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Association of *Lewis* and *Secretor* gene polymorphisms and *Helicobacter pylori* seropositivity among Japanese-Brazilians

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Background. *Secretor* (*Se*) and *Lewis* (*Le*) genes are involved in the synthesis of Lewis b (Le^b) and type I antigens throughout the body, especially in the epithelial cells of gastric mucosa. *Helicobacter pylori* can attach to the gastric epithelial cells with the blood group antigen-binding adhesin, which binds to Le^b or H type I carbohydrate structures. In a previous study, a marked association between *H. pylori* seropositivity and polymorphism of the *Se* and *Le* genes was observed among Japanese outpatients of a gastroenterology clinic. The present work aims to investigate the associations between *Se* and *Le* gene polymorphisms and *H. pylori* infection among Japanese-Brazilians. ***Methods.*** The subjects consisted of 942 healthy volunteer Japanese-Brazilians, who were tested for the presence of anti-*H. pylori* IgG antibodies and genotyped for *Se* and *Le* polymorphisms. ***Results.*** The sex-age-adjusted odds ratios (aORs) for *H. pylori* seropositivity were 0.99 for the *Sese* genotype relative to the *SeSe* genotype (95% confidence interval [CI], 0.73–1.33), and 1.03 for *sese* relative to *SeSe* (95% CI, 0.71–1.48). On the other hand, the aOR for the subjects with the *le* allele (*Lele* or *lele*) relative to the *LeLe* genotype was 1.48 (95% CI, 1.07–1.79). When the *Se* and *Le* genotypes were analyzed in combination according to risk group, no statistically significant association was observed. ***Conclusions.*** These results are inconsistent with previous work and may have been modulated by an external factor or some other unidentified factor. Japanese-Brazilians are genotypically the same as Japanese, but their lifestyle is adapted to that of Brazil. Further investigations are necessary to clarify this influence on susceptibility to *H. pylori* infection.

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Introduction

Chronic infection with *Helicobacter pylori* is commonly associated with gastroduodenal diseases in humans, including stomach cancer.[1,2] *H. pylori* infection depends largely on poor sanitary conditions, and children acquire the bacterium as infants, mainly from family.[3,4] The bacteria persist in the gastric mucus layer, leading to chronic atrophic gastritis and gastric cancer.[5,6] The infection activates the immune system, and the antibody serum titer persists during the course of infection. However, some individuals do not develop persistent infection even under the same poor sanitary conditions, suggesting that host factors do play an important role in the infection and its maintenance. For example, *H. pylori* infection studies have revealed that the bacteria attach to the gastric mucosa with the blood group antigen-binding adhesin, BabA.[7,8] BabA binds to both Lewis^b and H type I carbohydrate structures, leading to a series of pathogenic processes in the gastric epithelial cells.[9] Blood group antigens (Lewis and ABH antigens) are carbohydrate structures widely expressed in many tissues throughout the body, especially in the epithelial cells of gastric mucosa. The secretor status is defined by the presence of ABH antigens in body fluids and secretions such as saliva and gastric juice. Lewis (Lewis^a, Le^a, and Lewis^b, Le^b) and ABH antigens are closely interrelated; they are produced from a common precursor antigen (type I precursor) by the action of the products of Lewis (*Le*) and secretor (*Se*) genes. Type I precursor is converted in H type I antigen by the Se enzyme, and then converted to Le^b by the action of the Le enzyme. The Le enzyme also metabolizes the type I precursor to Le^a antigen.[10] Low activity of the Se enzyme and high

activity of the Le enzyme is considered to prevent the synthesis of H type I and Le^b, which may result in a reduced chance of being infected by *H. pylori*.

The *Se* gene and the *Le* gene have many different polymorphisms (already described) and, in this way, they have different alleles. So, different alleles may lead to functional or nonfunctional genes. In individuals who have functional *Le* and *Se* genes, all the type I precursor is transformed in H type I antigen, and they express Le^b and ABH antigens in the foveolar epithelium and in the gastric juice as well. On the other hand, an individual who has nonfunctional alleles of the *Le* and *Se* genes (*se* and *le*) fails to produce Le^b antigen and ABH blood antigens. Finally, individuals who have the nonfunctional *Le* gene do not express Le^a or Le^b, but they express ABH blood antigens only if they have the *Se* gene. In the Japanese population, *sej* and *se5* alleles of the *Se* gene and *le1* and *le2* alleles of the *Le* gene products are described to have low or no activity.[11–13]

Ikehara et al.[14] reported an association of low seropositivity of anti-*H. pylori* IgG antibody and low expression of *Se* alleles and high expression of *Le* alleles, indicating that *Se* and *Le* genotypes affect susceptibility to *H. pylori* infection. However, in a recent report, Hamajima et al.[15] described no consistency in the association of *H. pylori* infection and *Se* and *Le* polymorphisms, suggesting that even in the same ethnic group (Japanese) different subject sources lead to different results that may be the result of an unidentified effect modification.

