Original Article J. Clin. Biochem. Nutr., **40**, 62–65, January 2007

Absence of Common Polymorphisms of Toll Like Receptor 4 (TLR4): Asp299Gly, Thr399Ile in Patients with Gastroduodenal Diseases in Japan

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Received 24 July, 2006; Accepted 22 August, 2006

Summary **Host genetic factors may play a key role in determining the long-term outcome of the *Helicobacter pylori* (*H. pylori*) infection. Toll-like receptor 4 (TLR4) mediated recognition of lipo-polysaccharide (LPS) is required for efficient recognition of gram-negative bacterial infections. The aim of this study is to investigate the effects of common polymorphisms of TLR4 Asp299Gly, Thr399Ile in patients with gastroduodenal diseases in Japanese population. The study was performed in 149 gastric cancer (GC) cases (mean age 64.0 ± 12.4, M:F = 109:40) and 94 patients without evidence of GC (mean age 64.1 ± 12.3, M:F = 65:25) as the control group. TLR4 Asp299Gly, Thr399Ile were determined by Polymerase chain reaction-length of polymorphisms (PCR-RFLP) in all the patients. Asp299Gly, Thr399Ile were not detected in all 243 patients enrolled in this study. In conclusion, our data suggest that TLR4 Asp299Gly, Thr399Ile are very rare in Japanese population and thus they may not be a important factor in determining the outcome of *H. pylori* infected individuals in Japan.**

***Key Words*: gastric cancer, *Helicobacter pylori*, toll-like receptor 4, polymorphism**

Introduction

Helicobacter pylori (*H. pylori*) infection is now accepted as a crucial event in the development of peptic ulcer disease and atrophic gastritis, and it is implicated in the development of gastric carcinoma [*1–5*]. However, there are distinct differences in the extent of gastric mucosal inflammation and atrophy among *H. pylori* infected patients, and only a small group of them develop peptic ulcer disease and gastric cancer. It suggests that some genetic factors may play important roles in long term outcome of *H. pylori* infection [*6*].

Toll-like receptor 4 (TLR4) is important mediator of the inflammatory response in the first line of host defense by recognition of Lipopolysaccharide (LPS) [*7–9*]. LPS, a major component of the outer cell wall of gram-negative bacteria, is a potent and well-characterized inducer of inflammation. After forming a complex with the LPS- binding protein, LPS interacts with a membrane molecule, CD14 and MD2 [*10–12*]. Together with TLR4, this complex induces second-messenger and signal transduction pathways [*13*]. These signals in turn activate transcription factors, mainly nuclear factor-κB (NFκB) and cytokines [*10*, *14*].

Recently it was reported that TLR4 expression by gastric epithelium was more stronger in *H. pylori* gastritis than in the non in inflamed gastric mucosa [*15*]. It was also reported that TLR4 regulated the response of gastric epithelial cells to *H. pylori* infection and *H. pylori* LPS-induced transcription of several genes depends on the activation of NFκB [*16*]. These findings support a sentinel role for TLR4 in the mucosal immunity to *H. pylori.*

Genetic studies of TLR4 have identified polymorphisms.

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Arbour *et al*. [*17*] demonstrated 2 cosegregating polymorphisms in the extracellular domain of the TLR4 gene. The first is an A-G substitution at nucleotide 896 from the start codon of the TLR4 gene, which results in an aspartic acid-to-glycine substitution at position 299 of the amino acid sequence (Asp299Gly). A cosegregating polymorphism that results in a thereonine-to-isoleucine substitution at position 399 of the amino acid sequence (Thr399Ile) also was identified. These polymorphisms are associated with functional changes, as demonstrated by decreased airway responsiveness after LPS stimulation [*18*].

Because of the important roles that TLR4 play with respect to LPS binding and signaling, we hypothesized that polymorphisms in the TLR4 gene may be important factors for determining the long term outcome of the *H. pylori* infection.

In the present study, we investigated the effects of TLR4 (Asp299Gly, Thr399Ile) polymorphisms on gastroduodenal diseases in Japanese population.

Subjects and Methods

Study Population

We studied 243 patients attending the gastroenterology division of Fujita Health University Hospital (Aichi, Japan) from January 2005 to January 2006. Gastroscopy was done in all of the patients and they were divided into two groups for assessment of TLR4 polymorphisms. There were 149 gastric cancer (GC) patients with a mean age of 64.0 (29–91) years and a male:female (M:F) ratio of 109:40 and 94 patients with no evidence of GC (mean age of 64.1 [30–90] years old and M:F = 65:29) as the control group. GC was diagnosed histologically and was classified according to Lauren's classification [*18*]. Detailed information was obtained about staging and anatomic location (Table 1). The Ethics Committee of Fujita Health University School of Medicine approved the protocol and written informed consent was obtained from all of the subjects.

Detection of H. pylori

H. pylori infection status was determined by histology, culture, the rapid urease test (RUT), or the serum titer of antibodies against *H. pylori.* Infection was diagnosed when at least one of these tests was positive.

