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Original article

ABO blood type, *Lewis* and *Secretor* genotypes, and chronic atrophic gastritis: a cross-sectional study in Japan

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Abstract

Background. **The H type I structure, synthesized by the secretor (Se) enzyme in gastric foveolar cells, and its metabolite, Lewis b (Le^b) antigen, mediate the adhesion of *Helicobacter pylori* (*H. pylori*) to the gastric epithelium, whereas *H. pylori* does not bind to modified forms of Le^b specific for blood types A and B. Such host factors as *Le* and *Se* genotypes and ABO blood type may affect the establishment of *H. pylori* infection and, once infected, the risk of chronic atrophic gastritis.**

Methods. **We investigated the cross-sectional relation of ABO blood type and *Le* and *Se* genotypes to gastric atrophy, assessed by serum pepsinogen levels, in Japanese residents from two sources.**

Results. **Among the 151 *H. pylori*-positive participants of the *H. pylori* eradication program, odds ratios (ORs) for gastric atrophy, adjusted for age, sex, and smoking, were elevated for blood types A (OR = 5.35; 95% confidence interval (CI), 2.11–13.58) and B (OR = 4.79; 95% CI, 1.77–12.93) relative to type O. ORs for blood types A and B were also elevated in *H. pylori*-negative subjects. These associations were not observed among 250 *H. pylori*-positive health check-up examinees. The *Le* genotype was not associated with gastric atrophy in either study population. The *se/se* genotype was associated with statistically nonsignificant elevation of gastric atrophy risk in both populations.**

Conclusions. **The present data showed a strong association of blood types A and B with gastric atrophy in one, but not the other, study population. Discrepant results between the two populations warrant further investigation.**

Key words ABO blood-group system · Atrophic gastritis · Polymorphism (genetics)

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Introduction

Stomach cancer is the second most frequent cancer in the world, accounting for a large proportion of cancer cases in Asia, Latin America, and some countries in Europe [1]. Evidence from pathological and epidemiological studies strongly supports a multistep model of gastric carcinogenesis, in which the gastric epithelium undergoes sequential changes from normal to chronic atrophic gastritis to intestinal metaplasia to dysplasia, before developing an invasive neoplasm of an intestinal type [2,3].

A higher incidence of stomach cancer in blood type A individuals than in those with blood type O was noticed as early as in the 1960s [4]. The prevalence of chronic atrophic gastritis, intestinal metaplasia, and dysplasia is also higher in subjects with blood type A than in type O individuals [5,6].

Infection with *Helicobacter pylori* (*H. pylori*) has been established as a risk factor for gastric adenocarcinoma [7]. *H. pylori* infection induces inflammatory responses of the gastric epithelium [8]. This inflammation, in turn, is a hypothesized mechanism by which *H. pylori* infection leads to atrophic gastritis and gastric cancer [8,9]. The adherence of *H. pylori* to the human gastric epithelial lining can be mediated by the blood-group antigen-binding adhesin (BabA) that targets human fucosylated blood group antigens H type I (type O substance) and Lewis b (Le^b) [10–12]. The presence of the *babA2* gene, encoding for BabA, in the *H. pylori* genome is crucial for *H. pylori*-related pathogenesis [12], and correlates with the activity of gastritis in the infected stomach [13].

In the gastric foveolar cells, the Secretor (Se) enzyme synthesizes H type I antigen from its precursor, and the Lewis (Le) enzyme converts H type I antigen into Le^b antigen [14] (Fig. 1). We have shown an association between several DNA sequence variants of the *Se* and

