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Original article

Significant association between *PTPN11* polymorphism and gastric atrophy among Japanese Brazilians

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Abstract

Background. **Helicobacter pylori, especially the cytotoxin-associated antigen A (*cagA*)-positive strains, plays a crucial role in the development of gastric atrophy and gastric cancer. CagA delivered into gastric epithelial cells combines with src homology 2 domain-containing protein tyrosine phosphatase-2 (SHP-2), possibly leading to atrophy/cancer. Our previous study found that a single-nucleotide polymorphism (SNP; IMS-JST057927) of the *PTPN11* gene encoding SHP-2, was associated with gastric atrophy among *H. pylori*-seropositive subjects. This study aimed to examine the reproducibility of the association among Japanese residing in a different circumstance.**

Methods. **The subjects were 918 healthy adult Japanese Brazilians from four different areas in Brazil. Blood was sampled from March to May 2001. The target SNP in intron 3 of *PTPN11* was genotyped by polymerase chain reaction with confronting two-pair primers (PCR-CTPP). Gastric atrophy was evaluated with serum pepsinogens (PGs); PG I, less than 70 ng/dl and PG I/II ratio, less than 3.**

Results. **The genotype frequency of *PTPN11* was in Hardy-Weinberg equilibrium: 65.5% for *G/G*, 30.4% for *G/A*, and 4.1% for *A/A*. The *PTPN11* polymorphism had no significant association with *H. pylori* seropositivity. Among the *H. pylori*-seropositive subjects, the odds ratios (ORs) of gastric atrophy were 0.93 (95% confidence interval [CI], 0.59–1.47) for the *G/A* genotype and 0.31 (95% CI, 0.10–0.95) for the *A/A* genotype, compared with the *G/G* genotype.**

Conclusions. **The present study reproduced the significant association between the *A/A* genotype and reduced risk of gastric atrophy among Japanese outside Japan. According to the Japan Single Nucleotide Polymorphisms (JSNP) database (db)SNP data, the *G* allele is very frequent among Japanese and rare in Caucasians. This fact may partly explain the distribution of gastric atrophy/cancer in the world.**

Key words *Helicobacter pylori* · Gastric atrophy · *PTPN11* polymorphism · SHP-2 · Japanese Brazilians

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Introduction

Helicobacter pylori, a micro-aerophilic spiral-shaped bacterium colonizing the human stomach, is estimated to inhabit at least half of the world's human population. A higher risk of the development of gastric cancer has been reported in subjects with positive serologic tests for *H. pylori* [1]. The World Health Organization and International Agency for Research on Cancer consensus group stated, in 1994, that there was sufficient epidemiological and histological evidence to classify *H. pylori* as a definite carcinogen [2]. The unique geographic distribution of the cancer could be partly explained by the bacterium [3]. The predominant phenotype predisposing to the cancer is characterized by severe gastric atrophy, corpus-predominant gastritis, or both, along with intestinal metaplasia [4]. The channel of infection is considered to be through oral-oral and fecal-oral routes. The infection chance largely depends on the sanitary conditions, especially in childhood [5]. Infection with *H. pylori* strains carrying the cytotoxin-associated antigen A (*cagA*) gene has been considered to be more strongly associated with gastric adenocarcinoma [6]. Recent studies have suggested that combinations of host genetic traits and bacterial virulence determine the severity of the gastric damage and the eventual clinical outcome of *H. pylori* infection [7–9]. There is no doubt that the frequency of *H. pylori* exposure is the deterministic factor for the infection, but genetic traits of the host could also affect the susceptibility to *H. pylori* infection and its persistence [10,11].

The risk of gastric cancer would rise depending on the severity of gastric atrophy, as a result of the interactions between bacterial virulence and proinflammatory genetic traits [12,13]. The *cagA*-positive *H. pylori* strains are associated with higher grades of gastric inflammation and are more virulent than the *cagA*-negative strains [14]. CagA proteins are classified into two major subtypes, East-Asian type and Western type

[3,15]. The grade of gastric atrophy (or gastric cancer risk) is higher in patients infected with East-Asian *cagA*-positive strains than in those with *cagA*-negative strains or Western *cagA*-positive strains. Interestingly enough, the grade of atrophy varies even among patients with East Asian strains [16].

