RESEARCH COMMUNICATION

Associations between a PTPN11 Polymorphism and Gastric Atrophy - Opposite in Uzbekistan to that in Japan

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Abstract

Src homology 2 domain-containing protein tyrosine phosphatase-2 (SHP-2) of gastric epithelial cells interacts with cagA from *Helicobacter pylori* (*H. pylori*). Our previous studies found the AA genotype of a G/A single nucleotide polymorphism at intron 3 (rs2301756) of PTPN11 gene, which encodes SHP-2, to be associated with a lower risk of gastric atrophy. The present study aimed to examine the association with gastric atrophy among the subjects of a case-control study of peptic ulcer disease (PUD) conducted in the Uzbek Republic. Cases were 95 patients (61 males and 34 females) with PUD aged 16 to 85 years. Controls were 102 hospital volunteers (42 males and 60 females) including 42 patients with miscellaneous diseases, aged 15 to 75 years. Gastric atrophy was evaluated with serum pepsinogens (PG1<70ng/ml and PG1/PG2<3). Polymorphisms of PTPN11 at intron 3 (rs2301756) and intron10 (rs12229892) were genotyped with PCR with confronting two-pair primers (PCR-CTPP). Anti-cagA IgG antibody was detected in 93.7% of cases and 77.5% in controls. Gastric atrophy was observed in 24.2% of the PUD patients and 33.3% in the controls. The A allele at intron 3 was completely linked to the G allele at intron 10. The age, sex, and group (cases and controls) adjusted odds ratio of gastric atrophy was 0.18 (95% confidence interval, 0.04-0.86) for intron 3 GG genotype relative to AA genotype. Since the finding was opposite to that among Japanese, the *H. pylori* strains and/or lifestyle in Uzbekistan might modify the association.

Key Words: cagA - gastric atrophy - PTPN11 polymorphism - Uzbekistan

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Introduction

Helicobacter pylori (*H. pylori*) is an established cause of gastric cancer, through the development of gastric atrophy and preceding precancerous lesions. CagA injected from *H. pylori* to endothelial cells play a main role to the gastric carcinogenesis (Blaser et al., 1995; Kuipper et al., 1995; Shimoyama et al., 1998). It combines src homology 2 domain-containing protein tyrosine phosphatase-2 (SHP-2) to transduce the signal to other molecules relating atrophy and carcinogenesis (Hatakeyama, 2004).

PTPN11 gene encoding SHP-2 has a number of polymorphisms. The majority of PTPN11 polymorphisms have a minor allele with a very low frequency, and some of the polymorphisms are tightly linked to other polymorphisms. Accordingly, the candidate polymorphisms for Asians are two G/A single nucleotide polymorphisms (SNPs); one at intron 3 (rs2301756 or IMS-JST057927) and the other at intron 10 (rs12229892). In our previous studies among Japanese and Japanese Brazilians, those with AA genotype at intron 3 showed a significantly reduced risk of gastric atrophy (Goto et al., 2006; Kawai et al., 2006). The present study aimed to examine the association in the data collected for a case-control study on peptic ulcer disease (PUD) in Uzbekistan Republic (Abdiev et al., in press). The study was approved by Ethics Committee of Nagoya University School of Medicine (approval number 459).

Materials and Methods

Study subjects were described in our previous study (Abdiev et al., in press). Briefly, in total 197 participants (103 males and 94 females) were enrolled for the case-control study on PUD from January to March of 2007. The PUD cases consisted of 95 patients (85 duodenal ulcer patients and 10 gastric ulcer patients), who visited the Republic Research Center of Emergency Medicine in Tashkent for seeking treatment during the enrollment period. All the cases were diagnosed endoscopically at the Center before their enrollment. They were

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consecutively invited to the study. The controls were arbitrarily invited 102 volunteers including 60 students/staffs and 42 patients (14 with acute appendicitis, 17 with acute cholecystitis, 4 with hernia, 3 with pancreatitis, 2 with echinococcus infection, 1 with pneumonia, and1 with nephritis) of the Center. They were 160 Uzbeks, 24 Russians, 4 Koreans, and 9 persons of other ethnicities. Anti-cagA antibody was positive among 93.7% of cases and 77.5% of controls (Abdiev et al., in press).

