Polymorphisms of Two *Fucosyltransferase* Genes (*Lewis* and *Secretor* Genes) Involving Type I Lewis Antigens Are Associated with the Presence of Anti-*Helicobacter pylori* IgG Antibody[1]

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Abstract

Helicobacter pylori **attach to the gastric mucosa with adhesin, which binds to Lewis b (Le^b) or H type I carbohydrate structures. The *Secretor* (*Se*) gene and *Lewis* (*Le*) gene are involved in type I Le antigen synthesis. The present study was performed to investigate the possibility that *Se* and *Le* gene polymorphisms alter the risk of *H. pylori* infection. Two hundred thirty-nine participants were genotyped for *Se* and *Le* and tested for the presence of anti-*H. pylori* IgG antibodies. Using the normal gastric mucosa from 60 gastric cancer patients, we assessed immunohistochemically whether type I Le antigen expression depended on the *Se* and *Le* genotypes. The *H. pylori* infection rate was positively associated with the number of *Se* alleles (*se/se* group, 45.1%; *Se/se* group, 64.6%; and *Se/Se* group, 73.3%) and negatively associated with the number of *Le* alleles (*le/le* group, 76.4%; *Le/le* group, 68.3%; and *Le/Le* group, 55.6%). When the subjects were classified into three groups [low risk, (*se/se*, *Le/Le*) genotype; high risk, (*Se/Se*, *le/le*), (*Se/Se*, *Le/le*), and (*Se/se*, *le/le*) genotypes; moderate risk, other than low- or high-risk group], the odds ratio relative to the low-risk group was 3.30 (95% confidence interval, 1.40–7.78) for the moderate-risk group and 10.33 (95% confidence interval, 3.16–33.8) for the high-risk group. Immunohistochemical analysis supported the finding that *Se* and *Le* genotypes affected the expression**

of *H. pylori* adhesin ligands. We conclude that *Se* and *Le* genotypes affect susceptibility to *H. pylori* infection.

Introduction

Helicobacter pylori infection is known to be linked with duodenal and gastric ulcers (1), gastric adenocarcinomas of the distal stomach (2), and low-grade B-cell lymphoma of mucosa-associated lymphoid tissue (3), and *H. pylori* is classified as a definite carcinogen for gastric cancer by the International Agency for Research on Cancer (4). Using an animal experiment model, we have also proved an association between *H. pylori* infection and gastric cancer in that *H. pylori* infection enhanced glandular stomach carcinogenesis (5), and the eradication of *H. pylori* diminished these enhancing effects (6).

Recent progress in the molecular analysis of *H. pylori* infection has clearly revealed that the bacteria attach to the gastric mucosa with the blood group antigen-binding adhesin, BabA (7, 8). A clinical relevance has been shown for the *babA2* gene encoding BabA adhesin with regard to *H. pylori*-related diseases (9). BabA binds to both Le^b [3] [Gal (α1.2Fuc)β1.3GlcNAc(α1.4Fuc)-R] and H type I blood group carbohydrate structures [H type I structures; Gal (α1.2Fuc) β1.3GlcNAc-R] expressed on the foveolar epithelium of the gastric mucosa (Fig. 1; Ref. 7).

We have been studying Le blood type antigens using biochemical and molecular biological methods and have obtained the following results concerning type I Le antigen synthesis from a series of previous studies. Individuals homozygous for nonfunctional alleles of *Le* gene (*le/le*) fail to express type I Le antigen in the entire body (Le negative; Refs. 10, 11). In the human fucosyltransferase family, only Le enzyme (FUT3, Fuc-T III) exhibits fucose transfer activity toward a type I precursor (Galβ1.3GlcNAc-R) or H type I structure with α1.4 linkage (Fig. 1; Refs. 10, 12, 13). Se enzyme (FUT2, Fuc-T II) exhibits fucose transfer activity toward the type I precursor with α1.2 linkage (Fig. 1) and is responsible for Le^b expression on erythrocytes, solely determines the secretor status, and makes a marked contribution to Le^b expression in colorectal tissues (10, 14–16). Individuals homozygous for nonfunctional alleles of the *Se* gene (*se/se*) fail to express ABH blood antigens in secreted fluids (nonsecretors; Refs. 14, 17), whereas those very rare individuals homozygous for nonfunctional alleles of the *H* gene (*h/h*) fail to express ABH blood antigens on erythrocytes (18, 19). The type I Le antigen synthetic pathway in secretor tissues is shown in Fig. 1.