Attachment of *cagA*-positive *H. pylori* to cultured human gastric epithelial cells induces cell spreading and elongation accompanying cytoskeletal rearrangements, termed the "hummingbird phenotype". This change is preceded by the phosphorylation of CagA, which is injected through the type-IV secretion system during *H. pylori* and gastric epithelial cell interaction, and is associated with increased cell motility, such as cell scattering [17–20]. CagA-dependent morphological transformation of gastric epithelial cells requires SHP-2 (src homology 2 domain-containing protein tyrosine phosphatase-2) [21]. SHP-2 plays a key role in the intracellular signalling elicited by a number of growth factors, hormones, and cytokines [22,23]. It is widely expressed in both embryonic and adult tissues, and is required in several developmental processes, including gastrulation and mesodermal patterning [24,25].

Elucidation of the mechanism of interaction between CagA protein and SHP-2 has made it possible to speculate that *PTPN11* functional polymorphisms may affect the response of gastric epithelial cells, leading to atrophy and carcinoma, in individuals infected with *cagA*-positive *H. pylori*. Several single-nucleotide polymorphisms (SNPs) have been identified in *PTPN11*. A particularly prevalent SNP is in intron 3 (G/A), identified as IMS-JST057927 in the Japan Single Nucleotide Polymorphisms (JSNP) database (http://snp.ims.u-tokyo.ac.jp; RefSeq ID: rs2301756). In our previous study among Japanese, subjects possessing the *A* allele, especially those subjects with the *A*/*A* genotype, showed a significantly reduced risk for gastric atrophy [26]. The purpose of the present study was to confirm the association among Japanese Brazilians from four different areas in Brazil, who have the same genetic background as Japanese in Japan. Because the infection is mainly from family members, the *H. pylori* strains among Japanese Brazilians seem to be the same as those among Japanese in Japan. The reproduced association under a different circumstance will indicate that the strains and host genetic factors are important components for gastric atrophy.

Subjects, materials, and methods

Study population

The subjects were apparently healthy adult Japanese Brazilians, who voluntarily participated in this study, from four different areas (São Paulo, Curitiba, Mogi das Cruzes, and Mirandopolis) in Brazil. They were enrolled through associations of Japanese Brazilians, such as Kenjinkais (associations named for each prefecture of Japan), Japanese cooperative societies, country clubs of Japanese, and other non-profit Japanese Brazilian associations. After written informed consent had been obtained, lifestyle data and blood samples were taken in the rooms of each association on the occasions of festivals and sports meetings (Undoukai), from March to May 2001. Those with a history of disease such as ulcer and gastric cancer were not excluded, but the participants were all superficially in good health. Six applicants aged less than 30 years or more than 69 years of age were excluded from the analysis. The remaining 963 subjects of full Japanese ancestry were the subjects of the present study. There were 656 subjects in São Paulo, 90 in Curitiba, 110 in Mogi das Cruzes, and 107 in Mirandopolis. Excluding several subjects with samples with degraded DNA and samples that were unavailable for a pepsinogen (PG) test, we ultimately used 918 subjects (384 males and 534 females) for the statistical analysis. There were 93 immigrants (Issei), 729 second-generation (Nisei), and 96 third-generation (Sansei) [27,28].

This study was approved by both the Ethics Committee of Aichi Cancer Center, Japan (protocol no. 11-6), and the Ethics Committee of the School of Medicine of the University of São Paulo, Brazil (protocol no. 393/01), where the study was initiated, as well as the Ethics Committee of Nagoya University Graduate School of Medicine (protocol no. 317).

Genotyping

A 10-ml sample of peripheral blood was obtained from each subject, and DNA was extracted by a simple salting-out procedure [29]. The IMS-JST057927 SNP was genotyped by polymerase chain reaction with confronting two-pair primers (PCR-CTPP) [26]. The primers were: F1, 5′ GAT TGG GCA ATG GAC GA; R1, 5′ AAT GAC CAC TAA ACT TCT TAA ATG AGC; F2, 5′ CAT TTG TCT CTA AAG GAC TGT GGA; and R2, 5′ AAT CTG CAT CCC ATG CAG. Genomic DNA was applied in a volume of 25 µl with 0.12 mM dNTPs, 25 pmol of each primer, 0.5 units of AmpliTaq Gold (Perkin-Elmer, Foster City, CA, USA), and 2.5 µl 10 × PCR buffer including 15 mM $MgCl_2$. PCR was performed at 95 °C for 10 min for the initial denaturation, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 62 °C for 1 min, and extension at 72 °C for 1 min. The final extension was at 72 °C for 5 min. All PCR products were resolved directly by electrophoresis on a piece of 2% agarose-Tris-borate-EDTA (TBE) gel containing ethidium

bromide, and DNA bands were visualized by ultraviolet transillumination.