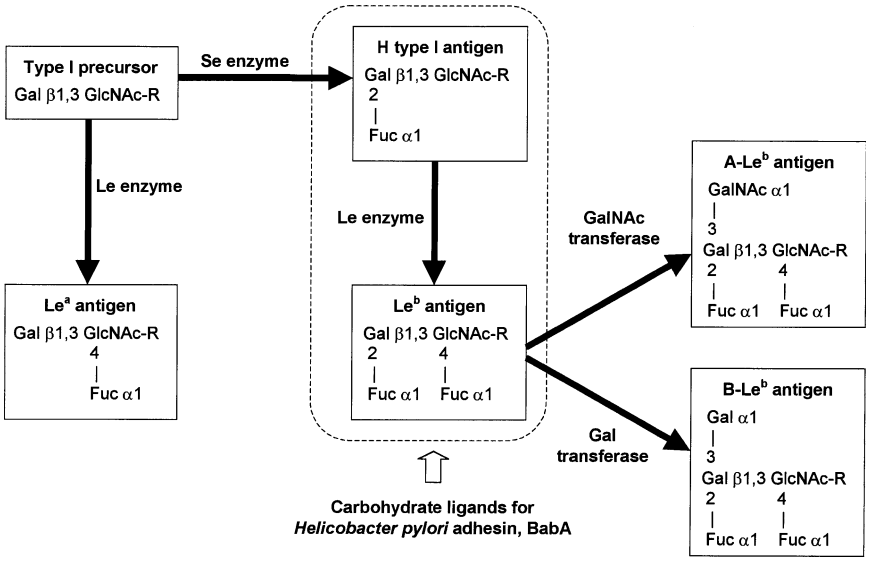

Fig. 1. A schematic drawing of the biosynthesis pathway for H type I and Lewis (Le) antigens in the gastric foveolar cells. H type I and Le^b antigens, but not Le^a, A-Le^b, or B-Le^b, have been shown to bind to the blood-group antigen-binding adhesin (BabA) produced by *Helicobacter pylori*. [10] *Fuc*, Fucose; *Gal*, galactose; *GalNAc*, *N*-acetyl galactosamine; *GlcNAc*, *N*-acetyl glucosamine

Le genes that correspond to varying enzymatic activity [15–17] and the seroprevalence of *H. pylori* antibody [14]. This finding suggested that the *Se* and *Le* genotypes of the host might affect the colonization of *H. pylori* in the stomach, and the subsequent establishment of long-term infection. An interaction between the bacterial protein BabA and the human Le^b antigen may also influence the development of *H. pylori*-associated gastric pathology, such as chronic atrophic gastritis, among individuals infected with *H. pylori*. Le^b antigen takes part in the adherence of *H. pylori* to gastric mucosa, whereas A-Le^b and B-Le^b structures (modified forms of Le^b with α1,3-linkage of *N*-acetylgalactosamine and galactose, respectively, to the terminal galactose residue of Le^b found in blood type A and B individuals; Fig. 1) neither bind to the bacteria in solution nor inhibit bacterial adherence in situ [10]. Therefore, we hypothesized that the risk of chronic atrophic gastritis might be influenced by the ABO blood type as well as the *Le* and *Se* genotypes.

To address this hypothesis, we investigated the association of ABO blood type and *Le* and *Se* genotypes with chronic atrophic gastritis, assessed by serum pepsinogen levels, among Japanese residents recruited through two health-related programs. Since atrophic gastritis is relatively rare among those who are not infected with *H. pylori*, the present analysis focused primarily on *H. pylori*-positive subjects.

Materials and methods

Study subjects

The subjects were drawn from two sources: (a) 241 participants of an *H. pylori* eradication (HPE) program at the Aichi Cancer Center in Nagoya, Japan, who were recruited between March and December 1999 [18,19]; (b) 454 residents who underwent a health check-up examination (HCE) provided by a municipal health center in Nagoya, Japan, in August and September 2000 [20]. To be eligible for this study, subjects had to have no history of cancer, provide written consent to participate in the study, and complete a self-administered questionnaire on their medical history, family history of cancer, and lifestyle habits.

The study protocols were approved by the Ethics Committee of the Aichi Cancer Center.

Questionnaire

Each subject was asked to fill out a brief self-administered questionnaire at the time of recruitment. Questionnaires for the *H. pylori* eradication program and the health check-up examination had different formats, but both asked for essentially the same information on the history of cancer in parents and siblings, smoking, and alcohol consumption.

In addition, information about personal medical history and medication use in the past year was obtained from the participants of the *H. pylori* eradication program. Twenty-three (9.5% of 241) participants reported a history of gastric or duodenal ulcer disease. An additional 23 (9.5% of 241) subjects reported the use of medication for gastritis or dyspepsia. Thirty-nine (84.8%) of these 46 subjects tested positive for *H. pylori* serology. No attempt was made to verify self-reported histories of ulcer disease or medication use.