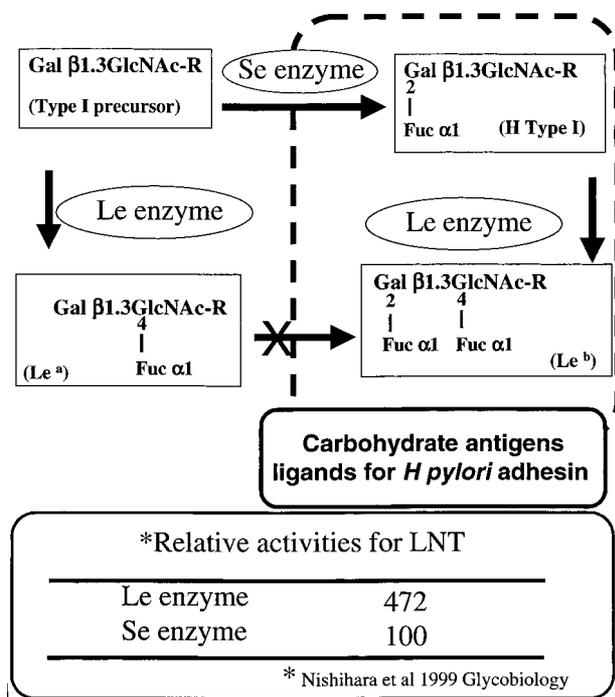Since the first report of nonfunctional *H* alleles in Cauca-

Fig. 1. Type I Le antigen synthetic pathway and the involvement of Se and Le enzyme. Se enzyme transfers fucose to type I precursor with $\alpha 1.2$ linkage and Le enzyme transfers fucose to H type I antigen with $\alpha 1.4$ linkage to synthesize Le[b] antigen. BabA binds both H type I and Le[b] antigens (*boxed* with *dotted line*). Se enzyme cannot transfer fucose to the galactose residue of Le[a] antigen. Le enzyme exhibits about five times as high relative activities for lacto-*N*-tetraose (*LNT,* Galβ1.3GlcNAcβ1.3Galβ1.4Glc) as Se enzyme.

sians (18), some ethnic-specific nonfunctional point mutations have been reported not only in the *H* gene but also in the *Se* and *Le* genes. In the *Se* gene in the Japanese population (14, 20), we found the *sej* (also called *se2*) allele with an A385T (Ile[129] to Phe) missense mutation with 40% frequency. This mutation results in much lower $\alpha 1.2$-fucosyltransferase activity (14). Koda *et al.* (20) reported other inactive alleles in the Japanese, *i.e., se3* (C571T; Arg[191] to terminal codon), *se4* (C628T; Arg[210] to terminal codon), and *se5*, attributable to recombinant fusion between the *Se* gene and the *Sec1* pseudogene. However, our recent population study indicated the frequencies of *sej* and *se5* alleles to be 40.6 and 4.7%, respectively, whereas *se1*, *se3,* and *se4* alleles were not found in any of 600 Japanese analyzed (21). Therefore, *sej/sej*, *sej/se5,* and *se5/se5* individuals can be regarded essentially as the nonsecretors in Japan. On the other hand, we also found only three missense mutated *Le* alleles, *le1* (T59G; Leu[20] to Arg and G580A; Gly[170] to Ser; Ref. 20), *le2* (T59G and T1067A; Ile[356] to Lys), and *le3* (T59G only), in the Japanese population (10, 15). The *le3* allele exhibited as much $\alpha 1.3/4$-fucosyltransferase activity as the wild-type allele *in vitro*, whereas both *le1* and *le2* showed very low activity compared with the wild-type *Le* allele (15). As a result, *le1/le1, le1/le2,* and *le2/le2* individuals can be regarded essentially as the Le-negative individuals in Japan.

In a previous biochemical analysis, we demonstrated that the amount of Le[b] antigen was affected by the number of *Se* alleles (16). In our population study, we proved that *Se* genotypes affected the serum level of sialyl-Le[a] antigen (CA19.9; NeuAcα2.3 Galβ1.3GlcNAc-R) in individuals without malignancy attributable to the competition between Se enzyme and

Galβ1.3GlcNAc α2.3-sialyltransferases (21). Considering the type I Le antigen synthetic pathway, it is possible that the type I precursor structure for acceptor substrate is used by Se and Le enzymes, with some competition between the two. Therefore, we expected that both *Se* and *Le* gene polymorphism would affect the susceptibility to *H. pylori* infection because of the impact of these polymorphisms on the synthesis of adhesin ligands. An increased number of *H. pylori* causes an inflammatory reaction in gastric mucosa through the up-regulation of IL-8 by nuclear factor-κB activation (22) via an intracellular signal pathway involving IKK, NIK, TRAF2, and TRAF6 (23). This may be reflected in the augmentation of anti-*H. pylori* antibody in patient serum. Therefore, we tested the presence of anti-*H. pylori* IgG antibody for the purpose of evaluating the association between Le blood genotype and *H. pylori* infection.

