteristics

Characteristics	Total=400 (%)
Male/female (N)	136/264
Median age±SD (years)	44.6±15.9
H. pylori infection	
Negative	196 (49)
Positive	204 (51)
TLR2 rs3804099	
TT	256 (64)
TC	69 (17.25)
CC	75 (18.75)
TLR2 rs3804100	
TT	296 (74)
TC	92 (23)
CC	12 (3)
TLR4 rs10759932	
TT	292 (73)
TC	90 (22.5)
CC	18 (4.5)
Gastric mucosal morphological patterns	
Chronic gastritis	312 (78)
Internal metaplasia	38 (9.5)
Gastric atrophy	26 (6.5)
Gastric cancer	24 (6)

Association of TLR polymorphism with gastric lesions

For determination of the association between genotype and gastric lesions, a total of 400 patients were divided into two groups: 312 patients of the non-malignant (chronic gastritis) group and 88 patients of the premalignant (IM and GA) and/or malignant (GC) group. As shown in Table 2, TLR4 *rs10759932*, C/C homozygous genotype was associated with premalignant/malignant and had a significantly increased risk of premalignant/malignant development (OR=2.48, 95% CI=1.96-4.62, p=0.015) (Table 2).

Association of TLR polymorphism with H. pylori *susceptibility*

The association between TLR polymorphism and *H. pylori* infection was divided by genotypes (T/T, T/C, and C/C), dominant model (T/T vs. T/C+C/C), and recessive model (T/T+C/C vs. C/C). A significant association was observed between the recessive model of TLR4 *rs10759932* and *H. pylori* status. The recessive model showed a decreased risk of *H. pylori* susceptibility (adjusted OR=0.52, 95% CI=0.38-0.82, p=0.046) (Table 3).

Association of H. pylori *infection with risk of gastric lesions by recessive model*

We further evaluated the association between *H. pylori* infection and risk of gastric lesions in subpopulations stratified by the recessive model. Table 4 shows the results. In subjects without *H. pylori* infection, the recessive model was associated with an increased risk of non-malignant or chronic gastritis (OR=3.46, 95% CI=2.25-5.67,

Table 2. Association between TLR genotype and gastric lesions

Polymorphism	Gastric lesions			p*
	Non-malignant	Premalignant (GA/IM)/malignant (GC)	OR (95% CI)	
TLR2 *rs3804099*				
TT	194 (62.18)	62 (70.46)	0.73 (0.61-0.87)	0.192
TC	56 (17.95)	13 (14.77)	0.82 (0.57-0.95)	0.261
CC	62 (19.87)	13 (14.77)	0.61 (0.38-0.77)	0.183
TLR2 *rs3804100*				
TT	230 (73.72)	66 (75)	0.78 (0.62-0.91)	0.838
TC	70 (22.43)	22 (25)	0.69 (0.43-0.87)	0.746
CC	12 (3.85)	0	-	-
TLR4 *rs10759932*				
TT	216 (69.23)	76 (86.36)	0.75 (0.59-0.83)	0.471
TC	90 (28.85)	0	-	-
CC	6 (1.92)	12 (13.64)	2.48 (1.96-4.62)	0.015*

GA: gastric atrophy; IM: intestinal metaplasia; GC: gastric cancer
*Significance is set at p<0.05