Laboratory assays

Blood sample collection and processing. Peripheral blood was drawn from each participant of the *H. pylori* eradication program at the time of enrollment. The serum was separated and used in laboratory tests for anti-*H. pylori* antibodies, ABO blood type, and pepsinogens. For the health check-up examinees, residual plasma samples saved after routine laboratory tests were used for this study. DNA for the determination of the *Le* and *Se* genotypes was extracted from buffy coat with the use of the QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA, USA).

Circulating pepsinogen levels and the assessment of chronic atrophic gastritis. Concentrations of pepsinogens I and II (PG1 and PG2) in sera from the HPE subjects and plasma from the HCE subjects were measured by radioimmunoassay using a commercially available kit (DINABOT, Tokyo, Japan). According to the PG1 level and PG1/PG2 ratio, each subject was assigned to one of three categories of gastric atrophy as follows: (i) severe (PG1 $<$ 30 ng/ml and PG1/PG2 ratio $<$2); (ii) moderate (PG1 $<$ 70 ng/ml and PG1/PG2 ratio $<$3, excluding those classified as "severe"); (iii) none or mild (all others). Since the number of subjects classified as having severe atrophy was small (21 participants of the *H. pylori* eradication program and 47 health check-up examinees), we combined the severe and moderate categories of atrophy and compared them with the none or mild category in the statistical analysis.

Detection of an H. pylori *antibody.* An enzyme-linked immunosorbent assay (ELISA) for anti-*H. pylori* IgG antibody, using the high-molecular-weight campylobacter-associated protein (HM–CAP) test (Enteric Products, Westbury, NY, USA), was performed on sera from the HPE subjects and plasma from the HCE subjects. An ELISA value of 2.3 or higher was considered *H. pylori*-positive. With the use of the same cut-off point, this assay had previously shown 86% sensitivity and 77% specificity in a study of 251 subjects aged 40–69 years with no history of gastrectomy [21].

ABO blood type. ABO blood type was determined for 240 participants of the *H. pylori* eradication program and 450 health check-up examinees whose serum or plasma samples were available. Three aliquots of each serum or plasma specimen were mixed with red blood cells of known blood types, A, B, or O. The assay results were scored by two individuals who did not know each other's assessment. The two scorers agreed on the assay results of all but two specimens. These two subjects (both in the HCE group) were excluded from the statistical analysis involving the ABO blood type.

Genotyping for the Lewis (Le) *and* Secretor (Se) *genes.* The *Le* genotype was determined for the T59G polymorphic site. The thymine (T) in the wild type allele (*Le*) is replaced with guanine (G) in the *le1*, *le2*, and *le3* alleles. The *le1* and *le2* alleles confer very low enzymatic activity relative to the *Le* [17] and *le3* [22] alleles. Since the *le3* allele, which confers about the same enzymatic activity as the *Le* allele, is very rare in the Japanese population [23], we regarded a G at the T59G site as representing either the *le1* or the *le2* allele. Thus, the TT, TG, and GG genotypes were scored as the *Le*/*Le*, *Le*/*le*, and *le*/*le* genotypes, respectively. For the *H. pylori* eradication program participants, the T59G genotype was determined as previously described [14]. For the health check-up examinees, the polymerase chain reaction with confronting two-pair primers (PCR–CTPP) [24,25] was used for the T59G genotyping. The gel-filtered oligonucleotide primers (Hokkaido System Science, Sapporo, Japan) used in the PCR–CTPP were: F1, 5′-CCA TGG ATC CCC TGG GTG; R1, 5′-CCA CCA GCA GCT GAA ATA GCC; F2, 5′-CGC TGT CTG GCC GCA CT; R2, 5′-GAA GGT GGG AGG CGT GAC TTA. Genomic DNA (30–100 ng) was used for each 25-μl reaction with 0.18 mM dNTPs, 12.5 pmol of each primer, 0.5 units of AmpliTaq Gold, and 2.5 μl GeneAmp 10× PCR buffer containing 15 mM $MgCl_2$ (Perkin-Elmer, Foster City, CA, USA). PCR parameters included 10 min initial denaturation at 95°C, followed by 35 cycles of 1 min at 95°C, 1 min at 66°C, 1 min at 72°C, and a 5-min final extension at 72°C. Amplified DNA was visualized on a 2% agarose gel containing ethidium bromide. The 329-bp and 81-bp bands represented the T allele and G allele, respectively, while a common band of 373 bp appeared for both alleles.