Today, *H. pylori* still infects, chronically, over half of the world's population, in part because of the development of a unique set of virulence factors, including adhesin Bab1.[9] On the other hand, with more sanitary living conditions that come with increasing socioeconomic status, *H. pylori* infection prevalence decreases. In Japan, the prevalence of *H. pylori* infection in individuals aged more than 40 years is as high as that in developing countries (over 70%)[16,17] and it is associated with the development of severe atrophic and metaplastic gastritis and, probably, with the high incidence and mortality of gastric cancer.[18] The prevalence rates of *H. pylori* infection among Japanese-Brazilians are similar to those in residents of Japan.[19,20] In our previous work, we investigated *H. pylori* seropositivity and lifestyle factors among 963 Japanese-Brazilians, and we observed an inverse association between infection and length of education, while fruit intake was positively associated with *H. pylori* infection.[20]

The present study aimed to correlate *H. pylori* infection and *Le* and *Se* genotypes in Japanese-Brazilians. As Japanese migrants or their descendants belong to the same ethnic group, of Japanese, the genotype results may be comparable. When living in a different country, Japanese migrants acquire a different lifestyle, with the intake of different foods that may influence the development of some diseases. We found that the *Le* genotype was associated with *H. pylori* infection, but polymorphism of the *Se* genotype was not. These results are inconsistent with previous work, and may have been influenced by external factors or by some other unidentified factor. Japanese-Brazilians have a lifestyle adapted to that of Brazil, which may influence *H. pylori* infection susceptibility.

Methods

Study subjects

The subjects of our study were apparently healthy adult Japanese Brazilian volunteers from four different cities, São Paulo, Curitiba, Mogi das Cruzes, and Mirandopolis, who were enrolled from March to May 2001.[20] Those with a history of disease such as ulcer and stomach cancer were not excluded. Japanese migrants and their descendants try to keep Japanese traditions through Japanese cooperative societies, country clubs, and other non-profit Japanese associations. After a first contact with these associations, 12 in São Paulo, 4 in Curitiba, 1 in Mogi das Cruzes, and 1 in Mirandopolis responded to the first call within a predetermined period. With the approval of the directors of the associations, the members were invited, through a standardized letter informing them of the study objectives, the procedures, and confidentiality, to take part in the study. The total number of applicants was 967; the individuals were aged 33 to 69 years, and comprised first to fourth generations. Six applicants younger than 33 years or older than 69 years were excluded.

*DNA extraction and anti-*H. pylori *antibody test*

A 10-ml peripheral blood sample was obtained from each participant. Plasma samples were separated after centrifugation and frozen at −20°C, following the same protocol as that in our previous study.[20] For the identification of *H. pylori*-infected participants, an anti-*H. pylori* IgG antibody test, high molecular-weight *Campylobacter*-associated-protein (HM-CAP) enzyme-linked immunosorbent assay (ELISA; Detaminor *H. pylori* antibody; Enteric Products, Westbury, NY, USA) was performed.[21] The test was conducted at SRL (Tokyo, Japan), where routine measurements of IgG antibody have been established. A value of 2.3 EV (ELISA value) or over was regarded as positive for *H. pylori* infection.

DNAs were extracted from the blood by a salting-out method[22] and utilized for polymorphism analysis.

Se *and* Le *genotyping analysis*

The *Se* gene has six alleles: *Se1* (357C, 385A, 571C, and 628C), *Se2* (357T, 385A, 571C, and 628C), *sej* (357T, 385T, 571C, and 628C), *se3* (357C, 385A, 571T, and 628C), *se4* (357C, 385A, 571C, and 628T), and *se5* (conversion with pseudogene). Because *Se1* and *Se2* code for the full-activity enzyme, while *se3* and *se4* are very rare among Japanese,[10] the genotyping was conducted to distinguish A385T for *sej* and the pseudogene for *se5* from *Se* (*Se1* and *Se2*). Polymorphism was analyzed by polymerase chain reaction with confronting two-pair primers (PCR-CTPP).[23,24] The primers were as follows (5′-3′): se5 F0, ttt cac tgc cac cag cac ctg; se385 F1, atc aaa ggc act ggg acc cag; Se385 R1, gga cgt act ccc ccg gga t; sej F2, tgg agg agg aat acc gcc act; and sej R2, gtc ccc tcg gcg aac atg g. The underlined letters are the bases for the A385T polymorphism, and the primer se5 F0 was set for the pseudogene.

