

Impact of *PSCA* Variation on Gastric Ulcer Susceptibility

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Abstract

Peptic ulcer is one of the most common gastrointestinal disorders with complex etiology. Recently we conducted the genome wide association study for duodenal ulcer and identified disease susceptibility variations at two genetic loci corresponding to the *Prostate stem cell antigen (PSCA)* gene and the *ABO blood group (ABO)* gene. Here we investigated the association of these variations with gastric ulcer in two Japanese case-control sample sets, a total of 4,291 gastric ulcer cases and 22,665 controls. As a result, a C-allele of rs2294008 at *PSCA* increased the risk of gastric ulcer with odds ratio (OR) of 1.13 (P value of 5.85×10^{-7}) in an additive model. On the other hand, SNP rs505922 on *ABO* exhibited inconsistent result between two cohorts. Our finding implies presence of the common genetic variant in the pathogenesis of gastric and duodenal ulcers.

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Introduction

Peptic ulcer is the most common disease in the gastrointestinal tract with symptoms of nausea, vomiting, and abdominal pain, and sometimes causes bleeding and perforation with acute peritonitis. Lifetime prevalence of peptic ulcer is 10–15% in the Japanese and 4–10% in Caucasians [1–3]. Approximately 70% of gastric ulcer patients and 90% of duodenal ulcer patients are associated with *H. pylori* infection [4]. Since eradication of *H. pylori* by antibiotics in combination with proton pump inhibitor can effectively cure peptic ulcer [5], *H. pylori* is shown to be the major cause of peptic ulcer. Although nearly 50% of individuals on the earth are infected with *H. pylori*, most of them remain asymptomatic indicating that the clinical outcome after the *H. pylori* infection varies substantially between individuals. These inter-individual diversities are affected by various factors including bacteria subtypes, host response, and their interaction. *Duodenal ulcer promoting gene A (dupA)* in *H. pylori* was indicated to induce interleukin (IL)-8 that increases the risk of duodenal ulcer and decreases the risk of gastric cancer [6,7]. Nonsteroidal anti-inflammatory drugs (NSAIDs) and smoking are known risk factors for peptic ulcer [8,9]. In addition to these bacterial and environmental factors, host genetic factors had been implicated to have some roles in the risk of peptic ulcer. Proband-wise concordance rate of peptic ulcer in monozygotic twins was as high as 23.6% while that in dizygotic twins was 14.8%. Several candidate gene approaches revealed the possible association of

genetic variations in *IL-6*, *IL-8*, *IL-10* [10], *TNF*, *LTA* [11], and *COX1* [12] with peptic ulcer risk.

In our previous genome wide association study (GWAS) of duodenal ulcer using a total of 7,035 cases and 25,323 controls, we identified the significant association of genetic variations at *PSCA* (*prostate stem cell antigen*) and the *ABO* blood group with duodenal ulcer [13]. The C allele of rs2294008 at *PSCA* increased the risk of duodenal ulcer (odds ratio (OR) of 1.84 with P value of 3.92×10^{-33}) in a recessive model, while it decreased the risk of gastric cancer (OR of 0.79 with P value of 6.79×10^{-12}) as reported previously [14]. Our functional analyses revealed that the T allele of SNP rs2294008 creates an upstream translational initiation codon and add the signal peptide sequences at the N-terminal portion, resulting in alteration of the protein subcellular localization from cytoplasm to cell surface. SNP rs505922 on *ABO* was also associated with duodenal ulcer in a recessive model (OR of 1.32 with P value of 1.15×10^{-10}). Since *H. pylori* infection and non-steroidal anti-inflammatory drugs induce gastroduodenal mucosal injury which would cause duodenal and gastric ulcer, we examined the role of variants in the *PSCA* and *ABO* genes on gastric ulcer risk among Japanese population.