For the *Se* gene, six alleles have been identified in the Japanese population [16,23]: *Se1* (wild type), *Se2*, *sej*, *se3*, *se4*, and *se5*. When compared with the *Se1* and *Se2* alleles, the *sej*, *se3*, *se4*, and *se5* alleles confer little or no enzymatic activity. The *se3* and *se4* alleles are extremely rare in the Japanese population [16,23]. Therefore, the genotyping was performed to distinguish the *sej* and *se5* alleles from the *Se1* and *Se2* alleles. Subjects with at least one copy of the *Se1* or *Se2* allele were regarded as having the *Se*/− (i.e., *Se*/*Se* or *Se*/*se*) genotype. All the others were considered to have the *se*/*se* genotype. The *sej* allele was identified based on a thymine (T) replacing an adenine (A) at the A385T polymorphic site. The *se5* allele (fusion gene of the *Se* gene and a pseudogene) was identified by a PCR-based method, as previously described [16,23]. Genotyping for the *Se* gene was performed as previously described for the *H. pylori* eradication program participants [14], and by duplex PCR–CTPP [26] for the health check-up examinees.

Statistical analysis

Statistical analysis was performed using the SAS statistical package (release 8.1, SAS Institute, Cary, NC, USA) and the EpiInfo software (version 6.04b, Center for Disease Control and Prevention, Atlanta, GA, USA).

The mean age of the two study populations was compared by *t*-test [27]. The frequency distributions of sex, smoking status, *H. pylori* serology, ABO blood type, *Le* and *Se* genotypes, and gastric atrophy were compared between the two populations by χ^2 test [27]. The association between covariates and gastric atrophy (moderate or severe vs none or mild) was examined by logistic regression analysis [28]. Ten-year age groups and three categories of smoking status (never, former, and current) were used in the logistic regression analysis.

Results

The characteristics of the 241 *H. pylori* eradication (HPE) program participants and 454 health check-up examinees (HCE) are summarized in Table 1. The age of the study subjects ranged from 39 to 69 years for the HPE group, and from 35 to 85 years for the HCE group. The mean age was slightly lower for the HPE than for the HCE group (56.8 vs 58.4 years, $P = 0.037$). The proportion of women was 51.0% and 72.3% for the HPE and HCE groups, respectively. Most subjects (82.8%) in the HCE group had never smoked, reflecting a female predominance in this population, whereas 22.8% and 19.1% of the HPE subjects were current and former smokers, respectively. The prevalence of *H. pylori* positivity was slightly higher in the HPE group than in the HCE group (62.7% vs 55.1%, $P = 0.054$). The distributions of the ABO blood type and *Lewis* (*Le*) and *Secretor* (*Se*) genotypes showed no statistically significant difference between the HPE and HCE groups.

According to our definition based on serum PG1 and PG2 levels, 96 (39.8%) of the HPE subjects and 158 (34.8%) of the HCE subjects had severe or moderate gastric atrophy (Table 1). The prevalence of severe or moderate gastric atrophy was significantly higher in *H. pylori*-positive subjects than in their *H. pylori*-negative counterparts in both groups: 51.0% vs 21.1% for the HPE group ($P < 0.001$) and 54.9% vs 10.3% for the

Table 1. Characteristics of the study subjects by source of recruitment

Variable	*H. pylori* eradication program participants	Health check-up examinees	*P* value[a]
Number of subjects[b]	241	454	
Age in years (mean ± standard deviation)	56.8 ± 7.9	58.4 ± 11.9	0.037
Sex			
Male	118 (49.0%)	126 (27.8%)	<0.001
Female	123 (51.0%)	328 (72.3%)	
Smoking			
Never	140 (58.1%)	376 (82.8%)	<0.001
Former	46 (19.1%)	10 (2.2%)	
Current	55 (22.8%)	68 (15.0%)	
H. pylori antibody			
Negative	90 (37.3%)	204 (44.9%)	0.054
Positive	151 (62.7%)	250 (55.1%)	
ABO blood type			
O	67 (27.9%)	113 (25.7%)	0.86
A	87 (36.3%)	165 (37.6%)	
B	62 (25.8%)	110 (25.1%)	
AB	24 (10.0%)	51 (11.6%)	
Lewis genotype			
Le/*Le*	123 (51.0%)	231 (55.8%)	0.47
Le/*le*	99 (41.1%)	151 (36.5%)	
le/*le*	19 (7.9%)	32 (7.7%)	
Secretor genotype			
Se/*Se*	61 (25.5%)	134 (29.6%)	0.35
Se/*se*	127 (53.1%)	215 (47.5%)	
se/*se*	51 (21.3%)	104 (23.0%)	
Gastric atrophy			
None or mild	145 (60.2%)	296 (65.2%)	0.21
Moderate	75 (31.1%)	113 (24.9%)	
Severe	21 (8.7%)	45 (9.9%)	