Reactions were performed with 100 ng of genomic DNA, 0.18 mM of each dNTP, 12.5 pmol of each primer, 10% glycerol, 0.5 units of AmpliTaq Gold, 2.5 µl of GeneAmp 10× PCR buffer containing 15 mM $MgCl_2$ (Perkin-Elmer, Foster City, CA, USA), in a final volume of 25 µl. PCR conditions were 10 min initial denaturation at 95°C, followed by 35 cycles of 1 min at 95°C, 1 min at 61°C, and 1 min at 72°C, and a final extension at 72°C for 5 min. Amplified PCR products were analyzed in 2% agarose gel electrophoresis containing ethidium bromide. The amplified bands with 284 bp, 216 bp, and 353 bp represent the *Se* allele (385A), the *sej* allele (385T), and the *se5* allele, respectively, while a common band of 460 bp appeared for the *Se* and *sej* alleles.

The *Le* gene has four alleles: *Le* (59T, 508G, and 1067T), *le1* (59G, 508A, and 1067T), *le2* (59T, 508G, and 1067A), and *le3* (59G, 508G, and 1067T). T59G was genotyped to distinguish *le1* and *le2* from *Le* by PCR-CTPP, because *le3* is very rare (0.5% out of 800 alleles) for Japanese.[11] The primer sequences (5′-3′) were: F1, cca tgg atc ccc tgg gtg; R1, cca cca gca gct gaa ata gcc; F2, cgc tgt ctg gcc gca ct; and R2, gaa ggt ggg agg cgt gac tta. Reactions were performed with 100 ng of genomic DNA, 0.18 mM of dNTPs, 25 pmol of each primer, 0.5 units of AmpliTaq Gold, 2.5 µl of GeneAmp 10× PCR buffer containing 15 mM $MgCl_2$ (Perkin-Elmer), in a final volume of 25 µl. PCR conditions were the same as those for the *Se* genotyping, except for annealing at 66°C. Amplified products were a 329-bp band for the T allele (*Le*) and an 81-bp band for the G allele (*le1* and *le2*), while a common band of 373 bp appeared for both alleles.

PCR-CTPP failed to amplify 19 samples of DNA and these were excluded from this study. We analyzed data for a total of 942 individuals.

Statistical analysis

The 95% confidence intervals (95% CI) of the percentages were calculated by assuming a binomial distribution. Sex-age-adjusted odds ratios (ORs) and 95% CI were calculated according to an unconditional logistic model. The antibody-positive rate was tested by the χ^2 test. Goodness-of-fit of genotype distribution to Hardy-Weinberg equilibrium was examined by the "genhwcci". All these calculations were conducted using the computer program STATA version 7 (STATA, College Station, TX, USA).

Ethical issues

This project has the approval of the Ethics Committees of the School of Medicine of the University of São Paulo and the National Ethics Committee in Brazil, and the Ethics Committee of the Aichi Cancer Center in Japan.

Results

Anti-*H. pylori* IgG seropositivity results of the participants according to sex and age are shown in Table 1. The ages ranged from 33 to 69 years. The overall rate of *H. pylori* infection was 50% for men and 47.3% for women. This difference between the sexes was not significant ($P = 0.41$). Nevertheless, there was a statistically significant difference in *H. pylori* seropositivity with increase of age ($P = 0.01$).

The *Se* and *Le* genotypes were determined for all subjects of the study. The genotype frequencies of the *Le* and *Se* gene polymorphisms were calculated, and both them were in Hardy-Weinberg equilibrium ($P = 0.50$ for *Se*, and $P = 0.96$ for *Le*). When the two sexes were combined, a χ^2 test for 2 (seronegative vs seropositivity) by 3 (genotypes) showed no statistically significant association between *H. pylori* seropositivity and the *Se* genotype ($P = 0.97$), but there was an association for the *Le* genotype ($P = 0.02$), as shown in Table 2. When the *Se* and *Le* genotypes were combined and classified as low-, moderate-, and high-risk groups according to previous works (low risk, *sese* and *LeLe*; high risk, *SeSe* and *lele*, *SeSe* and *Lele*, and *Sese* and *lele*; moderate risk, combinations other than low- and high-risk groups),[14,15] the *H. pylori* infection rate differences were not statistically significant (Fig. 1).

