Original Article

A single nucleotide polymorphism in *Prostate Stem Cell Antigen* is associated with endoscopic grading in Kyoto classification of gastritis

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The risk allele of a single nucleotide polymorphism (SNP) rs2294008 in the *Prostate stem cell antigen* (*PSCA*) gene is strongly associated with gastric cancer. Although the Kyoto classification score is believed to be an indicator of gastric cancer risk, it lacks supporting genetic evidence. We investigated the effect of this risk allele of *PSCA* SNP on the Kyoto score. Participants without a history of gastric cancer or *Helicobacter pylori* (*H. pylori*) eradication underwent esophagogastroduodenoscopy, *H. pylori* evaluation, and SNP genotyping. The Kyoto score is the sum of scores obtained from endoscopy-based atrophy, intestinal metaplasia, enlarged folds, nodularity, and diffuse redness. The Kyoto score is novel in the light of scoring for gastritis. A total of 323 patients were enrolled (number of individuals with genotype CC: 52; CT: 140; TT: 131, average age: 50.1 years, male: 50.8%). The patient baseline characteristics including age, sex, body mass index, smoking, drinking, family history of gastric cancer, and *H. pylori* status had no association with *PSCA* SNP. The Kyoto score was higher in T (CT or TT genotype; risk allele) carriers than in CC carriers. Atrophy, enlarged folds, and diffuse redness scores were higher in T allele carriers (risk allele) than in CC genotype individuals. In multivariate analysis, the Kyoto score was independently associated with *PSCA* SNP (OR: 1.30, *p* = 0.012). Thus, the Kyoto score was associated with a genetic predisposition.

Key Words: genetic polymorphism, *Helicobacter pylori* infection, gastric cancer, gastritis, endoscopy

G astric cancer is the third leading cause of cancer mortality worldwide. The main environmental factor resulting in gastric cancer is *Helicobacter pylori* (*H. pylori*) infection and the virulence of this bacterium.[1–5] Genetic predisposition to gastric cancer is associated with inherited cancer syndromes and cancer-associated single nucleotide polymorphisms (SNPs). Previous genome-wide association studies have identified gastric cancer susceptibility in *Prostate Stem Cell Antigen* (*PSCA*), *Mucin 1* (*MUC1*), *PLCE1*, 3q13.31, 5p13.1, 5q14.3, 6p21.1, *ATM*, 12q24.11-12, 20q11.21, and blood type A.[6–12] Among these genes, an SNP in *PSCA* has been found to be strongly associated with gastric cancer. The T allele of rs2294008 in *PSCA* is a risk

for gastric cancer, especially for diffuse type cancer [diffuse gastric cancer; odds ratio (OR) 4.2, *p*<0.001, intestinal gastric cancer; OR 1.6, *p* = 0.004].[6] We previously reported that the rs2294008 polymorphism was associated with the gastritis development in gastric cancer pathogenesis and was not involved in *H. pylori* infection *per se* or in the progression from severe gastritis to gastric cancer.[13] We have also previously shown that *PSCA* expression was decreased in severe gastritis compared with mild gastritis only among T allele carriers, who had CT or TT genotype. In contrast, the A allele of rs4072037 in *MUC1* was shown to be associated with diffuse gastric cancer (OR 1.6, *p*<0.001). The combined genotype of rs2294008 and rs4072037 could identify individuals with a high risk of gastric cancer development.[7] The association between these *PSCA* and *MUC1* SNPs and endoscopic findings is still unclear.

In endoscopic findings, the Kyoto classification of gastritis has been reported to correlate with *H. pylori* infection and the risk for gastric cancer.[14–18] Shichijo *et al.*[14] evaluated the usefulness of the Kyoto classification for risk stratification of gastric cancer. The severity of atrophy, intestinal metaplasia, diffuse redness, age, and male sex were all associated with increased risk of gastric cancer. Sugimoto *et al.*[15] also presented the relationship between total Kyoto classification score and gastric cancer risk. Although the Kyoto classification score is believed to be related to gastric cancer risk, it lacks genetic supporting evidence.

In this study, we investigated the effect of *PSCA* and *MUC1* genes on the Kyoto classification.

Methods

Study design and participants. This research project was approved by the institutional review board at the Institute of Medical Science, University of Tokyo on September 21, 2013 (approved no. 25-34-0921). All participants provided written informed consent.

This cohort study consisted of participants who underwent esophagogastroduodenoscopy (EGD) at Toyoshima Endoscopy

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doi: 10.3164/jcbn.20-67
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J. Clin. Biochem. Nutr. | January 2021 | vol. 68 | no. 1 | 73–77

Clinic from December 2013 to January 2016. EGDs were performed either for screening and evaluation of present symptoms, or for surveillance of previous esophagogastroduodenal disease. In this study, we enrolled patients who were suspected of having *H. pylori* infection based on EGD analysis. The inclusion criteria included patients aged 20 years or more without a history of gastric cancer, surgical gastrectomy, or *H. pylori* eradication. The exclusion criteria included a diagnosis of gastric cancer based on an EGD at the time of enrollment, an unidentified status of *H. pylori*, a severe concomitant illness, or withdrawal of agreement.

The following demographic characteristics were collected: age, sex, body mass index, smoking history, habitual drinking, first-degree family history of gastric cancer, use of non-steroidal anti-inflammatory drugs, and use of proton pump inhibitors.[19] A score of at least 400 on the Brinkman index was defined as positive smoking history. Consumption of at least one alcoholic drink per day was defined as habitual drinking.