Table 3. Association between recessive genotype and *H. pylori* infection

Polymorphism	*H. pylori* status		OR (95% CI)	p
	Negative	Positive		
TLR2 rs3804099				
TT	131 (66.84)	125 (61.27)	1	0.721
TC	41 (20.92)	28 (13.73)	0.96 (0.62-1.29)	0.543
CC	24 (12.24)	51 (25)	0.86 (0.58-0.97)	0.261
Dominant				
TT	131 (66.84)	125 (61.27)	1	0.462
TC+CC	65 (33.16)	79 (38.73)	0.68 (0.59-0.91)	0.158
Recessive				
TT+TC	172 (87.76)	153 (75)	1	0.663
CC	24 (12.24)	51 (25)	0.78 (052-0.91)	0.328
TLR2 rs3804100				
TT	149 (76.02)	147 (72.06)	1	0.843
TC	43 (21.94)	49 (24.02)	0.98 (0.83-1.25)	0.924
CC	4 (2.04)	8 (3.92)	0.74 (0.53-0.93)	0.548
Dominant				
TT	149 (76.02)	147 (72.06)	1	0.614
TC+CC	47 (23.98)	57 (27.94)	0.79 (0.56-0.97)	0.627
Recessive				
TC+CC	47 (23.98)	57 (27.94)	1	0.283
CC	4 (2.04)	8 (3.92)	0.84 (0.63-0.97)	0.365
TLR4 rs10759932				
TT	139 (70.92)	153 (75)	1	0.442
TC	53 (27.04)	37 (18.14)	0.58 (0.43-0.82)	0.624
CC	4 (2.04)	14 (6.86)	0.62 (0.43-0.92)	0.068
Dominant				
TT	139 (70.92)	153 (75)	1	0.441
TC+CC	57 (29.08)	51 (25)	0.87 (0.63-0.98)	0.472
Recessive				
TT+TC	192 (97.96)	190 (93.14)	1	0.793
CC	4 (2.04)	14 (6.86)	0.52 (0.38-0.82)	0.046*

*Significance is set at p<0.05

Table 4. Risk of gastric lesions associated with combined effect of recessive model and *H. pylori* infection

Gastric mucosal pathology	*H. pylori*	TLR4 rs10759932		OR (95% CI)	p
		TT+TC	CC		
Non-malignant	-	186 (59.62)	5 (1.60)	3.46 (2.25-5.67)	0.001*
	+	116 (37.18)	5 (1.60)	2.28 (1.24-3.57)	0.001*
Pre-/malignant	-	17 (17.71)	0	-	-
	+	68 (70.83)	11 (11.46)	1.83 (1.16-2.84)	0.027*

*Significance is set at p<0.05

p=0.001). Meanwhile, in subjects with *H. pylori* infection, the recessive model was associated with an increased risk of both non-malignant (OR=2.28, 95% CI=1.24-3.57, p=0.001) and premalignant/malignant (OR=1.83, 95% CI=1.16-2.84, p=0.027).

DISCUSSION

To our knowledge, this is the first study to assess the SNPs of TLR2 *rs3804099* and *rs3804100* and TLR4 *rs10759932* in association with risk of gastric lesion development and *H. pylori* susceptibility. In the present study, we found that TLR4 *rs10759932*, but not TLR2 *rs3804099* and *rs3804100*, was associated with risk of premalignant/malignant and *H. pylori* susceptibility. TLR4 *rs10759932*, C/C homozygous genotype had an increased risk of premalignant gastric lesions. The C/C homozygous might change the expression level or structure of the TLRs that modify the signaling pathway. Meanwhile, the recessive model was associated with a reduced risk of *H. pylori* susceptibility. These may have a protective effect on host susceptibility to *H. pylori* infection, or functional impairment of TLR4 is crucial during *H. pylori*-induced gastric diseases.

We further tested the combined effects between the recessive model of TLR4 *rs10759932* and *H. pylori* infection on risk of gastric lesions. The results showed a significant association with an increased risk of non-malignant and premalignant/malignant in patients with or without *H. pylori* infection. *H. pylori* infection appears to contribute to chronic gastritis, and premalignant/malignant supported the development of GC involved in *H. pylori* infection. However, no multiplicative or additive interaction of SNP *H. pylori* was addressed. Additionally, the results of *H. pylori* positive patients revealed an approximately 1.25-fold reduced risk of premalignant compared with non-malignant. Thus, these analyses suggested that there was an interaction between the recessive model TLR4 *rs10759932* and *H. pylori* infection.