Table 3 shows the sex-age-adjusted ORs for *H. pylori* seropositivity. The *Se* genotype showed no significant association with *H. pylori* seropositivity (OR, 0.99; 95% CI, 0.73–1.33 for *Sese* genotype relative to *SeSe* genotype; and OR, 1.03; 95% CI, 0.71–1.48 for *sese* relative to *SeSe*). On the other hand, the crude and sex-age-adjusted OR for the subjects with the *le* allele (*Lele* or

Table 1. Distribution of age and sex according to anti-*Helicobacter pylori* antibody status

	Total				
	n (%)	*H. pylori*[a]	*H. pylori*[b]	*H. pylori*[c]	*P* value
Sex					
Men	392 (41.6)	196	196	50.0	0.41
Women	550 (58.4)	290	260	47.3	(χ^2 = 0.68; df = 1)
Age (years)					
33–39	79 (8.4)	49	30	38.0	
40–49	270 (28.7)	144	126	46.7	0.01
50–59	348 (36.9)	187	161	46.3	(χ^2 = 11.2; df = 3)
60–69	245 (26.0)	106	139	56.7	
Total	942 (100)	486	456	48.4	

[a] Anti-*H. pylori* antibody test seronegative
[b] Anti-*H. pylori* antibody test seropositive
[c] Percentage seropositive on anti-*H. pylori* antibody test

Table 2. Distribution of *Se* and *Le* genotypes according to anti-*Helicobacter pylori* antibody status

	Total				
	n (%)	*H. pylori*[a]	*H. pylori*[b]	*H. pylori*[c]	*P* value
Secretor gene					
SeSe	290 (30.8)	149	141	48.6	0.97
Sese	456 (48.4)	237	219	48.0	(χ^2 = 0.06, df = 2)
sese	196 (20.8)	100	96	49.0	
Lewis gene					
LeLe	427 (45.3)	238	189	44.3	0.02
Lele	415 (44.1)	193	222	53.5	(χ^2 = 7.70, df = 2)
lele	100 (10.6)	55	45	45.0	
Total	942 (100)	486	456	48.4	

[a] Anti-*H. pylori* antibody test seronegative
[b] Anti-*H. pylori* antibody test seropositive
[c] Percentage seropositive on anti-*H. pylori* antibody test

Table 3. Odds ratio (ORs) and 95% confidence intervals (95% CIs) of *Helicobacter pylori* seropositivity

	OR (95% CI)	
Genotype	cOR[a]	aOR[b]
Secretor gene		
SeSe	1 (Reference)	1 (Reference)
Sese	0.98 (0.73–1.31)	0.99 (0.73–1.33)
sese	1.01 (0.70–1.46)	1.03 (0.71–1.48)
Sese/sese	0.99 (0.75–1.30)	1.00 (0.76–1.32)
Lewis gene		
LeLe	1 (Reference)	1 (Reference)
Lele	1.45 (1.10–1.90)	1.48 (1.13–1.95)
lele	1.03 (0.66–1.60)	1.03 (0.66–1.60)
Lele/lele	1.36 (1.05–1.75)	1.38 (1.07–1.79)
Combined risk group[c]		
Low risk	1 (Reference)	1 (Reference)
Moderate risk	0.97 (0.62–1.50)	0.97 (0.63–1.52)
High risk	1.14 (0.69–1.87)	1.14 (0.69–1.89)

[a] Crude OR
[b] Sex-age-adjusted OR
[c] Low-risk group: *sese* and *LeLe*; high-risk group: *SeSe* and *lele*, *SeSe* and *Lele*, and Sese and lele; and moderate risk group: combinations other than low-, and high-risk groups