Genotyping

Genomic DNA was extracted from non-cancerous gastric biopsy tissue or peripheral blood using the standard phenol/chloroform method. Then Asp299Gly, Thr399Ile polymorphisms of TLR4 were determined using PCR and subsequent cleavage by NcoI and HinfI restriction endnucleases as previously described [*19*].

Results

Study population

A total of 149 GC patients and 94 control subjects without evidence of GC participated in this study. Their characteristics are summarized in Table 2. There were no significant differences between these groups in the distribution of age and sex, but the *H. pylori* infection rate was significantly higher in the GC patients ($p = 0.0002$). The control group included 25 patients gastric ulcer (26.7%), 7 patients with duodenal ulcer (7.4%) and patients with gastric + duodenal ulcer (2.1%). They were all *H. pylori* positives.

TLR4 genotypes

Asp299Gly, Thr399Ile polymorphisms of TLR4 were typed in all 243 subjects. However, in this study, Asp299Gly and Thr399Ile polymorphisms of TLR4 were not detected in all the 243 subjects.

Discussion

H. pylori LPS has not been considered to play a major

Table 1. Clinicopathologic characteristics of GC

Variables (n)	
Histrogic subtypes	
Intestinal type	85
Diffuse type	64
Tumor location	
Cardia	6
Non cardia	143
Upper third	4
Middle third	84
Lower third	55
Tumor stage	
Early stage	62
Advanced stage	73

Table 2. Characteristics of subjects

	Gastric cancer (GC) cases	Patients without GC	*p*
Subjects (n)	149	104	
Sex [male/female (%/%)]	109/40 (73/27)	65/29 (69/31)	0.53$
Mean age ± SD (y)	64.0 ± 12.4	64.1 ± 12.3	0.76*
H. pylori infection positive ratio (%)	90.6	72.3	0.002$

$$x^2$ test, *Mann-Whitney U test

role in the pathogenesis of gastritis because its biological activity is lower than that of the LPS of pathogens such as *Escherichia coli* (*E.coli*) and *Salmonella species* (*Salmonella spp*) [*20*, *21*]. But in contrast, several studies have demonstrated significant roles of *H. pylori* LPS in the induction of gastritis. Teshima *et al.* [*22*, *23*] have shown that *H. pylori* LPS stimulates NF-κB activation markedly in cultured guinea pig gastric mucosal cells and leads to abundant release of superoxide anion from the cells. In addition, the severity of atrophic gastritis induced by long term infection of *Haemobartonella felis* in CH3/He mice is significantly higher than that in LPS-non responder CH3/HeJ mice [*24*], which have an inactivating point mutation in TLR4 [*25*]. More recently, Karhukorpi *et al*. [*26*] reported elevated serum levels of a soluble form of the CD14 molecule in *H. pylori* infected individuals. Soluble CD14 binds to LPS and facilitates its signaling, and this study has shown that CD14 promoter polymorphism is associated with the disease outcome. All of these findings suggest that *H. pylori* LPS may be a potent mediator in modulating *H. pylori* infection induced gastritis and it may be reasonable to speculate that inter individual difference of innate immune response to *H. pylori* LPS is a important factor in determining the long term out come of the *H. pylori* infection.

TLR4-mediated recognition of LPS is required for efficient recognition of Gram-negative bacterial infections and some studies shows important roles that TLR4 plays with respect to LPS binding and signaling even in *H. pylori* infection [*16*].

In 2000, Arbor *et al.* reported that TLR4 polymorphisms Asp299Gly, Thr399Ile lead to a LPS-hyporesponsive phenotype in either human primary airway epithelial cells or alveolar macrophages [*17*]. Concerning host genetic factors, polymorphisms in TLR4 show an increased risk to develop a septic shock with gram negative microorganisms [*27*], premature birth [*28*], graft versus host disease after hematopoietic stem cell transplantation [*29*], and inflammatory bowel disease [*30*]. But in this study, we did not detect 299Gly or 399Ile alleles in patients with gastroduodenal diseases in Japanese population. All 243 subjects did not have 299Gly and 399Ile polymorphisms of TLR4. Noguchi *et al*. screened 32 Japanese asthmatics and did not detect 299Gly or 399Ile polymorphisms of TLR4 [*31*]. Similar results has also been reported in other studies in China [*32*, *33*]. 299Gly and 399Ile polymorphisms of TLR4 may be very rare at least in Japanese and Chinese population and thus may not be an important factor in determining the outcome of the *H. pylori* infected patients in this area. Recently, Ohara *et al.* reported the potential association between TLR4 Thr 135 Ala polymorphism at leucine-rich repeat (LRR) of toll-like receptor 4 and risk of diffuse type gastric cancer in Japanese population [*34*]. This polymorphism but not 299Gly or 399Ile may influence the function of TLR4 and modify the risk of *H. pylori* related gastrodduodenal diseases in Japanese population.

In conclusion, we have shown that 299Gly and 399Ile polymorphisms of TLR4 are very rare in patients with gastroduodenal diseases in Japan. Further genetic studies will be needed to resolve the impact of the TLR4 polymorphisms in the susceptibility of gastroduodenal diseases in Japanese population.

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