[a] For comparisons between the two groups based on a *t* test for age and a χ^2 test for all the other variables
[b] The numbers of subjects for each variable do not necessarily add up to the total number of subjects owing to missing data

Table 2. Logistic regression analysis for gastric atrophy in *H. pylori*-positive subjects

Variable	Participants of the *H. pylori* eradication program ($N = 151$)			Health check-up examinees ($N = 250$)		
	Prevalence of gastric atrophy[a]	OR[b]	95% CI[c]	Prevalence of gastric atrophy	OR	95% CI
Age (years)						
30–39	NA[d]	NA	NA	3/7 (42.9%)	1.00	Reference
40–49	7/20[e] (35.0%)	1.00	Reference	12/23 (52.2%)	1.51	0.26–8.66
50–59	24/52 (46.2%)	1.40	0.43–4.54	37/61 (60.7%)	2.29	0.46–11.45
60–69	46/79 (58.2%)	2.94	0.94–9.16	56/113 (49.6%)	1.52	0.31–7.40
70–79	NA	NA	NA	26/42 (61.9%)	2.63	0.49–14.05
80–89	NA	NA	NA	3/4 (75.0%)	9.69	0.40–237.2
Sex						
Male	40/82 (48.8%)	1.00	Reference	44/83 (53.0%)	1.00	Reference
Female	37/69 (53.6%)	2.02	0.83–4.91	93/167 (55.7%)	1.05	0.53–2.08
Smoking						
Never	42/82 (51.2%)	1.00	Reference	113/208 (54.3%)	1.00	Reference
Former	16/30 (53.3%)	1.69	0.58–4.92	1/5 (20.0%)	0.13	0.01–1.55
Current	19/39 (48.7%)	1.55	0.58–4.12	23/37 (62.2%)	1.40	0.61–3.23
ABO blood type						
O	10/38 (26.3%)	1.00	Reference	34/67 (50.8%)	1.00	Reference
A	37/58 (63.8%)	5.35	2.11–13.58	53/91 (58.2%)	1.46	0.76–2.82
B	24/40 (60.0%)	4.79	1.77–12.93	29/55 (52.7%)	1.06	0.51–2.20
AB	6/14 (42.9%)	2.52	0.67–9.58	16/26 (61.5%)	1.50	0.58–3.83

[a] Atrophy includes both "moderate" and "severe" categories. The denominators for each variable do not necessarily add up to 151 or 250 owing to missing data
[b] Odds ratio mutually adjusted for the variables in the table
[c] Confidence interval
[d] No subjects in the age group (see footnote e)
[e] Includes one 39-year-old subject

HCE group ($P < 0.001$). Because our research question concerned biological interactions between host factors (ABO blood type and *Le* and *Se* genotypes) and *H. pylori*, and severe or moderate gastric atrophy was scarce among *H. pylori*-negative subjects, the following analysis was primarily restricted to *H. pylori*-positive subjects. In logistic regression analysis, the "severe" and "moderate" categories of gastric atrophy were combined and compared with the "none or mild" category.

Table 2 shows the prevalence of severe and moderate gastric atrophy by age, sex, smoking status, and ABO blood type, as well as the results of logistic regression analysis, for *H. pylori*-positive subjects in both the HPE and HCE groups. In the HPE group, the prevalence odds ratios (ORs) for gastric atrophy, adjusted for age, sex, and smoking status, were statistically significantly elevated for blood types A (OR = 5.35; 95% confidence interval (CI), 2.11–13.58) and B (OR = 4.79; 95% CI, 1.77–12.93) relative to type O. OR for type AB was also elevated but not statistically significantly so (OR = 2.52; 95% CI, 0.67–9.58). An elevated risk associated with blood types A and B was not observed in the analysis of 250 *H. pylori*-positive subjects in the HCE group. Increasing age was associated with an elevated prevalence of gastric atrophy in both groups, although the 95% CIs of age-group-specific ORs did not exclude unity. OR estimates for sex and smoking were not consistent between the two groups.