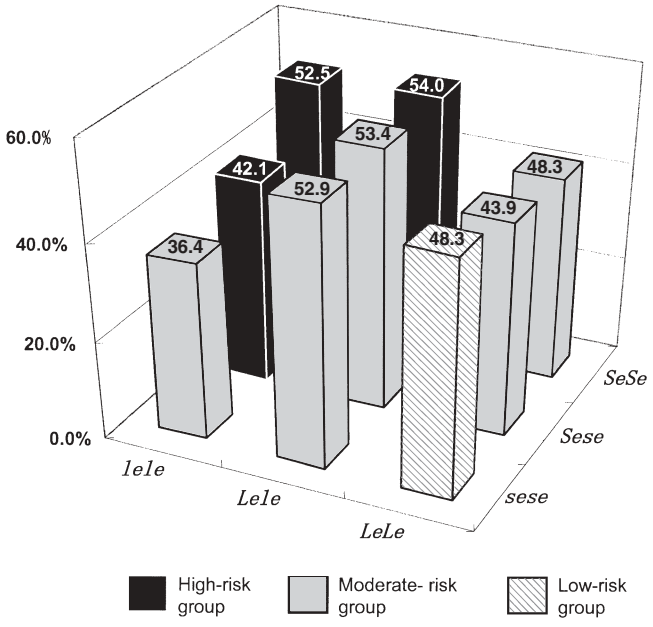

Fig. 1. *Helicobacter pylori* infection frequency (percentage) according to combination of *Se* and *Le* genotypes. The low-risk group includes individuals with *sese* and *LeLe* genotypes; the high-risk group includes those with *SeSe* and *lele*, *SeSe* and *Lele*, and *Sese* and *lele*; and the moderate-risk group includes those with combinations other than low-, and high-risk groups

lele) was significantly higher than that for those with *LeLe* (OR, 1.48; 95% CI, 1.13–1.95, and OR, 1.38; 95% CI, 1.07–1.79, respectively).

Discussion

Japanese migrants and their descendants living in Brazil were analyzed to determine the association of *H. pylori* infection and *Le* and *Se* gene polymorphisms. Genotyping of *Le* and *Se* genes by PCR-CTPP[23,24] is an efficient method to determine indirectly the kind of phenotype concerning blood type Lewis antigen expression. Although there are six possible alleles for the *Se* gene and four possible alleles for the *Le* gene, the method is used to distinguish the most common alleles among Japanese,[10] as our study individuals were all first to fourth generations of Japanese living in Brazil. In this way, our results may be compared to other Japanese studies.

Our results were not consistent with those observed in previous studies reported by Ikehara et al.[14] and Hamajima et al.[15] We also examined the association of *H. pylori* seropositivity and *Se* and *Le* genotypes and we found an association between the *le* allele and *H. pylori* seropositivity. A possible explanation for these different results may be the kind of population chosen for the studies. In the study of Ikehara et al.,[14] the subjects analyzed were outpatients who underwent gastroscopy at Aichi Cancer Center Hospital. There was a positive association with the number of *Se* alleles and a negative association with the number of *Le* alleles. When the subjects were classified into three groups by combination of the genotypes, strong associations were also observed. In the study of Hamajima et al.,[15] healthy checkup examinees and first visit outpatients from the same hospital were examined. Different and opposite results were observed by Hamajima et al.[15] compared with Ikehara et al.,[14] with an association of *H. pylori* infection and *se/se* genotype relative to *Se/Se* genotype, but no association when the individuals were analyzed according to *Se* and *Le* genotypes in combined risk groups. We found similar results when the individuals were analyzed in combined risk groups. The subjects of our study were apparently healthy individuals who were not recruited in a hospital, so they differed from the subjects analyzed in the previous works.

Virulence factors of *H. pylori* can confer a phenotype that tends to cause disease in the host, indicating that the bacterial genotype, as well as the host genotype, is important for infection. Vacuolating cytotoxin, for example, is encoded by the *vacA* gene, and seems to be essential for *H. pylori* colonization in an animal model.[25] The adhesin BabA, on the other hand, found in some *H. pylori* strains, is responsible for the binding to H type I and Le^b antigens.[9] The bacterial histo-blood group antigen-binding phenotype was also associated with the presence of cag pathogenicity island in clinical isolates of *H. pylori*.[8] Expression of BabA was also associated with duodenal ulcer formation.[26] Although we have not determined the genotype of *H. pylori* in our population study group, Tatemichi et al.[27] determined seropositivity against CagA antibodies in a case-control study of non-cardia gastric cancer among Japanese-Brazilians and non-Japanese Brazilians. They verified a higher level of CagA antibody titer in non-Japanese Brazilians with cancer than in Japanese-Brazilians with cancer, suggesting that ethnic differences may exist in *H. pylori* strains.[27] But no study has been done previously comparing the genotypes of *H. pylori* strains that infect Japanese and Japanese-Brazilian populations.