Tests for H. pylori antibody and pepsinogens

Anti-*H. pylori* IgG antibody test, performed by SRL (Tokyo, Japan), with high-molecular-weight Campylobacter-associated-protein (HM-CAP) enzyme-linked immunosorbent assay (ELISA; Enteric Products, Westbury, NY, USA), was used for the identification of *H. pylori*-infected participants [30,31]. According to the commonly used definition, an ELISA value (EV) of 2.3 or higher was regarded as *H. pylori*-seropositive. Gastric atrophy was evaluated with serum PGs and defined as PG I, less than 70 ng/dl and PG I/II ratio, less than 3. Especially, we defined severe atrophy as PG I, less than 30 ng/dl and PG I/II ratio, less than 2. These parameters for atrophy are in wide use in Japan and they have been validated against histological confirmatory studies. The PGs were measured with the "Eiken" Pepsinogen I and II kit (Eiken, Tokyo, Japan) at Mitsubishi Kagaku, BCL (Tokyo, Japan).

Statistical analysis

Hardy-Weinberg equilibrium was tested for the *PTPN11* polymorphism. Frequencies were compared by a χ^2 test. Odds ratios (ORs), adjusted for sex and age, with 95% confidence intervals (CIs), were estimated by a logistic model. All calculations were performed with the Stata 7 computer program (StataCorp, College Station, TX, USA).

Results

Characteristics of the subjects and allele frequency of the PTPN11 polymorphism

The characteristics of the subjects are summarized in Table 1. The mean age and SD were 52.7 years and 9.0 years, respectively. Females accounted for 58.2% of the study subjects. Roughly half of all the subjects were infected with *H. pylori*, while about 20% of the subjects had gastric atrophy. The genotype frequency of the *PTPN11* polymorphism was 65.5% for *G*/*G*, 30.4% for *G*/*A*, and 4.1% for *A*/*A*, which was in Hardy-Weinberg equilibrium ($\chi^2 = 0.61$; $P = 0.44$). The allele frequency was 80.7% for the *G* allele and 19.3% for the *A* allele.

As described in our previous article [26], *H. pylori* seropositivity was not associated with sex, but increased with age. There was no significant association between the *PTPN11* polymorphism and the seropositivity, although the OR of the *A*/*A* genotype was 1.68 (Table 2).

Table 1. Characteristics of the subjects and the *PTPN11* IMS-JST057927 polymorphism

	n[a]	Frequency (%)
All subjects	918	
Mean age, years (±SD)	52.7 (±9.0)	
Generation		
Immigrants	93	10.1
Second	729	79.4
Third	96	10.5
Sex		
Male	384	41.8
Female	534	58.2
Helicobacter pylori antibody		
Negative	471	51.3
Positive	447	48.7
Gastric atrophy		
Negative	741	80.7
Positive	177	19.3
Severe atrophy	33	3.6
PTPN11 IMS-JST057927		
Genotype		
G/*G*	601	65.5
G/*A*	279	30.4
A/*A*	38	4.1
Allele		
G		80.7
A		19.3

[a] Number of subjects, except where mean age is shown

PTPN11 polymorphism and gastric atrophy

There were 447 *H. pylori*-seropositive subjects, among whom 148 (33.1%) had gastric atrophy. On the other hand, there were 29 (6.2%) subjects with gastric atrophy among the 471 seronegative subjects. The difference in the prevalence was statistically significant ($P < 0.001$).

Table 3 shows the genotype distribution according to seropositivity and gastric atrophy. No severe atrophy cases were found among those with the *A*/*A* genotype. Accordingly, the OR of severe atrophy was not estimable for the *A*/*A* genotype. Similarly, the OR of gastric atrophy among the seronegative subjects was not calculable for the *A*/*A* genotype. The *G*/*G* genotype was most frequent for those with severe atrophy in both the seropositive and seronegative subjects, although the distributions of genotype were not significant by 3×3 χ^2 tests.

The age-sex adjusted OR of gastric atrophy in all *H. pylori*-seropositive subjects was 0.93 (95% CI, 0.59–1.47) for the *G*/*A* and 0.31 (95% CI, 0.10–0.95) for the *A*/*A*, compared to the *G*/*G* genotype. This trend became clear in calculating the ORs in the second- and third-generation subjects. The ORs for these subjects were 1.10 (95% CI, 0.68–1.79) for *G*/*A* and 0.08 (95% CI, 0.01–0.60) for *A*/*A* (Table 4). When the ORs were calculated for all subjects, they were 0.82 (95% CI, 0.57–

Table 2. Odds ratios of *PTPN11* IMS-JST057927 polymorphism for *H. pylori* seropositivity