In 1999, we began an *H. pylori* eradication intervention study to assess *H. pylori* eradication therapy. In the present study, we report the correlation between the *Le* and *Se* genotypes and *H. pylori* infection, indicated by the augmentation of anti-*H. pylori* IgG antibody, in the Japanese population.

Materials and Methods

Participants. We began an intervention study in 1999 to eradicate *H. pylori*. Participants were from 40 to 69 years of age with no history of gastrectomy. All gave informed consent for the use of their clinical pathological specimens for this research prior to undergoing gastroscopy at Aichi Cancer Center Hospital. Participants found to have a malignancy were excluded from the present study. DNA and serum samples from 239 participants were examined for *H. pylori* infection and the determination of the genotypes. Of the 239 participants, 95 (39.7%) said they were taking medication for 105 diseases or conditions, including 23 cases of gastric/duodenal ulcer, 23 of so-called gastritis, 16 of hypertension, 7 of diabetes mellitus, 7 of pain including arthritis and lumbago, 6 of hyperlipidemia, and 23 other miscellaneous conditions. The remaining 60.3% were disease free. We determined the *Se* and *Le* genotypes of another 150 DNA samples from gastric cancer patients who had been diagnosed and had undergone total gastrectomy in the past at Aichi Cancer Center Hospital, after their written informed consent to genotyping was obtained. The patients with early gastric cancer and long-term survivors were included. Sixty surgically resected normal gastric mucosa specimens from these patients were available for evaluating immunohistochemically whether type I Le antigen expression was dependent on the *Le* and *Se* genotypes. Because the stomach cancer patients were prevalent cases, they were not appropriate for examining the genotypes for stomach cancer risk but were applicable for the study of association between phenotype and genotype.

Determination of *Se* and *Le* Genotype. Because the *se3* and *se4* alleles were not found in our previous analysis of >600 Japanese samples (21), only A385T mutations in the *sej* and the *se5* alleles were assessed. The *Le* genotype was assigned based on detection of three missense mutations, T59G, G508A, and T1067A, in the *Le* gene (10). Aliquots of 7 ml of peripheral blood were obtained from the participants with 2Na-EDTA, and the buffy coat was separated to extract genomic DNA using a QIAamp DNA Blood Mini kit (Qiagen, Inc., Valencia, CA). The detection method for *sej, le1, le2,* and *le3* alleles was based on the PCR-RFLP described in detail previously (21). In brief, the full-length open reading frame of the *Se* or *Le* gene was amplified with a specific primer set and subjected to the second PCR reaction for the PCR-RFLP. The second PCR products

Table 1 Frequency of *Le* and *Se* genotype subgroups

Group	1	2	3	4	5	6	7	8	9
Le genotype	*Le/Le*	*Le/le*	*Le/Le*	*Le/le*	*Le/Le*	*Le/le*	*le/le*	*le/le*	*le/le*
Se genotype	*se/se*	*se/se*	*Se/se*	*Se/se*	*Se/Se*	*Se/Se*	*se/se*	*Se/se*	*Se/Se*
No. of patients	27	21	63	54	34	23	3	10	4
Frequency (%)	11.3	8.8	26.4	22.6	14.2	9.6	1.3	4.2	1.7
Population[a] (%)	8.5	8.5	24.3	24.3	14.8	9.8	1.5	4.3	4.3

[a] Narimatsu *et al.* (21).

were digested with *Alu*I to detect A385T mutations of the *sej* allele, with *Pvu*II to G508A of *le1*, with *Hin*dIII to T1067A of *le2*, and with *Msp*I to T59G of *le1, le2,* and *le3*. The *se5* allele was detected by simple PCR with a specific primer set as reported previously (20, 21). The specifically amplified DNA fragment was subcloned in pCR II vector (TA Cloning kit; Invitrogen, Carlsbad, CA), and the nucleotide sequence was determined with an ABI 310 genetic analyzer.

Detection of *H. pylori* Infection. Two-ml serum samples were subjected to testing for anti-*H. pylori* IgG antibodies with the high molecular weight campylobacter-associated protein (Enteric Products, Inc., Stony Brook, NY) ELISA conducted by SRL Co., Ltd. (Tokyo, Japan). Cases with ELISA values >2.2 were defined as positive for *H. pylori* infection, in accordance with global standards as in the suppliers' manual. Many epidemiological studies in countries including Japan have adopted this method as a standard tool for detecting individuals infected with *H. pylori* (2, 24–29). The causal relationship between stomach cancer and *H. pylori* has been established largely depending on epidemiological studies using the antibody rather than biopsy samples (2, 24–26, 28, 29).