Results of studies about the association of *H. pylori* infection and Lewis phenotypes and secretor status are controversial. In Lewis-negative individuals, the secretor genotype does not affect the Lewis phenotype, but in Lewis-positive individuals, the non-secretor genotype generates the Le(a+b−) phenotype, the secretor genotype causes the Le(a−b+) phenotype, and the partial secretor genotype gives rise to the Le(a+b+) phenotype.[28] Both Le(a−b+) and Le(a+b+) individuals are considered to be in the high-risk group for *H. pylori* infection. Klaamas et al.[29] observed that the *H. pylori* seronegativity was associated with the Le(a+b−) phe-

notype and non-secretor status in blood donors. The Le(a+b−) phenotype and non-secretor status were also associated with pepic ulcer,[30] esophageal adenocarcinoma,[31] and susceptibility to *H. pylori* infection. On the contrary, Unlauft et al.[32] failed to establish a correlation between *H. pylori* infection and Le^b or H type I antigen. In a cross-sectional study of patients with dyspeptic symptoms, Mattos et al.[33] did not find any correlation between *H. pylori* infection and Lewis blood group or secretor phenotypes. The secretor status and *H. pylori* infection rate were not correlated in other studies that analyzed patients with dyspepsia,[34] those with mucosa-associated lymphoid tissue (MALT) lymphoma,[35] and those with gastroduodenal ulcer.[36]

A question that must be considered is that differences among populations may exist concerning *Le* and *Se* genotypes and *H. pylori* infection. Other host genetic factors may also influence the susceptibility to *H. pylori* infection, such as polymorphism in the genes involved in the inflammatory response to bacterial infection, such as the interleukin-1β (*IL-1*β) gene.[37,38] Furthermore, some lifestyle factors, such as smoking habit, may influence the susceptibility to *H. pylori* infection according to the genotype for *IL-1*β gene polymorphism.[38,39] In relation to *Le* polymorphism, Matsuo et al.[40] verified that smoking cessation and a non-functional *le* allele in *Le* polymorphism may affect the success rate of *H. pylori* eradication. We analyzed a possible association of smoking habit and *Le* and *Se* polymorphism in our casuistics, but no correlation was found (data not shown).

Studies of Japanese migrants and their descendants in Brazil can offer important information, as they are genetically similar to the original population living in Japan, but they have been influenced by external factors and they have adapted their lifestyle to that of Brazil. Studies of the lifestyle of first- and second-generation Japanese in Brazil have pointed to different smoking and drinking habits, with lower rates compared to in those Japanese in Japan.[41] Dietary patterns of Japanese migrants in Brazil were studied by Cardoso et al.,[42] and these authors observed changes in the kind and amount of food intake, as well as daily energy intake, which was quite close to that of the general Brazilian population. Differences in dietary patterns constitute a major component of the environmental changes experienced by migrant populations.

The subjects of the present study were previously analyzed for *H. pylori* seropositivity and sex, age, generation, and lifestyle factors.[20] There was no significant difference in *H. pylori* seropositivity among the generations, at 52.2% for the first generation (Issei), 48.2% for the second generation (Nisei), and 42.7% for the third generation (Sansei) ($\chi^2 = 1.98$; $P = 0.372$). Fruit intake was associated with *H. pylori* seropositivity, with an OR of 1.38 (95% CI, 1.05–1.83) for less frequent intake relative to everyday intake. Educational level was also associated with the *H. pylori* positivity rate, with an OR of 0.61 (95% CI, 0.42–0.89) for those individuals with 12 years or more of schooling relative to those with 8 years or less.[20] These data together indicate the influence of other factors associated with *H. pylori* infection.

In the present study, the sex-age-adjusted OR of *le/le* was almost identical to that of *Le/Le* and was less than that of heterozygote individuals. There are two possible explanations for this finding. The first one is that the OR for *le/le* was randomly underestimated in relation to that for *Le/le*, because the 95% CI included the point estimated for *Le/le*. The second explanation is that *Le/le* was truly a risky genotype in comparison with both homozygote groups, but this explanation is not biologically plausible. The combined OR for *Le/le* and *le/le* may reflect an underlying impact of the *le* allele on *H. pylori* seropositivity.

In the present study, the *Le* genotype was associated with *H. pylori* infection, but polymorphism of the *Se* genotype was not. These results are inconsistent with the results of previous work[14,15] and may have been modulated by external factors or some other unidentified factor. Although Japanese-Brazilians are genotypically similar to Japanese, they have a lifestyle adapted to that of Brazil. Further investigations will be necessary to analyze better this influence on susceptibility to *H. pylori* infection.

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