Approximately 7.5 ml of whole blood was drawn from each person. Serum separated from the blood was immediately stored at -20°C until analysis. Anti-cagA IgG antibody was measured with cagA IgG EIA WELL (Radim SpA, Rome, Italy, 10.0 units or higher was regarded as seropositive). Serum pepsinogens (PG) were measured by chemiluminescence enzyme immunoassay (CLEIA). Gastric mucosal atrophy was grouped into "none" (PG1>70ng/ml or PG1/PG2>3), "mild" (PG1<70ng/ml and PG1/PG2<3, excluding "severe" cases), or "severe" (PG1<30ng/ml and PG1/PG2<2).

DNA was extracted from 2.5 ml of heparinized blood with a BioRobot M48 Workstation (QIAGEN Group, Tokyo). Two PTPN11 G/A polymorphisms (rs2301756 and rs12229892) were genotyped with a polymerase chain reaction with confronting two-pair primers (PCR-CTPP) (Hamajima et al., 2003). The primers were F1: GGA TTA CAG GCA TAA GCC AC, R1: GAC CAC TAA ACT TCT TAA ATG AGC, F2: CAT TTG TCT CTA AAG GAC TGT GGA, and R2: CTC TGG CTC TCT CGT ACA AGA for rs2301756 at intron 3, and F1: CAG CGA GCT CAC TGA GTC C, R1: CAA TTC CAG GCA CTC ACA CTC AC, F2: AGA GAT ACC CAA GGG CCA CA, and R2: ATC TGC CTG CCT CGA CC for rs12229892 at intron 10. Amplification conditions were 10 minutes of initial denaturation at 95°C, followed by 30 cycles of 1 minute at 95°C, 1 minute at 64°C (rs2301756) or 68°C (rs12229892) and 1 minute at 72°C, then a 5 minute final extension at 72°C. The separately amplified DNA was visualized on a 2% agarose gel with ethidium bromide staining. The amplified DNA of rs2301756 at intron 3 was 201bp for the G allele, 339bp for the A allele, and 490bp for the common band at intron 3, and that of rs12229892 at intron 10 was 131bp, 185bp, and 275bp, respectively.

For statisical assessment, the differences in percentage were examined with a Fisher's exact test. The 95% confidence intervals (CIs) for percentages were calculated based on binomial distributions. Logistic regression analysis was performed for estimating odds ratios (ORs) and 95% CIs. Age adjustment in the logistic analysis was done as a continuous variable. The calculations were achieved using the STATA version 7 (Stata Corp, College Station, TX).

Results

Since the background of study subjects was described in our previous paper (Abdiev et al., in press), only sex, age range, nationality, and cagA antibody positivity are shown in Table 1. Except one PUD case, all subjects were successfully genotyped. The substantial differences in the genotype frequency were not observed between 42 controls with miscellaneous diseases and 60 healthy controls. The genotype frequencies for the polymorphisms at intron 3 and intron 10 were in Hardy-Weinberg equilibrium among the 102 controls; p=0.250 and p=0.603, respectively. The A allele was 0.725 (95%CI, 0.659-0.785) at intron 3 and 0.049 (95%CI, 0.024-0.088) at intron 10 among 102 controls. Among 83 Uzbek controls, the A allele was 0.729 (95%CI, 0.655-0.795) and 0.042 (95%CI, 0.017-0.085), respectively. Among 4 Koreans, it was 0.125 (95%CI, 0.003-0.527) and 0.500 (95%CI, 0.157-0.843), respectively.