Results

A total of 4,291 gastric ulcer cases and 22,665 controls without having the past history of duodenal ulcer or continuous NSAID intake were recruited from the BioBank Japan and the Aichi

Cancer Center (**Table 1**). We then genotyped SNP rs2294008 and rs505922 in two case-control sample sets and examined the association with gastric ulcer in three genetic models (additive, recessive, and dominant model) (**Table 2**). To increase the statistical power of this study, we used subjects with either of 22 diseases as control samples. Therefore we evaluated the confounding effect of disease mix control samples used in this analysis. SNPs rs2294008 and rs505922 did not show significant association between case-mix controls ($n = 19,884$) and healthy volunteers ($n = 2,781$) (**Table S1**). In addition, both SNPs did not show the significant deviation from HWE (Hardy-Weinberg equilibrium) in each disease group. Therefore disease mix controls seem not to largely affect the association result in our analysis.

The results of association analyses revealed that gastric ulcer patients had a higher frequency of C allele at rs2294008 than the control group in both sets (39.7% vs 36.9% and 40.1% vs 37.0%, respectively). A meta-analysis of the two studies showed the significant association of rs2294008 in an additive model with no evidence of heterogeneity ($P = 5.85 \times 10^{-7}$ with OR of 1.13), although the association was not statistically significant among Aichi Cancer Center cohort probably due to smaller sample size. Risk alleles (C allele at rs2294008) in the two sample sets were consistent between duodenal ulcer and gastric ulcer, indicating the role of *PSCA* variation as common genetic factors for peptic ulcer. However impact of this variation on gastric ulcer risk was not as strong as those on duodenal ulcer reported previously [13].

On the other hands, SNP rs505922 showed inconsistent results between two cohorts. A T allele of rs505922 increased gastric ulcer risk in all three genetic models in BioBank Japan cohort. However, gastric ulcer patients exhibited lower frequency (53.5%) of a T allele than the healthy controls (55.1%) in the Aichi Cancer Center cohort. Therefore, further association analysis is essential to determine the role of *ABO* variations on gastric ulcer susceptibility.

Since we have genotyping results of 1,862 gastric ulcer cases and 17,482 controls analyzed by Illumina Human Hap610-Quad genechip, we conducted whole genome screening using these sample set. Although 62 SNPs exhibited suggestive associations with P values of less than 1×10^{-4} , no SNPs cleared genome wide significant threshold (**Table S2** and **Figure S1**). Thus, our sample set did not have sufficient statistical power to detect gastric ulcer susceptibility loci by GWAS.

We also investigated the association of previously reported genes with gastric ulcer (**Table 3**). We selected 32 SNPs at five gene loci that had been genotyped by Illumina Human Hap610-Quad genechip. As a result, two loci at *LTA* and *PTGS1* indicated suggestive association ($P = 1.64 \times 10^{-3}$ and 0.0376), although these

associations were not statistically significant after Bonferroni's correction ($P < 0.00156 = 0.05/32$). Thus further analyses are necessary to elucidate the role of these variations on gastric ulcer.

Discussion

The development of gastric ulcer is determined by the interplay between gastric acid secretion and mucosal resistance, however their underlying pathogenesis has not been fully elucidated. Gastric mucus, a gelatinous material secreted by gastric mucous cells, serves as an unstirred layer through which the diffusion of acid and pepsin is reduced. We here found that variation in the *PSCA* gene was significantly associated with gastric ulcer. *PSCA* was initially identified as a tumor antigen that was highly expressed in prostate, bladder, and pancreatic cancer tissues [15,16]. Since tumor cells treated with anti-*PSCA* antibody exhibited a growth suppressive effect [17,18], cell surface-*PSCA* is considered to play an important role in cell proliferation. In contrast, down-regulation of *PSCA* in gastric and esophageal cancer tissues was also reported [19,20]. Thus the role of *PSCA* in carcinogenesis is still controversial [21]. These diverse effects of *PSCA* among various cancer types might be partially explained by the effect of genetic variation. Individuals carrying the T allele at rs2294008 express *PSCA* proteins with an additional fragment of nine amino acids at the N-terminal portion [13]. On the other hand, individuals carrying the C allele at rs2294008 express a shorter *PSCA* protein which lacks the signal peptide and is predicted to be localized in the cytoplasm without glycosylation [22]. We also found that the cytosolic shorter *PSCA* protein was more susceptible to proteasomal degradation than the long *PSCA* protein at the cell-surface. Since *PSCA*-derived peptides were reported to be a target of T-cell-based immunotherapy for advanced prostate cancer [23], the shorter *PSCA* protein would cause the activation of CD4-positive and/or CD8-positive T cells and subsequently promote epithelial mucosal injury [24]. In contrast, the long *PSCA* protein at the cell surface might facilitate mucosal repair by enhancing epithelial cell proliferation. In addition, T allele of SNP rs2294008 was shown to be associated with higher mRNA and protein expression [25]. Thus the impact of *PSCA* on gastric ulcer and carcinogenesis could be regulated by the *PSCA* variation.