Immunohistochemical Analysis of Detecting Type I Le Antigen. We performed immunohistochemical analysis with monoclonal antibodies, anti-Le[a] antigen (7LE; Seikagaku Co., Tokyo, Japan), and anti-Le[b] antigen (BG-6; Signet Pathology Systems, Inc., Dedham, MA; Ref. 9). Sections were cut at 4 μm, deparaffinized with xylene, and rehydrated through ethanol. The sections were treated with 0.3% (v/v) H_2O_2 in methanol for 15 min to block endogenous peroxidase and washed three times in PBS. They were then incubated for 30 min with blocking agent [0.5% normal swine serum (Dako A/S, Copenhagen, Denmark), 0.1% NaN_3 in PBS] at room temperature to eliminate nonspecific staining. Sections were incubated overnight at 4°C with monoclonal antibodies. Control staining reactions included replacement of the primary antibodies with normal mouse serum (Nippon Bio-Supply Center, Tokyo, Japan). After washing out primary antibody, sections were rinsed three times with PBS for 5 min at room temperature. Binding was visualized with the streptavidin-biotin technique (Vectastain ABC kit; Vector Laboratories, Inc., Burlingame, CA), and nuclei were counterstained with Mayer's hematoxylin.

Statistical Analysis. For the purpose of estimating the *H. pylori* infection risk for the functional *Se* allele located in 19q13.3, we categorized the participants into three groups: *Se/Se, Se/se,* and *se/se,* homozygous for the functional *Se* alleles, heterozygous for the functional and nonfunctional allele (*Se/sej Se/se5*), and homozygous for the nonfunctional alleles (*sej/sej, sej/se5* and *se5/se5*), respectively. We also grouped the participants depending on the *Le* alleles located in 19p13.3. As mentioned above, the *Le* genotypes were separated into three groups: group *Le/Le,* consisting of *Le/Le* and *Le/le3*; group *Le/le,* consisting of *Le/le1* and *Le/le2*; and group *le/le,* consist-

Table 2 *Se* and *Le* genotypes and ELISA values for anti-*H. pylori* IgG antibodies

ELISA 0–1.7	*H. pylori* infection	*Se/Se n* (%)	*Se/se n* (%)	*se/se n* (%)
	(−)	15 (22.2)	40 (31.5)	25 (49.0)
1.8–2.2	(−)	2 (4.4)	5 (3.9)	3 (5.9)
2.3–3.9	(+)	14 (20.0)	12 (9.5)	5 (9.8)
4.0–6.5	(+)	30 (53.3)	70 (55.1)	18 (35.3)
Total		61 (100.0)	127 (100.0)	51 (100.0)

ELISA 0–1.7	*H. pylori* infection	*Le/Le n* (%)	*Le/le n* (%)	*le/le n* (%)
	(−)	46 (37.1)	30 (30.6)	4 (23.5)
1.8–2.2	(−)	9 (7.3)	1 (1.0)	0 (0)
2.3–3.9	(+)	17 (13.7)	11 (11.2)	3 (17.6)
4.0–6.5	(+)	52 (41.9)	56 (57.1)	10 (58.8)
Total		124 (100.0)	98 (100.0)	17 (100.0)

ing of *le1/le1, le1/le2,* and *le2/le2*. Statistical analysis was performed with the computer program STATA Version 6 (Stata Corp., College Station, TX). The categorical data were examined with a χ^2 test, and the ORs of the *H. pylori* infection were estimated using an unconditional logistic model. The adjustment for sex, age as a continuous variable, and genotypes as dummy variables were conducted using the logistic model, as well as a significance test for trends in ORs allocating consecutive values for genotypes. The 95% CIs for the percentage were calculated based on binomial distribution.

Results

The Prevalence of *H. pylori* Infection and *Se* and *Le* Genotyping. *Se* and *Le* gene polymorphisms were clearly determined by PCR-RFLP and specific PCR. Representative PCR products were subcloned and sequenced to confirm the specific PCR amplification. Participants were divided into three genotype groups according to the *Se* and *Le* alleles (for *Se*: *Se/Se, Se/se,* and *se/se*; for *Le*: *Le/Le, Le/le,* and *le/le*). As shown in Table 1, the allele frequency of *Se* and *Le* alleles among the participants was similar to our previous study [a 2 × 9 table for the independent test, χ^2 = 4.82 with d.f. = 8, P = 0.777; 2 × 3 tables for *Se* (χ^2 = 1.19, d.f. = 2, P = 0.553) and for *Le* (χ^2 = 1.19, d.f. = 2, P = 0.489); Ref. 21]. The genotype frequencies for *Se* and *Le* genes were independent of each other (χ^2 = 0.78, with d.f. = 4 for a 3 × 3 table; P = 0.941).