Genotype	*n*[a]	*H. pylori* +	*H. pylori* + (%)	Odds ratio[b]	95% CI[c]
G/*G*	601	296	49.3	Reference	
G/*A*	279	127	45.5	0.86	0.65–1.15
A/*A*	38	24	63.2	1.68	0.85–3.32

[a] Number of subjects
[b] Adjusted for sex and age
[c] Confidence interval

Table 3. *PTPN11* IMS-JST057927 genotype distribution according to *H. pylori* seropositivity and grade of gastric atrophy (GA)

	H. pylori-seropositive			*H. pylori*-seronegative		
Genotype	GA (–)	GA (+)	GA (++)	GA (–)	GA (+)	GA (++)
G/*G*	194 (64.9%)	85 (68.5%)	17 (70.8%)	284 (64.3%)	14 (70.0%)	7 (77.8%)
G/*A*	85 (28.4%)	35 (28.2%)	7 (29.2%)	144 (32.6%)	6 (30.0%)	2 (22.2%)
A/*A*	20 (6.7%)	4 (3.2%)	0 (0.0%)	14 (3.2%)	0 (0.0%)	0 (0.0%)
Total	299 (100%)	124 (100%)	24 (100%)	442 (100%)	20 (100%)	9 (100%)

GA (–), GA (+), and GA (++), no atrophy, moderate atrophy, and severe atrophy, respectively

Table 4. Genotype frequencies of *PTPN11* polymorphism and odds ratios of gastric atrophy in the *H. pylori*-seropositive subjects

Generation	Genotype	*n*[a]	Gastric atrophy	(%)	Odds ratio[b]	(95% CI[c])	*P*
All	*G*/*G*	296	102	(34.5)	Reference		
	G/*A*	127	42	(33.1)	0.93	(0.59–1.47)	0.760
	A/*A*	24	4	(16.7)	0.31	(0.10–0.95)	0.040
	G/*A*+*A*/*A*	151	46	(30.5)	0.80	(0.52–1.23)	0.308
Immigrants	*G*/*G*	34	18	(52.9)	Reference		
	G/*A*	13	3	(23.1)	0.17	(0.34–0.86)	0.032
	A/*A*	3	3	(100.0)			0.243[d]
Second and third	*G*/*G*	262	84	(32.1)	Reference		
	G/*A*	114	39	(34.2)	1.10	(0.68–1.79)	0.693
	A/*A*	21	1	(4.8)	0.08	(0.01–0.60)	0.014

[a] Number of subjects
[b] Adjusted for sex and age
[c] Confidence interval
[d] Calculated by Fisher's exact test

1.19) and 0.40 (95% CI, 0.14–1.17), respectively. There were no substantial differences in the ORs for *A*/*A* between males and females; 0.41 and 0.24 among the seropositives, and 0.53 and 0.32 among all subjects, respectively. The age was also unrelated to the OR for *A*/*A*; when the subjects were divided, at age 53 years, into two groups of similar sizes, the OR was 0.52 for the younger seropositives and 0.28 for the older seropositives. The corresponding ORs for all subjects, including the seronegatives, were 0.58 and 0.36. These ORs of *A*/*A* relative to *G*/*G* according to sex and age groups were not significant because of the reduction of sample size.

Discussion

The *PTPN11* SNP named as IMS-JST057927 in the JSNP database, had a significant association with the risk of gastric atrophy among *H. pylori*-seropositive Japanese in our previous study [26]. In the present study, we found additional evidence of the association between the *PTPN11* polymorphism and gastric atrophy in Japanese Brazilians. Among *H. pylori*-seropositive subjects, the OR of gastric atrophy was 0.31 (95% CI, 0.10–0.95) for the *A*/*A* genotype compared with the *G*/*G* genotype, though the *G*/*A* genotype showed no significant result (OR, 0.93; 95% CI, 0.59–1.47). Because a similar mark-

Table 5. Ethnic variations in allele frequency of the *PTPN11* polymorphism (IMS-JST057927 in JSNP; rs2301756 in NCBI dbSNP)

Ethnic group	n[a]	Allele frequency		Genotype frequency			ss no.
				G/*G*	*G*/*A*	*A*/*A*	
Japanese	1484	*G* = 0.802	*A* = 0.198		No data		3247842
Chinese	48	*G* = 0.917	*A* = 0.083	0.833	0.167	0.000	23392631
African American	46	*G* = 0.348	*A* = 0.652	0.043	0.609	0.348	23392631
Caucasians	46	*G* = 0.065	*A* = 0.935	0.000	0.130	0.870	23392631
	120	*G* = 0.125	*A* = 0.875	0.000	0.250	0.750	11044821
Our studies							
Japanese[b]	902	*G* = 0.822	*A* = 0.178	0.678	0.286	0.035	
Japanese Brazilians[c]	1836	*G* = 0.807	*A* = 0.193	0.655	0.304	0.041	

[a] Number of sample chromosomes
[b] Goto et al. [26]
[c] Present study

edly reduced risk was observed only for the *A*/*A* genotype in our previous study among Japanese in Japan (OR, 0.09 for the *A*/*A* genotype and OR, 0.70 for the *G*/*A* genotype), the *A* allele may work recessively against gastric atrophy. This trend became clear in the second and third generations in the present study.