H. pylori plays an important role in the development of gastritis, peptic ulcers, and gastric cancer, and the eradication of *H. pylori* was shown to reduce the recurrence of gastric ulcer [26] and prevent the onset of gastric cancer [27]. Since vertical transmission during childhood is the major source of infection, family history of *H. pylori* infection or *H. pylori*-related diseases is a risk factor for *H. pylori* infection [28–30]. In addition, recent accumulated evidences

Table 1. Characteristics of study population.

Samples	Source	Platform	Number of samples	Female (%)	Age (mean +/- SD)
Gastric ulcer ^a	BioBank Japan	Illumina HumanHap 610	1,862	32.0 (%)	66.0+/-10.7
		Invader assay	2,004	35.3 (%)	66.5+/-11.7
	Aichi Cancer Center	TaqMan	425	48.7 (%)	55.6+/-12.3
Control ^a	BioBank Japan ^b	Illumina HumanHap 610	17,482	54.1 (%)	62.2+/-13.0
		Illumina HumanHap 550	3,309	66.0 (%)	43.8+/-16.2
	Aichi Cancer Center	TaqMan	1,874	38.7 (%)	53.7+/-14.6

^aSubjects with a history of gastric cancer or duodenal ulcer were excluded from cases and controls.

^bControl samples consist of patients with colon cancer, breast cancer, diabetes, arteriosclerosis obliterans, atrial fibrillation, brain infarction, drug response, amyotrophic lateral sclerosis, liver cancer, liver cirrhosis, osteoporosis, fibroid, cervical cancer, chronic hepatitis B, ovarian cancer, pulmonary tuberculosis, keloid, drug eruption, hematological cancer, uterus cancer, heat cramp, endometriosis, and 907 healthy volunteers.

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Table 2. Association of PSCA and ABO SNPs with gastric ulcer.

SNP	Case			Control			Additive			Recessive			Dominant				
	CC	CT	TT	RAF ^a	CC	CT	TT	RAF ^a	P^b	OR ^b (95% C.I.)	P_{Het}^c	P^b	OR ^b (95% C.I.)	P_{Het}^c	P^b	OR ^b (95% C.I.)	P_{Het}^c
rs2294008	##	###	###	###	###	###	###	###	2.55 × 10 ⁻⁶	###	(1.07–1.18)	6.12 × 10 ⁻⁴	1.18	(1.07–1.30)	3.52 × 10 ⁻⁵	###	(1.08–1.25)
8q24/PSCA																	
ACC	70	201	154	###	235	917	722	###	9.09 × 10 ⁻²	###	(0.98–1.33)	3.10 × 10 ⁻²	1.38	(1.03–1.84)	###	###	(0.89–1.37)
meta ^d									5.85 × 10 ⁻⁷	###	(1.08–1.18)	8.94 × 10 ⁻⁵	1.20	(1.09–1.31)	2.60 × 10 ⁻⁵	###	(1.08–1.24)
rs505922	##	###	###	###	###	###	###	###	9.39 × 10 ⁻⁴	###	(1.03–1.14)	7.72 × 10 ⁻⁴	1.13	(1.05–1.22)	5.26 × 10 ⁻²	###	(1.00–1.19)
9q34/ABO																	
ACC	97	201	127	###	379	925	570	###	0.407	###	(0.81–1.09)	0.829	0.97	(0.77–1.23)	0.232	###	(0.67–1.10)
meta ^d									3.88 × 10 ⁻³	###	(1.02–1.12)	1.75 × 10 ⁻³	1.12	(1.04–1.20)	0.141	###	(0.98–1.15)