The prevalence of *H. pylori* infection defined by ELISA values was 73.3% in the *Se/Se,* 64.6% in the *Se/se,* and 45.1% in the *se/se* groups, whereas it was 55.6% in the *Le/Le,* 68.3% in the *Le/le,* and 76.4% in the *le/le* groups (Table 2).

The crude OR (0.32; 95% CI, 0.14–0.70) and the ORs adjusted by sex and age (OR, 0.35; 95% CI, 0.15–0.80), and sex and age genotype (OR, 0.34; 95% CI, 0.15–0.78) were significantly lower for *se/se* than *Se/Se* participants (Table 3). On the contrary, *le/le* individuals showed a 2.59 times higher crude OR than *Le/Le* individuals, although the difference was not statistically significant (Table 3). The estimated *H. pylori* infection rate decreased with the number of *Se* alleles (adjusted P, trend = 0.012), whereas it increased with the number of *Le* alleles (adjusted P, trend = 0.012; Table 3).

***H. pylori* Infection Rate and the Combination of *Se* and *Le* Genotypes.** Fig. 2 shows the *H. pylori* infection rate for the individuals with different *Se* and *Le* genotypes. They were classified into three groups (low-, moderate-, and high-risk groups), based on the infection rate and number of alleles

Table 3   ORs and 95% CIs of *Se* and *Le* genotypes for *H. pylori* infection

Genotype	Infection[a] +	Infection[a] −	Crude OR (95% CI)	Adjusted OR[b] (95% CI)	Adjusted OR[c] (95% CI)
Se/Se	44	17	1.0	1.0	1.0
Se/se	82	45	0.70 (0.36–1.37)	0.79 (0.39–1.58)	0.75 (0.37–1.53)
se/se	23	28	0.32 (0.14–0.70)	0.35 (0.15–0.80)	0.34 (0.15–0.78)
			P trend = 0.004	*P* trend = 0.013	*P* trend = 0.012
Le/Le	69	55	1.0	1.0	1.0
Le/le	67	31	1.72 (0.99–3.00)	1.95 (1.08–3.50)	1.99 (1.10–3.62)
le/le	13	4	2.59 (0.80–8.39)	2.80 (0.81–9.74)	2.82 (0.79–9.99)
			P trend = 0.023	*P* trend = 0.013	*P* trend = 0.012

[a] Infection (+) is defined by an ELISA value >2.3.
[b] Adjusted for sex and age.
[c] Adjusted for sex, age, and genotype.

(Fig. 2). The combined rate was 33.3% (95% CI, 16.5–54.0%; $n = 34$) for the low-risk group, 62.3% (54.7–69.5%; $n = 175$) for the moderate-risk group, and 83.8% (68.0–93.8%; $n = 37$) for the high-risk group. The sex and age-adjusted OR relative to the low-risk group was 3.30 (1.40–7.78) for the moderate-risk group and 10.33 (3.16–33.80) for the high-risk group. As shown in Table 4, the trend test revealed a highly significant value (*P* trend <0.001). These observations and interpretations indicate the possibility that both *Le* and *Se* genotypes result in a greater number of *H. pylori* attaching effectively to the gastric mucosa, thereby augmenting the production of anti-*H. pylori* antibody.

Le[a] and Le[b] Antigen Expression Depends on *Se* and *Le* Genotype. In many studies, type I Le antigens were detected on the gastric foveolar epithelium, depending on the secretor and Le phenotype, with some exceptions (30–32). We performed immunohistochemical analysis with the specific monoclonal antibodies for the purpose of confirming whether type I Le antigen expression was dependent on *Se* and *Le* genotypes as a potential mechanism to explain the effects observed. The 60 gastric cancer patients selected consisted of 40 *Se/−* and 20 *se/se* cases, including 30 *Le/−* and 10 *le/le* patients, and 15 *Le/−* and 5 *le/le* patients, respectively. As indicated in Fig. 3*A* by the strong positive staining, Le[b] antigen was clearly detected on the foveolar epithelium of most (*Se/−, Le/−*) patients but rarely detected in (*se/se, Le/−*; Fig. 3*B*) and (*se/se, le/le*) patients (Fig. 3*D*). In (*Se/−, le/le*) patients, focal and small amounts of Le[b] antigen expression were seen in foveolar epithelium (Fig. 3*C*). In contrast, Le[a] antigen was clearly detected on the foveolar epithelium of both *Se/−, Le/−* patients (Fig. 3*E*) and *se/se, Le/−* patients (Fig. 3*F*) but poorly detected in *Se/−, le/le* patients (Fig. 3*G*) or *se/se, le/le* patients (Fig. 3*F*). These studies indicate that type I Le antigens, expressed by gastric foveolar cells, are synthesized by Le and Se enzymes and raise the possibility that both *Le* and *Se* genotypes play a role in affecting *H. pylori* infection through BabA binding.