The results of logistic regression analysis for the *Le* and *Se* genotypes among *H. pylori*-positive subjects are shown in Table 3. The prevalence of gastric atrophy did not differ by *Le* genotype in either the HPE or HCE group. Subjects with the *se*/*se* genotype were more likely to have gastric atrophy than those with the *Se*/− (i.e., *Se*/*Se* or *Se*/*se*) genotype in both study populations, although the association was not statistically significant.

If blood types A and B are associated with a risk of gastritis, at least partly, through the modification of the Le^b antigen, the association may be more pronounced among subjects with an active Se enzyme (see Fig. 1). Therefore, we examined whether the association between the ABO blood type and gastric atrophy was stronger for a subset of *H. pylori*-positive subjects with the *Se*/− genotype in the HPE group (Table 4). Point estimates of adjusted ORs for blood types A, B, and AB relative to type O in this subset analysis were comparable with those in the analysis including all *H. pylori*-positive subjects presented in Table 2. A similar analysis restricted to *H. pylori*-positive subjects with the *Le*/− & *Se*/− genotype showed a stronger association of gastric atrophy with blood types A (OR = 8.12; 95% CI, 2.47–26.73) and B (OR = 6.84; 95% CI, 1.93–24.31). The

Table 3. Logistic regression analysis for the *Lewis* (*Le*) and *Secretor* (*Se*) genotypes and gastric atrophy in *H. pylori*-positive subjects

Variable	Participants of the *H. pylori* eradication program ($N = 151$)			Health check-up examinees ($N = 250$)		
	Prevalence of gastric atrophy[a]	OR[b]	95% CI[c]	Prevalence of gastric atrophy	OR	95% CI
Le genotype						
Le/*Le*	35/68 (51.5%)	1.00	Reference	64/123 (52.0%)	1.00	Reference
Le/*le*	34/68 (50.0%)	0.96	0.48–1.92	51/86 (59.3%)	1.36	0.77–2.42
le/*le*	8/15 (53.3%)	1.11	0.35–3.55	10/19 (52.6%)	0.91	0.33–2.50
Se genotype						
Se/*Se*	21/44 (47.7%)	1.00	Reference	37/67 (55.2%)	1.00	Reference
Se/*se*	40/82 (48.8%)	1.05	0.49–2.23	62/124 (50.0%)	0.91	0.49–1.68
se/*se*	15/23 (65.2%)	1.86	0.64–5.40	37/58 (63.8%)	1.55	0.73–3.25

[a] Atrophy includes both "moderate" and "severe" categories. The denominators do not necessarily add up to 151 or 250 for each variable owing to missing data
[b] Odds ratios are adjusted for age (10-year groups), sex, and smoking (never, former, or current), but not mutually for the variables in the table
[c] Confidence interval

Table 4. Logistic regression analysis for ABO blood type and gastric atrophy in subsets of *H. pylori*-positive subjects who participated in the *H. pylori* eradication program

ABO blood type	Subjects with the *Se*/− genotypes ($N = 125$)[a]			Subjects with the *Se*/− genotype who did not report the use of medication for gastrointestinal conditions ($N = 93$)[a]		
	Prevalence of gastric atrophy[b]	OR[c]	95% CI[d]	Prevalence of gastric atrophy	OR	95% CI
O	7/31 (22.6%)	1.00	Reference	7/22 (31.8%)	1.00	Reference
A	30/48 (62.5%)	6.32	2.16–18.44	25/36 (69.4%)	6.43	1.80–22.94
B	18/33 (54.6%)	5.15	1.64–16.17	14/23 (60.9%)	5.03	1.29–19.67
AB	6/13 (46.2%)	3.48	0.83–14.54	5/11 (45.5%)	2.11	0.41–10.78

[a] Information on the ABO blood type was not available for one subject
[b] Atrophy includes both "moderate" and "severe" categories
[c] Odds ratios are adjusted for age (10-year groups), sex, and smoking (never, former, or current)
[d] Confidence interval

number of subjects in each of the other combinations of the *Le* and *Se* genotypes (i.e., *Le*/− and *se*/*se*, *le*/*le* and *Se*/−, and *le*/*le* and *se*/*se*) was too small for separate analysis.