We analyzed 4,291 gastric ulcer cases and 22,665 controls.
^ars2294008 (C allele) and rs505922 (T allele).
^bP values were obtained using chi-square test. To calculate odds ratios (OR), non risk alleles were considered as references.
^cHeterogeneity across two stages was assessed by Cochran Q test.
^dOR and P values were obtained using the Mantel-Haenszel fixed-effects model in the meta analysis.
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revealed a number of risk factors of gastric cancer (T allele at rs2294008, blood type A, decreased gastric acid, intake of proton pump inhibitor/H₂ blocker, and *CagA* in *H. pylori* [31]) or peptic ulcer (C allele at rs2294008, blood type O, NSAID intake, *dupA* in *H. pylori*) [32]. In addition, *CYP2C19* genotype was associated with the response to triple anti-*H. pylori* therapy including proton pump inhibitor [33]. However, our previous analysis revealed that SNP rs2294008 and rs505922 did not associated with *H. pylori* prevalence [13]. Taking the above information into account, the estimation of disease risk and drug efficacy would enable us to determine the appropriate treatment protocol for *H. pylori* carriers.

Here we found that *PSCA* variant was significantly associated with gastric ulcer. In our previous analysis, *PSCA* variation did not associate with *H. pylori* prevalence [13]. Since *H. pylori* infection was associated with many diseases such as MALT lymphoma [34], idiopathic thrombocytopenic purpura [35], atrophic gastritis [36], and NSAID-induced gastric ulcer, it is very interesting to evaluate the effect of *PSCA* variation on these diseases. We hope our findings would contribute to the elucidation of disease pathogenesis as well as to the establishment of personalized medical treatments in the future.

Methods

Ethics Statement

This research project was approved by the ethical committees at the University of Tokyo, RIKEN, and Aichi Cancer Center. All participants provided written informed consent as approved by the ethical committees of the University of Tokyo and Aichi Cancer Center.

Study participants

The demographic details of study participants are summarized in Table 1. A total of 3,866 gastric ulcer patients, and 20,791 gastric ulcer negative controls were obtained from BioBank Japan that was initiated in 2003 with the funding from the Ministry of Education, Culture, Sports, Science and Technology, Japan [37]. In the BioBank Japan Project, DNA and serum of patients with 47 diseases were collected through collaborating network of 66 hospitals throughout Japan. The list of participating hospitals is shown in the following website (http://biobankjp.org/plan/member_hospital.html). A total of 425 gastric ulcer cases and 1,874 healthy controls were obtained from the Aichi Cancer Center. The diagnosis of gastric ulcer was based on clinical, endoscopic, and histological features. List of disease-mix control samples used in this study was shown in **Table S1**. We excluded patients with duodenal ulcer or gastric cancer from both cases and controls. Deregulation of *PSCA* was reported in many types of malignancy such as prostate, pancreatic, lung, bladder, gastric, cholangiocarcinoma, and esophageal cancer [14–16,20,38,39]. In addition, *ABO* locus was previously shown to be associated with various diseases such as myocardial infarction and pancreatic cancer [40,41]. Therefore, we excluded subjects with these diseases from case mix controls. We also excluded the subjects with continuous NSAID intake.

SNP Genotyping

Genotyping platforms used in this study are shown in Table 1. A total of 1,862 gastric ulcer cases and 20,791 gastric ulcer negative control samples were genotyped with Illumina Human Hap610-Quad or with Human Hap550v3. The other samples were genotyped by the Invader assay system (Third Wave Technologies, Madison, WI) or Taqman assay.

Table 3. Association of variations on candidate genes with Gastric ulcer.