Table 1. Characteristics of Peptic Ulcer Disease (PUD) Patients, Patients with Other Miscellaneous Diseases (patient controls), and Healthy Controls

Characteristic		PDU patients n=95 (%)	Patient controls n=42 (%)	Healthy controls n=60 (%)
Sex	Males	61 (64.2)	17 (40.5)	25 (41.7)
	Females	34 (35.8)	25 (59.5)	35 (58.3)
Age	Mean	45.8	36.7	31.3
	Range	16 - 86	17 - 75	15 – 65
Nationality				
	Uzbeks	79 (83.2)	34 (81.0)	49 (81.7)
	Russians	13 (13.7)	4 (9.5)	7 (11.7)
	Koreans	1 (1.1)	2 (4.8)	1 (1.7)
	Others	2 (2.1)	2 (4.8)	3 (5.0)
CagA antibody				
	Negative	89 (93.7)	30 (71.4)	49 (81.7)
	Positive	6 (6.3)	12 (28.6)	11 (18.3)
Gastric atrophy*				
	None	72 (75.8)	30 (71.4)	38 (63.3)
	Mild	19 (20.0)	10 (23.8)	21 (35.0)
	Severe	4 (4.2)	2 (4.8)	1 (1.7)
Intron 3				
	AA	48 (51.1)	23 (54.8)	33 (55.0)
	GA	30 (31.9)	14 (33.3)	22 (36.7)
	GG	16 (17.0)	5 (11.9)	5 (8.3)
Not genotyped		1	0	0
Intron 10				
	GG	81 (86.2)	36 (85.7)	56 (93.3)
	GA	13 (13.8)	6 (14.3)	4 (6.7)
	AA	0 (0.0)	0 (0.0)	0 (0.0)
Not genotyped		1	0	0

*Gastric atrophy; "None" for PG1>70ng/ml and PG1/PG2<3, "Mild" for PG1<70ng/ml and PG1/PG2<3 excluding the "Severe" cases, and "Severe" for PG1<30 and PG1/PG2<2

The age-sex-adjusted ORs of the polymorphisms for PUD were not significant; at intron3, 1.02 (95%CI, 0.51-2.05) for GA and 2.31 (95%CI, 0.89-6.02) for GG, relative to AA, and at intron 10, 2.04 (95%CI, 0.78-5.32) for GA, relative to GG.

Table 2 shows the linkage between the polymorphisms

Table 2. Linkage of PTPN11 between the Polymorphisms at Intron 3 (rs2301756) and Intron 10 (rs12229892)

Polymorphism at intron 3	n	Polymorphism at intron 10 GG	GA	AA
AA	104	104	0	0
GA	66	55	11	0
GG	26	14	12	0

One patient with peptic ulcer disease could not genotyped due to poor quality of DNA

Table 3. Sex-age-adjusted Odds Ratios (ORs) and 95% Confidence Intervals (95%CIs) of PTPN11 Polymorphism at intron 3 (rs2301756) for Gastric Atrophy (GA) Measured with Pepsinogens in the Uzbekistan Republic

Combined analysis						
Genotype	GA(-)	(+)	OR	95%CI	OR[*1]	95%CI
AA	69	35	1	Reference	1	Reference
GA	46	20	0.90	0.45-1.79	0.92	0.46-1.86
GG	24	2	0.16	0.03-0.72	0.18	0.04-0.86
GA+GG	70	22	0.63	0.33-1.21	0.68	0.35-1.31

Subgroup analysis								
Genotype	Among PUD patients				Among controls			
	GA(-)	(+)	OR	95%CI	GA(-)	(+)	OR	95%CI
AA	35	13	1	Reference	34	22	1	Reference
GA	20	10	1.38	0.48-3.85	26	10	0.64	0.24-1.69
GG	16	0	0.00	Uncalculable[*2]	8	2	0.38	0.07-2.20
GA+GG	36	10	0.77	0.29-2.06	34	12	0.57	0.22-1.44

*[1]Adjustment for groups of cases and controls *2 p=0.027 for the Fisher's exact test comparing GA(+) frequencies between GG and AA

at introns 3 and 10 among 196 subjects. The haplotype A-A was not observed, while the AA genotype at intron 10 was not detected due to the low allele frequency. Among 26 participants with GG genotype at intron 3, A allele at intron 10 was 0.231 (95%CI, 0.125-0.368).