In this study, the allele frequency of the *PTPN11* polymorphism was 80.7% for the *G* allele and 19.3% for the *A* allele. The frequency was similar to that in our previous study in Japan (82.2% for the *G* allele, 17.8% for the *A* allele). The IMS-JST057927 SNP has a RefSeq ID: rs2301756 in the National Center for Biotechnology Information (NCBI) dbSNP, and three NCBI Assay ID (ss)IDs. In researching these submitted data we found a quite interesting fact, that the allele frequency in Asian populations is obviously different from that in Western populations. In Japanese and Chinese, the high-risk *G* allele is dominant (Table 5). Because gastric cancer incidence is high in Japanese, Koreans, and Chinese, it seems that the ethnic difference may be determined partly by this polymorphism.

It is well documented that CagA is strongly associated with the risk of gastric atrophy/cancer. Although CagA antibody is not detected in the serum of all infected individuals, nearly 100% of *H. pylori* strains in Japan possess CagA [32]. Similarly, CagA antibody was not detected in all of the infected Japanese Brazilians [33], but it is likely that the great majority of *H. pylori* strains in the second and third generations were CagA-positive, because *H. pylori* infection usually occurs in childhood, through family members. Japanese Brazilians have maintained a Japanese culture, and their lifestyles remain unchanged. Accordingly, it seems reasonable that the association of the *PTPN11* polymorphism with gastric atrophy was observed in this population. It is rare that those with *cagA*-negative strains or those without *H. pylori* infection develop gastric atrophy, so that the effects of the polymorphism may be limited in these groups.

The function of the *PTPN11* polymorphism at intron 3 is still unknown. There are two possible biological explanations of the association with gastric atrophy. One explanation is that the polymorphism itself is functional. The G/A SNP is located 223 bp upstream of exon 4. Although the position is relatively far from the beginning of the exon, there is a probability that it causes a different splicing, which could make a less active SHP-2. The other explanation is a linkage to a functional polymorphism at the promoter region or at the coding region, which would influence SHP-2 activity. The function of the polymorphism remains to be elucidated.

It has been a mystery for a long time why only a proportion of individuals who are infected with CagA-positive *H. pylori* develop severe gastric diseases. We consider the hypothesis that the *G* allele of *PTPN11* may govern the genetic traits leading to the development of gastric atrophy via signal transduction from CagA may partly explain the mystery, though other mechanisms may be involved. CagA interacts with a number of other cellular proteins, such as C-terminal src kinase (Csk), Grb2, and c-Met hepatocyte growth factor receptor [21,34–36]. One report has shown that CagA mimics mammalian docking/scaffolding molecules such as Gab, one of the insulin receptor substrate family proteins [37]. The roles of these mechanisms should also be examined epidemiologically, through polymorphism studies.

Several other SNPs, such as those of interleukin-1B (*IL-1B*) and tumor necrosis factor-α (*TNF-A*), that have been considered to have an association with gastric atrophy. Interleukin 1β (IL-1β), a proinflammatory cytokine, and TNF α inhibit gastric acid secretion, and they are induced by *H. pylori*. However, in a previous study, we found no association between *TNF-A* gene polymorphisms and gastric atrophy [38]. Concerning the *IL-1B* gene, we examined the C-31T polymorphism among *H. pylori*-infected subjects in this study and found a trend that the *T* allele worked as a preventive

factor, but the result was not significant (OR, 0.60; 95% CI, 0.36–1.01 for the *C*/*T* genotype; OR, 0.63; 95% CI, 0.37–1.11 for the *T*/*T* genotype, compared with the *C*/*C* genotype).

In conclusion, a significant association between the *PTPN11* polymorphism and gastric atrophy was observed among *H. pylori*-seropositive Japanese Brazilians, which provided additional evidence of this association. However, as no other studies have reported on this association, except for ours, confirmation will be required for other populations infected with *cagA*-positive *H. pylori*.

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