SNP	Gene	relative loc	Chr	Position	Gastric ulcer	
					^a P	^b OR (95% C.I.)
rs3024505	<i>IL10</i>	1044	1	2.05E+08	0.817	### (0.71–1.31)
rs3024498	<i>IL10</i>	0	1	2.05E+08	0.569	### (0.55–3.01)
rs1554286	<i>IL10</i>	0	1	2.05E+08	0.911	### (0.93–1.08)
rs3021094	<i>IL10</i>	0	1	2.05E+08	0.178	### (0.98–1.12)
rs3024490	<i>IL10</i>	0	1	2.05E+08	0.902	### (0.93–1.07)
rs2222202	<i>IL10</i>	0	1	2.05E+08	0.975	### (0.74–1.33)
rs1800896	<i>IL10</i>	–1058	1	2.05E+08	0.766	### (0.87–1.20)
rs2844484	<i>LTA</i>	–3869	6	31644203	4.41×10^{-2}	### (1.00–1.15)
rs2009658	<i>LTA</i>	–1849	6	31646223	0.453	### (0.94–1.14)
rs2844482	<i>LTA</i>	–326	6	31647746	0.484	### (0.94–1.13)
rs1800683	<i>LTA</i>	–22	6	31648050	1.64×10^{-3}	### (0.83–0.96)
rs2229094	<i>LTA</i>	0	6	31648535	0.163	### (0.98–1.16)
rs2229092	<i>LTA</i>	0	6	31648736	0.295	### (0.90–1.42)
rs1041981	<i>LTA</i>	0	6	31648763	1.75×10^{-3}	### (0.83–0.96)
rs3093662	<i>TNF</i>	0	6	31652168	0.220	### (0.91–1.51)
rs3093668	<i>TNF</i>	383	6	31654474	0.335	### (0.87–1.51)
rs833068	<i>VEGFA</i>	0	6	43850505	0.427	### (0.96–1.10)
rs833069	<i>VEGFA</i>	0	6	43850557	0.401	### (0.96–1.10)
rs3025010	<i>VEGFA</i>	0	6	43855555	0.976	### (0.93–1.07)
rs3025033	<i>VEGFA</i>	0	6	43859053	0.282	### (0.96–1.14)
rs3025035	<i>VEGFA</i>	0	6	43859337	0.841	### (0.93–1.09)
rs6900017	<i>VEGFA</i>	4261	6	43866463	9.77×10^{-2}	### (0.99–1.15)
rs2069837	<i>IL6</i>	0	7	22734552	0.728	### (0.93–1.12)
rs2066992	<i>IL6</i>	0	7	22734774	0.953	### (0.93–1.09)
rs1554606	<i>IL6</i>	0	7	22735232	0.813	### (0.61–1.86)
rs10242595	<i>IL6</i>	2611	7	22740756	0.799	### (0.87–1.11)
rs1236913	<i>PTGS1</i>	0	9	1.24E+08	0.389	### (0.74–1.12)
rs1213266	<i>PTGS1</i>	0	9	1.24E+08	0.263	### (0.81–1.06)
rs4836885	<i>PTGS1</i>	0	9	1.24E+08	0.964	### (0.82–1.23)
rs6478565	<i>PTGS1</i>	0	9	1.24E+08	0.318	### (0.78–1.08)
rs10306163	<i>PTGS1</i>	0	9	1.24E+08	0.119	### (0.80–1.03)
rs10306202	<i>PTGS1</i>	1540	9	1.24E+08	3.76×10^{-2}	### (0.75–0.99)

We analyzed 1,862 gastric ulcer cases and 17,482 controls in this analysis. Chr., chromosome; Position in the NCBI Build 36.3.

^aP values were calculated by Cochran Armitage trend test.

^bOR, odds ratio was calculated by considering the major allele as the reference.

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Statistical Analysis

The association of SNPs rs2294008 and rs505922 with gastric ulcer was tested by chi-square test. The Odds ratios were calculated by considering the protective allele as the reference allele. The association of SNPs genotyped by Illumina Human Hap610-Quad with gastric ulcer was tested by multivariate logistic regression analysis upon adjusting for age at recruitment and gender using PLINK [42]. Heterogeneity across two stages was examined by Cochran Q test [43].

Supporting Information

Figure S1 Manhattan plot showing the genome-wide P values of association. The P values were obtained by logistic regression analysis upon adjustment for age and gender. The y-

axis represents the $-\log_{10}$ P values of 480,566 SNPs, and their chromosomal positions are shown on x-axis.

(TIF)

Table S1 Genotype frequency of two SNPs in disease mix controls.

(DOCX)

Table S2 The result of association analysis of Gastric ulcer in GWAS.

(DOCX)

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Author Contributions

Conceived and designed the experiments: CT K. Matsuda YN. Performed the experiments: CT K. Matsuo MK. Analyzed the data: CT AT NK HI. Contributed reagents/materials/analysis tools: HT YY KT KY. Wrote the paper: CT YN K. Matsuda.