Discussion

This study evaluated the association between *H. pylori* infection risk and both polymorphisms of the *Se* and *Le* genes involved in the BabA ligand carbohydrate antigen synthesis. We found that *H. pylori* infection, as determined by the presence of anti-*H. pylori* IgG antibody, was increased among individuals with a greater number of functional *Se* alleles and a
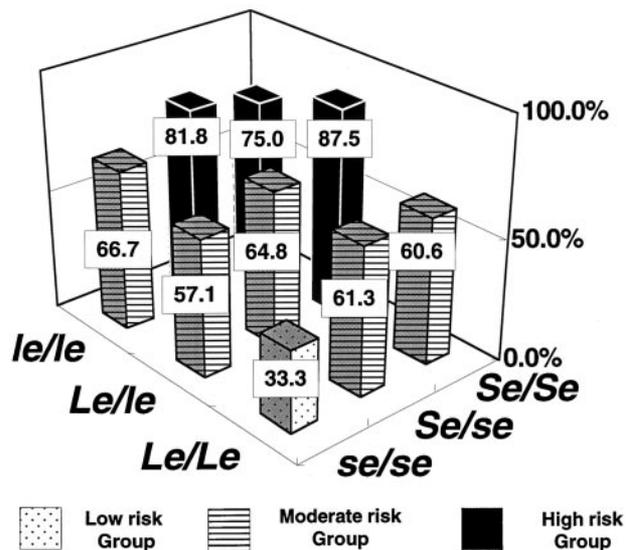

Fig. 2. *H. pylori* infection frequency (%) depending on *Se* and *Le* genotypes. The high-risk group includes individuals with genotypes that exhibit >70% frequency (■) and the low-risk group those with frequencies <50% (▦). The others were in the moderate risk group (▤).

Table 4   Crude, sex and age-adjusted ORs, and 95% CI for *H. pylori* infection defined by ELISA

Groups	Crude OR (95% CI)	Adjusted OR (95% CI)
Low risk	1.0	1.0
Moderate risk	3.30 (1.40–7.78)	3.34 (1.36–8.20)
High risk	10.33 (3.16–33.80)	10.21 (2.98–34.96)
	P trend <0.001	*P* trend <0.001

lesser number of *Le* alleles, indicating that the polymorphisms of these genes are an inherited risk factor in the Japanese population. We also demonstrated that Le[b] antigen expression was present in the gastric foveolar cells of *Se/−, Le/−* individuals but not in either *se/se, Le/−* or *Se/−, le/le* individuals.