It has been shown that TLR polymorphisms can influence *H. pylori* susceptibility. *H. pylori*-related diseases developed during modulation of pro-inflammatory or anti-inflammatory cytokines. Recent genetic association studies have suggested that chronic inflammation is a risk factor associated with the development of GC (19). TLR genotypes might alter cytokine production resulting in modulation of *H. pylori* susceptibility, chronic inflammation, and GC (20,21). TLR2 polymorphisms are associated with susceptibility to GC in various ethnic groups, such as Japanese, Chinese, and Caucasian population. TLR4 polymorphisms have also been associated with gastritis and GC in Europe, Asia, and America (22,23). In an ethnic Kashmiri population, a previous study observed a relationship between TLR4 polymorphisms and gastric diseases (24), and a weak association was seen in the Japanese population (25,26). Similar to other infectious pathogens, genetic polymorphism in the TLR4 gene reduces inflammation, causing less damage and persistent infection (27-29). However, TLR4 mRNA was up-regulated in *H. pylori*-infected gastric epithelial cell lines (30).

Thai patients might explain the typically benign outcome of *H. pylori*-related diseases. Our study provides additional evidence to further support the hypothesis that the outcome of the infection is largely influenced by the immune response or infectious susceptibility of the host. *H. pylori*-related host genetic factors independent of or combined with *H. pylori* infection appear to play important roles in modulating the risk of the development of gastric carcinogenesis. However, multicenter studies are needed for larger studies to test this hypothesis. The role of TLR genetic polymorphisms of GC should be extensively evaluated by studying the distribution of TLR polymorphisms in the Thai population. Further studies of TLR expression and *H. pylori* colonization with the assessment of the cytokine responses are needed to confirm the impact of downstream signaling on GC susceptibility.

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 the patients who participated in this study.

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Conflict of Interest: The authors have no conflict of interest to declare.

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References

1. Blaser MJ. The role of Helicobacter pylori in gastritis and its progression to peptic ulcer disease. Aliment Pharmacol Ther 1995; 9(Suppl 1): 27-30. [CrossRef]

2. Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of Helicobacter pylori infection. Clin Microbiol Rev 2006; 19: 449-90. [CrossRef]
3. Atherton JC. The pathogenesis of Helicobacter pylori-induced gastro-duodenal diseases. Annu Rev Pathol 2006; 1: 63-96. [CrossRef]
4. Basso D, Plebani M, Kusters JG. Pathogenesis of Helicobacter pylori infection. Helicobacter 2010; 15: 14-20. [CrossRef]
5. Albiger B, Dahlberg S, Henriques-Normark B, Normark S. Role of the innate immune system in host defence against bacterial infections: focus on the Toll-like receptors. J Intern Med 2007; 261: 511-28. [CrossRef]
6. Trejo-de la OA, Hernandez-Sancen P, Maldonado-Bernal C. Relevance of single-nucleotide polymorphisms in human TLR genes to infectious and inflammatory diseases and cancer. Genes Immun 2014; 15: 199-209. [CrossRef]
7. Cook DN, Pisetsky DS, Schwartz DA. Toll-like receptors in the pathogenesis of human disease. Nat Immunol 2004; 5: 975-9. [CrossRef]
8. Hu Y, Liu JP, Zhu Y, Lu NH. The Importance of Toll-like Receptors in NF-kappaB Signaling Pathway Activation by Helicobacter pylori Infection and the Regulators of this Response. Helicobacter 2016. [CrossRef]
9. Santini D, Angeletti S, Ruzzo A, et al. Toll-like receptor 4 Asp-299Gly and Thr399Ile polymorphisms in gastric cancer of intestinal and diffuse histotypes. Clin Exp Immunol 2008; 154: 360-4. [CrossRef]
10. Zeng HM, Pan KF, Zhang Y, et al. Genetic variants of toll-like receptor 2 and 5, Helicobacter pylori infection, and risk of gastric cancer and its precursors in a Chinese population. Cancer Epidemiol Biomarkers Prev 2011; 20: 2594-602. [CrossRef]
11. Zeng HM, Pan KF, Zhang Y, et al. [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 2011; 45: 588-92.
12. Castano-Rodriguez N, Kaakoush NO, Mitchell HM. Pattern-recognition receptors and gastric cancer. Front Immunol 2014; 5: 336.
13. Arbour NC, Lorenz E, Schutte BC, et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. Nat Genet 2000; 25: 187-91. [CrossRef]
14. Schmausser B, Andrulis M, Endrich S, et al. Expression and subcellular distribution of toll-like receptors TLR4, TLR5 and TLR9 on the gastric epithelium in Helicobacter pylori infection. Clin Exp Immunol 2004; 136: 521-6. [CrossRef]
15. Yokota S, Okabayashi T, Rehli M, Fujii N, Amano K. Helicobacter pylori lipopolysaccharides upregulate toll-like receptor 4 expression and proliferation of gastric epithelial cells via the MEK1/2-ERK1/2 mitogen-activated protein kinase pathway. Infect Immun 2010; 78: 468-76. [CrossRef]
16. Trejo-de la OA, Torres J, Perez-Rodriguez M, et al. TLR4 single-nucleotide polymorphisms alter mucosal cytokine and chemokine patterns in Mexican patients with Helicobacter pylori-associated gastroduodenal diseases. Clin Immunol 2008; 129: 333-40. [CrossRef]
17. Kutikhin AG. Impact of Toll-like receptor 4 polymorphisms on risk of cancer. Hum Immunol 2011; 72: 193-206. [CrossRef]
18. Tongtawee T, Dechsukhum C, Leeanansaksiri W, et al. Improved Detection of Helicobacter pylori Infection and Premalignant Gastric Mucosa Using Site Specific Biopsy: a Randomized Control Clinical Trial. Asian Pac J Cancer Prev 2015; 16: 8487-90. [CrossRef]
19. Bornschein J, Malfertheiner P. Gastric carcinogenesis. Langenbecks Arch Surg 2011; 396: 729-42. [CrossRef]
20. El-Omar EM, Ng MT, Hold GL. Polymorphisms in Toll-like receptor genes and risk of cancer. Oncogene 2008; 27: 244-52. [CrossRef]
21. Kutikhin AG. Association of polymorphisms in TLR genes and in genes of the Toll-like receptor signaling pathway with cancer risk. Hum Immunol 2011; 72: 1095-116. [CrossRef]
22. Castano-Rodriguez N, Kaakoush NO, Goh KL, Fock KM, Mitchell HM. The role of TLR2, TLR4 and CD14 genetic polymorphisms in gastric carcinogenesis: a case-control study and meta-analysis. PLoS One 2013; 8: e60327. [CrossRef]
23. Castano-Rodriguez N, Kaakoush NO, Pardo AL, Goh KL, Fock KM, Mitchell HM. Genetic polymorphisms in the Toll-like receptor signalling pathway in Helicobacter pylori infection and related gastric cancer. Hum Immunol 2014; 75: 808-15. [CrossRef]
24. Qadri Q, Rasool R, Afroze D, et al. Study of TLR4 and IL-8 gene polymorphisms in H. pylori-induced inflammation in gastric cancer in an ethnic Kashmiri population. Immunol Invest 2014; 43: 324-36. [CrossRef]
25. Tahara T, Arisawa T, Shibata T, Hirata I, Nakano H. Association of polymorphism of TLR4 and CD14 genes with gastroduodenal diseases in Japan. Inflammopharmacology 2007; 15: 124-8. [CrossRef]
26. Hishida A, Matsuo K, Goto Y, et al. Toll-like receptor 4 +3725 G/C polymorphism, Helicobacter pylori seropositivity, and the risk of gastric atrophy and gastric cancer in Japanese. Helicobacter 2009; 14: 47-53. [CrossRef]
27. Agnese DM, Calvano JE, Hahm SJ, et al. Human toll-like receptor 4 mutations but not CD14 polymorphisms are associated with an increased risk of gram-negative infections. J Infect Dis 2002; 186: 1522-5. [CrossRef]
28. Child NJ, Yang IA, Pulletz MC, et al. Polymorphisms in Toll-like receptor 4 and the systemic inflammatory response syndrome. Biochem Soc Trans 2003; 31: 652-3. [CrossRef]
29. Faber J, Meyer CU, Gemmer C, et al. Human toll-like receptor 4 mutations are associated with susceptibility to invasive meningococcal disease in infancy. Pediatr Infect Dis J 2006; 25: 80-1. [CrossRef]
30. Su B, Ceponis PJ, Lebel S, Huynh H, Sherman PM. Helicobacter pylori activates Toll-like receptor 4 expression in gastrointestinal epithelial cells. Infect Immun 2003; 71: 3496-502. [CrossRef]