Serum pepsinogen levels may be less reliable as a marker of gastric atrophy for subjects with history of peptic ulcer disease or recent use of medication for upper gastrointestinal symptoms. Therefore, we conducted an additional analysis of *H. pylori*-positive subjects with the *Se*/− genotype in the HPE group by excluding those subjects who reported a history of gastric or duodenal ulcer, or use of medication for gastritis or dyspepsia in the past year (Table 4). Elevated ORs associated with blood types A and B remained statistically significant.

We also estimated ORs for gastric atrophy associated with the ABO blood type in *H. pylori*-negative subjects. Among the 90 *H. pylori*-negative subjects in the HPE group, increased OR for gastric atrophy associated with blood types A and B, relative to type O, was observed after adjustment for age, sex, and smoking status: 4.48 (95%CI, 0.92–21.79) for type A and 5.53 (95%CI, 1.15–26.68) for type B. No such association was observed among the 204 *H. pylori*-negative subjects in the HCE group (data not shown).

Discussion

We found a statistically significantly elevated risk of gastric atrophy, determined by the serum PG1 level and PG1/PG2 ratio, associated with blood types A and B in the participants of the *H. pylori* eradication (HPE) program for both *H. pylori*-positive and -negative subjects after adjustment for age, sex, and smoking status. This association was not observed among the health check-up examinees (HCE) despite the similar prevalence of severe or moderate gastric atrophy in the two study

populations (among *H. pylori*-positive subjects, 39.8% in the HPE group and 34.4% in the HCE group).

Discrepant results between the two study populations regarding the association of blood types A and B with gastric atrophy may be due to the presence of effect modifiers that were not considered in the present analysis, confounding and other types of bias that operate differentially between the two populations, or apparently conflicting observations within the range of statistical variability for a true association that might be common between the two source populations. At least part of the discrepancy may be attributed to the difference in the source of the study subjects. Participants in the *H. pylori* eradication program were recruited from among the outpatients who sought an endoscopic examination of the upper gastrointestinal tract, whereas the health check-up examination at the municipal health center is provided to local residents who do not undergo periodic check-ups through employment. Subjects in the former group could be more likely to have had upper gastrointestinal symptoms or related health concerns than those in the latter group, even though the seroprevalence of *H. pylori* and the prevalence of gastric atrophy based on serum PG levels were similar between the two groups.

A limited number of epidemiological studies have addressed the association between the ABO blood type and preneoplastic lesions of the stomach. In an earlier study of 463 subjects in Colombia [5], the combined prevalence of intestinal metaplasia and chronic atrophic gastritis was 51% in subjects with blood type A and 39% in those with blood type O. In a more recent study of 3400 adults in China [29], subjects with blood type A were more likely to have intestinal metaplasia or dysplasia than other individuals. Using subjects with chronic atrophic gastritis or superficial gastritis as a reference group, OR (95% CI) was 1.28 (1.06–1.53) for intestinal metaplasia and 1.39 (1.12–1.73) for dysplasia, suggesting a higher probability of transition from superficial and chronic atrophic gastritis to intestinal metaplasia and dysplasia among individuals with blood type A than those with other blood types [29].

A biological interaction between the bacterial protein BabA and the human Le^b antigen suggests that the Le phenotype may affect the colonization of *H. pylori* in the stomach, the establishment of chronic infection, and the subsequent development of *H. pylori*-associated pathological conditions in the gastric epithelium. Boren et al. [10] showed that Le^b and H type I antigens, but not A-Le^b and B-Le^b structures, were involved in the adherence of *H. pylori* to gastric mucosa through BabA. These biochemical findings fit well with clinical observations made by Clarke et al. [30] that blood type O was overrepresented in duodenal ulcer patients compared with the general population, but run counter to the elevated risk of gastric atrophy among blood types A and B subjects observed in this study, and the higher risk of gastric cancer and preneoplasia in blood type A subjects than in their type O counterparts in previous studies [4–6]. Although both duodenal ulcer and gastric cancer (and its precursor lesions) are strongly associated with *H. pylori* infection, the epidemiology of the two conditions seems to be distinct [31], and thus it is possible that the host–pathogen interaction through BabA manifests differently in the pathogenesis of peptic ulcer and gastric preneoplasia.