A lower *H. pylori* infection frequency was associated with an increased number of nonfunctional *se* alleles (all *P* trends <0.05; Table 3; Fig. 2). According to the type I Le antigen synthetic pathway and our results from immunohistochemical analysis (Figs. 1 and 3), *se/se* individuals are unable to synthesize H type I structures, resulting in deficient Le[b] antigen expression on gastric foveolar cells, as shown in Fig. 3*B*. Therefore, these results could be interpreted as indicating that a decrease in both H type I structures and Le[b] antigen lead to a state in which *H. pylori* does not attach to the gastric mucosa with BabA. Higher *H. pylori* infection frequency was also associated with an increased number of *le* alleles (all *P* trends <0.05; Table 3; Fig. 2). As shown in Table 3 and Fig. 2, *Se* and *Le* genes affected the infection rate independently. Any interaction terms between the genotypes were not statistically significant by the logistic model. These results may be explained as that an increased number of *le* alleles makes it easier for the Se enzyme to use the type I precursor synthesizing H type I antigen in individuals with at least one *Se* allele, through the type I Le
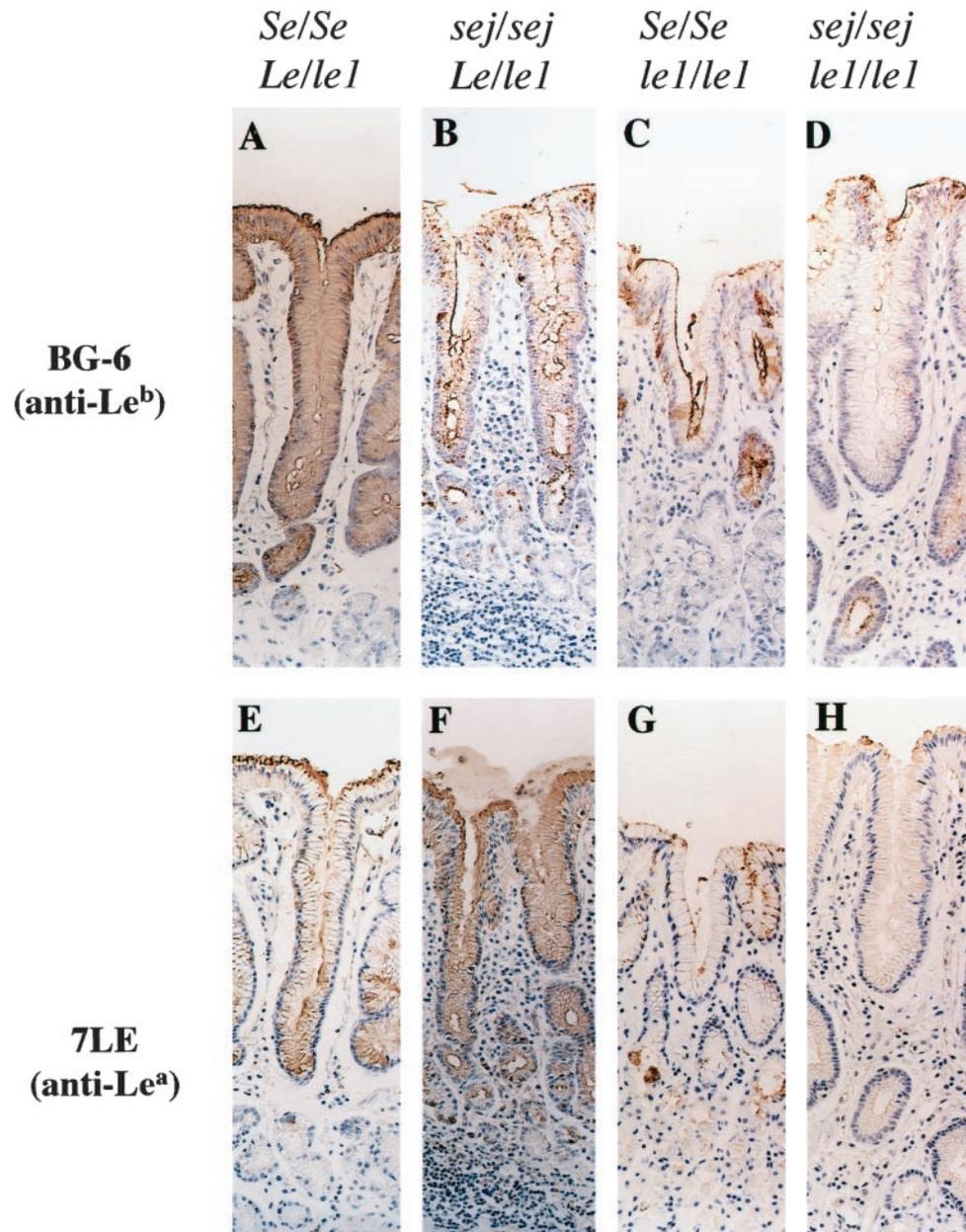
Fig. 3. Type I Le antigen expression depending on both *Se* and *Le* genotypes. Upper (*A–D*) and lower (*E–H*) panels were stained with anti-Le[b] and anti-Le[a] antibodies, respectively. *A* and *E* were from a *Se/Se, Le/Le* patient. *B* and *F* were from a *sej/sej, Le/le* patient. *C* and *G* were from a *Se/Se, le1/le1* patient. *G* and *H* were from a *sej/sej, le1/le1* patient.

antigen synthetic pathway (Fig. 1). This would be consistent with the significant correlation between the genotypes and the augmentation of anti-*H. pylori* antibody that is induced by the severe inflammatory reaction caused by nuclear factor-κB activation (22, 23).