The sex-age-adjusted ORs and 95%CIs of the polymorphism at intron 3 relative to AA genotype were listed in Table 3. In combined analysis including PUD cases and all controls, the GG genotype had a significantly low risk of gastric atrophy. The OR was 0.18 when the case-control group was adjusted. The subgroup analysis demonstrated that the risk reduction was larger among the PUD cases than among the control groups.

The findings were alsao similar when the analyses were conducted only for Uzbeks. For example, the sex-age-group adjusted OR for GG genotype was 0.10 (95%CI, 0.01-0.84). The results were also similar in the analysis for cagA antibody positive participants; the corresponding OR in this case was 0.21 (95%CI, 0.04-1.01, p=0.052).

Discussion

This study showed that the PTPN11 polymorphisms were not associated with the PUD risk in Uzbekistan Republic. The A allele at intron 3 was 0.725 among 102 controls and 0.699 among 196 whole subjects, being completely linked to the G allele at intron 10. The GG genotype at intron 3 had a significantly lower risk of gastric atrophy. This is the opposite phenomenon to that observed among Japanese (Goto et al., 2006; Kawai et al., 2006).

The A allele was the dominant allele of PTPN11 polymorphism at intron 3 among Caucasians (0.875 of 120 chromosomes), but not among Japanese (0.178 of 902 chromosomes) and Chinese (0.083 of 48 choromosomes) (Kawai et al., 2006). This study found that the A allele was 0.729 among 83 Uzbek controls, indicating that the allele frequency was close to Caucasians. The allele frequency was found to be only 0.125 among 4 Koreans in this study, indicating that genotyping errors seemed unlikely.

The linkage between polymorphisms at intron 3 (rs2301756) and at intron 10 (rs12229892) was confirmed in the present study. Since the minor allele at intron 10 was relatively rare, the statistical power to examine the association with gastric atrophy was not sufficient.

Our hypothesis is that gastric atrophy development after cagA positive *H. pylori* infection was rarer among those with AA genotype at intron 3 than among those with GG genotype. However, the present study in Uzbekistan Republic showed the opposite results. There were several interpretations. The first possible explanation is that cagA of *H. pylori* in Uzbekistan plays differently from that in Japan. There are two major types of cagA; Western types and East-Asian type (Hatakeyama, 2004). East-Asain cagA was reported to combine SHP-2 molecule more strongly than Western cagA. Among Western types, furthermore, three strains with different affinity to SHP-2 exist. The cagA type of *H. pylori* in Uzbekistan has not been reported. The second is that rs2301756 genotype may link another functional polymorphism, and the link is different between Uzbeks and Japanese. Since the polymorphisms around rs2301756 have been screened, the explanation may be unplausible. Third, the difference in lifestyle may modify the association. For example, many inconsistent findings have been reported for GSTM1 null/present polymorphism (Houlston, 1999). Those with the null genotype had a low risk of lung cancer in some studies, while in the other studies those were at a higher risk of lung cancer. Glutathione S-transferase μ reacts not only carcinogens but also anti-cancer substrates such as isothiocyanates. Since glutathione conjugates are excreted outside the cell, the GSTM1 null genotype may reduce risk among non-smokers eating enough vegetables, and increase risk among smokers. A cohort study in Shanghai showed that smoking adjusted relative risk of GSTM1 null genotype relative to GSTM1 present genotype for lung cancer was significantly below the unity among those with detectable urinary isothiocyanates and significantly above unity among those with undetectable urinary isothiocyanates (London et al., 2000). Although the biological mechanism for SHP-2 may not be analogous, such modification might be conceptually possible. Finally, the inconsistent results were obtained by chance. If so, repeated studies will provide us the answer.

In conclusion, the present study showed the allele frequency of PTPN11 polymorphisms among Uzbeks to be closer to that among Caucasians, and the AA at intron 3 was a higher risk genotype for gastric atrophy in Uzbekistan. The result is opposite to those among Japanese and the significance clearly should be examined by further studies.

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