There are several possible explanations for the higher risk of gastritis associated with blood types A and B than that associated with blood type O rather than the opposite direction of association that would fit the observations above described regarding Le^b and BabA in *H. pylori* adherence. First, not all *H. pylori* strains have the *babA* gene [12]. To our knowledge, the serological determination of whether a given individual is infected with a *babA*-positive *H. pylori* strain is still not possible. Since we have no access to systematically collected gastric tissue specimens for the subjects in this study, we cannot test whether the ABO–gastritis association differs between *babA*-positive and *babA*-negative *H. pylori* infection. Second, the host–pathogen interaction through BabA may not be as good an indicator of the long-term consequences of *H. pylori* infection (e.g., chronic atrophic gastritis) as of *H. pylori* adhesion. Further investigation to elucidate the pathobiological consequences of an interaction between *H. pylori* proteins (e.g., BabA) and host factors (e.g., ABO blood group) is warranted. Furthermore, the association between the host Le type and *H. pylori* infection we reported previously [14] has not always been consistent in other studies [32–34]. Third, *H. pylori* strains that colonize in the human stomach despite the less efficient adherence of *H. pylori* to the host epithelium expressing A-Le^b and B-Le^b may be more virulent in causing gastritis. Fourth, antigens and related molecules of the ABO blood group and the Lewis and Secretor systems may affect gastritis risk through a mechanism which is independent of *H. pylori* infection. The present findings among *H. pylori*-negative subjects of the HPE group, which also showed a significant association between blood types A and B and gastric atrophy, support this explanation.

In both study populations, we observed that the *se*/*se* genotype was associated with a slight, though not statistically significant, elevation of gastric atrophy risk when compared with the *Se*/− genotype. Since individuals with the *se*/*se* genotype, who lack an active Se enzyme, do not produce H type I or Le^b antigens that are known to interact with BabA (see Fig. 1), we would expect that the *se*/*se* subjects are less likely than the *Se*/− subjects to present *H. pylori*-associated gastric conditions mediated by BabA. The present data suggesting the opposite

direction of association, if any, seem to indicate that the hypothesis regarding the Se phenotype and *H. pylori*-associated gastritis needs to be revised. Also, the discrepancy between the predicted association and our data may be due in part to a misclassification of subjects with regard to the Le and Se status. We measured *Le* and *Se* genotypes at selected polymorphic sites as a means of making an inference about Le and Se phenotypes (i.e., enzyme activities). Although our assumptions about the genotype–phenotype correlation described in the Materials and Methods section (genotyping for the *Lewis* (*Le*) and *Secretor* (*Se*) genes) seem reasonable, genotypes may not be perfect indicators of phenotypes in the gastric tissue. For example, the *sej* allele of the *Se* gene, which we used as a marker of the nonsecretor phenotype, shows a weak but nonzero enzyme activity [15].

Some epidemiological considerations deserve attention in the interpretation of the present findings. For instance, our assessment of gastric atrophy was based on the serum PG1 level and PG1/PG2 ratio. No serological markers are both highly sensitive and specific indicators of histologically confirmed chronic atrophic gastritis [35]. In our previous assessment of chronic atrophic gastritis by serum pepsinogen levels in comparison with endoscopic findings as a gold standard, the criteria for the PG1 level and PG1/PG2 ratio used in the present study gave 87% sensitivity and 76% specificity for distinguishing subjects with moderate or severe atrophy from those with mild atrophy or none [36]. The less than perfect agreement between the atrophy status determined by serum pepsinogen levels and the histological assessment of gastric biopsies, the latter of which is itself subject to a sampling bias, leaves room for a misclassification of subjects with respect to the outcome variable of interest. If the misclassification was nondifferential (i.e., independent of the true atrophy status and ABO blood type), the ORs we observed might be an underestimate of a true association. Elevated ORs for gastric atrophy associated with blood types A and B in the HPE group persisted after excluding the subjects who reported a history of gastric or duodenal ulcer, or the use of medication for peptic gastritis or dyspepsia in the past year. As in any epidemiologic study, unmeasured or inadequately measured variables may have confounded the present analysis. The inclusion of alcohol consumption, a possible risk factor for gastritis, in logistic regression analysis resulted in little change in the OR estimates for the ABO blood type or *Le* and *Se* genotypes in both study populations.

In summary, the present data showed a strong association between blood types A and B with gastric atrophy in one study population regardless of *H. pylori* serology, but this was not corroborated in another population. Discrepant findings between the two study populations need to be resolved and warrant further investigation.

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