Although our results clearly indicated a significant correlation between *H. pylori* infection and both *Le* and *Se* genotypes of the host, some clinical studies have cast doubt on the link between *H. pylori* infection and the host Le blood type (32–34). The failure to find a correlation in these studies may have resulted from either the determination methods for host Le phenotype and/or the definition of *H. pylori* infection. For example, the hemagglutination test for determination of Le blood phenotype often results in mistyping because of specific biological conditions such as pregnancy (35), alcoholic cirrho-

sis (36), pancreatitis (36), hydatid cysts (37), and intestinal cancers (38). With immunohistochemical techniques, the Le phenotype might not have been accurately determined in previous studies because of the aberrant Le[b] antigen on foveolar epithelium, which might be caused by the H enzyme, caused by inflammation and intestinal metaplastic change (30, 32, 39). We obtained the same results with surgically resected gastric mucosa that had undergone these inflammatory changes. Furthermore, the different detection methods of *H. pylori* infection would make any correlation between Le blood type and *H. pylori* infection ambiguous. In one study, a significant correlation was found between the host Le blood type and *H. pylori* infection defined by the presence of anti-*H. pylori* antibody (33). No correlation was reported in other studies that used *H. pylori* culture or histological investigation to detect the

H. pylori infection (32, 34). The IARC classification of *H. py-lori* as a definite carcinogen for gastric cancer was based largely on epidemiological evidence in which anti-*H. pylori* IgG anti-body tests were commonly used for detection of *H. pylori* infection.[4] Therefore, an accurate Le blood type determination method using the genotypes and the anti-*H. pylori* IgG antibody test should be applied for the purpose of evaluating the asso-ciation between Le blood type and *H. pylori* infection in pa-tients with chronic gastritis, gastroduodenal ulcers, and gastric carcinogenesis. Taken together, our results indicate that host type I Le blood type is correlated with the augmentation of anti-*H. pylori* antibodies in the development of gastric cancer.

    H. pylori infection was not only observed in the *Se/−* group but also in the *se/se* group with less than the expected expression of adhesin ligand on gastric foveolar cells, for which we can propose several possible explanations: (*a*) *H. pylori*-positive gastritis is caused not only by *BabA2* gene-positive strains (9) but also by other adhesive factors, such as AlpA, AlpB adhesin, and the M_r 25,000 outer membrane protein of *H. pylori,* and by carbohydrate-carbohydrate interactions (40, 41); (*b*) Le antigens on lipopolysaccharides of *H. pylori* play a role in adhesion through the binding to lectin protein expressed on host gastric mucosa (40, 41); and (*c*) a slight fucose-transferring activity of the sej enzyme could synthesize small amounts of H type I structure in the gastric foveolar epithelial cells sufficient to retain *H. pylori* on the mucosa. In Fig. 3*B,* slight Le[b] antigen expression could be detected in some normal foveolar cells in *sej/sej* individuals using the highly sensitive immune-complex detection method. In our previous study, sej enzyme exhibited slight fucose transfer activity to the type I precursor structure (14).

    The frequency of the *se/se* phenotype as a nonsecretor is identical in most ethnic groups (~20%; Ref. 42), despite the difference in nonfunctional point mutations in the *Se* allele between Orientals and Caucasians. Henry *et al.* (43) and Yu *et al.* (44) proposed that individuals in the Orient homozygous for the *sej* allele could have a partial secretor status, whereas the nonfunctional *se1* allele of Caucasians did not exhibit fucose-transferring activity by nonsense mutation in *Se* allele. The wide distribution of *sej* (14, 44–47), the homozygote of which shows partial secretor status in Asian populations, may be somehow linked with the fact that *H. pylori* infection is more frequent in the Far East than in northern Europe or the United States (48). It may be true that improvement of environmental factors affects the *H. pylori* infection rate. The weak activity of the *sej* allele or other unidentified ethnic-specific *se* alleles might underline the high *H. pylori* infection frequency in Jap-anese, Blacks, and Hispanics, who still exhibit high incidences of infection, even after taking socioeconomic status into ac-count (48). Because both *le1* and *le2* alleles are found out not only in Asians but also in Africans and Caucasians (49), there is a slight possibility that inactivated alleles of *Le* gene modify the risk of *H. pylori* infection in an ethnic group-specific manner similar to the *sej* allele. However, more population studies on *Le* alleles among different ethnic groups should be performed to determine whether the geographical variation of *H. pylori* infection and gastric cancer risk might be explained by further correlation analysis of these gene polymorphisms, the frequency of *H. pylori* infection in other countries, and incidence of gastric cancer.

    In conclusion, our findings demonstrate that the *Se* and *Le*

genotypes affect the risk of *H. pylori* infection defined by the presence of anti-*H. pylori* IgG antibody. We are investigating whether the determination of the fucosyltransferases polymor-phisms is valuable in identifying patients at high risk of devel-oping duodenal ulcer, gastric ulcer, adenocarcinomas of the distal stomach, and low-grade B-cell lymphoma of mucosa-associated lymphoid tissue.

Acknowledgments

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Polymorphisms of Two *Fucosyltransferase* Genes (*Lewis* and *Secretor* Genes) Involving Type I Lewis Antigens Are Associated with the Presence of Anti- *Helicobacter pylori* IgG Antibody

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