***H. pylori* status.**   We divided participants into three categories according to the *H. pylori* status: positive, negative, and unidentified.[20,21] We defined positive bacterial culture, positive urea breath test, or positive hematoxylin-eosin (HE) staining as positive *H. pylori* status. We defined a combination of negative HE staining and negative culture or combination of negative HE staining and negative urea breath test as negative *H. pylori* status. When the patient was receiving proton pump inhibitors, the state of *H. pylori* infection was diagnosed based on the combination of negative HE staining and negative culture. We defined anything other than the above as unidentified *H. pylori* status. We cultured the gastric angulus mucosa collected by EGD. We pathologically estimated *H. pylori* infection by HE staining using the two points biopsy samples obtained from great curvature of corpus and antrum.

Kyoto classification of gastritis.   The Kyoto classification of gastritis, which is scored from 0 to 8, is based on the sum of scores of the following five endoscopic findings: atrophy, intestinal metaplasia (IM), enlarged folds, nodularity, and diffuse redness.[22] A high score represents increased risk for gastric cancer and *H. pylori* infection.[14–18] Gastric atrophy was classified according to the extent of mucosal atrophy, as described by Kimura and Takemoto. C-II and C-III of the Kimura-Takemoto classification were scored as 1, and O-I to O-III were scored as 2.[23–26] IM is observed as grayish-whitish and slightly opalescent patches. IM within the antrum was scored as 1, and IM extending into the corpus was scored as 2.[27] The presence of folds enlarged over 5 mm or more was scored as 1.[28] Nodularity is characterized by the appearance of multiple whitish elevated lesions mainly in the pyloric gland mucosa. The presence of nodularity was scored as 1. Diffuse redness refers to uniform redness involving the entire fundic gland mucosa. The presence of diffuse redness with regular arrangements of collecting venules (RAC) was scored as 1, and that without RAC was scored as 2.

EGDs were conducted by nine expert endoscopists, who met and discussed the EGD images prior to this study. Furthermore, the EGD images were retrospectively reviewed by the endoscopy unit director (OT). Discrepancies in diagnosis between endoscopists were resolved through discussions.

Genotyping the *PSCA* and *MUC1* SNPs.   DNA was isolated from peripheral blood leukocytes using QIAamp DNA mini kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. The samples were genotyped by the Invader assay system (Third Wave Technologies Madison, WI) using the purified DNA from peripheral blood. Aliquots of DNA from the patients were randomly assigned to a 96 well plate and subjected to SNP analysis.[13]

Statistical analysis.   The comparison of the values between the groups was performed using the chi-square test in categorical variables, the Mann-Whitney *U* test between the CC genotype and T carriers for *PSCA*, as well as the AA genotype and G carriers for *MUC1*, or the Kruskal-Wallis test among the three genotypes in continuous variables. We performed multivariate analysis to identify the factors that were independently associated with the *PSCA* SNP using a binominal logistic regression analysis. The odds ratios were calculated by considering the CC genotype as a reference. Significance was indicated by a *p* value less than 0.05. Calculations were carried out using statistical software Ekuseru-Toukei 2015 (Social Survey Research Information Co., Ltd., Tokyo, Japan).

Results

In total, 330 participants were enrolled. Four patients with gastric cancer, 2 with unidentified *H. pylori* status, and 1 who withdrew agreement were excluded. Finally, 323 participants were analyzed (number of genotype CC: 52; CT: 140; TT: 131; mean age: 50.1 years; male: 50.8%). Patient baseline characteristics, including age, sex, body mass index, smoking, drinking, family history of gastric cancer, and *H. pylori* status, had no association with rs2294008.

In univariate analysis, the Kyoto classification score of T carriers was higher than that of individuals with the CC genotype. In the Kyoto classification, the scores for atrophy, enlarged folds, and diffuse redness were higher in T carriers than in individuals with the CC genotype (Table 1). As shown in Fig. 1 according to the genotype, the T allele was related with a high Kyoto classification score (*p* = 0.006). In multivariate analysis, the Kyoto score was independently associated with rs2294008 (OR: 1.30, 95% confidence interval: 1.06–1.58, *p* = 0.012, Table 2).

There was no association between *MUC1* SNP and the Kyoto classification score (Supplemental Table 1* and Supplemental Fig. 1*).

Discussion

We found that the Kyoto classification score was independently associated with the *PSCA* SNP rs2294008T, but not with the *MUC1* SNP rs4072037A. Sugimoto *et al.*[15] presented the relationship between the total Kyoto classification score and gastric cancer risk. In their cross-sectional study, the total Kyoto classifications scores of patients with gastric cancer was significantly higher than those without gastric cancer (4.8 and 3.8, respectively). Our study showed the Kyoto score of *PSCA* T allele carrier was higher than that of CC genotype individual. This seems to indicate that endoscopic findings could reflect a genetic predisposition.

Several studies have demonstrated that endoscopic atrophy, which is a detail included in the Kyoto classification, is correlated with a risk for gastric cancer.[24,25,29,30] This study confirmed that the atrophy score in the new classification of the Kyoto score was correlated with *PSCA* SNP. Endoscopic atrophy is related to both diffuse type and intestinal type gastric cancer.[24,25] The *PSCA* SNP was also shown to be associated with pathological and serological (i.e., pepsinogen I/II ratio) atrophic gastritis, which partially supports our findings.[22,31–33]

This study also indicated that enlarged folds and diffuse redness are associated with the presence of the T allele and possibly contribute to the difference in the Kyoto classification score. First, enlarged folds are known to be associated with chronic active inflammation and their presence indicates a risk of diffuse gastric cancer.[34,35] Nishibayashi *et al.*[36] reported that the OR for gastric cancer increased with increasing fold width to a maximum of 35.5. Several studies have indicated that the molecular and biological features of *H. pylori*-related enlarged folds contribute to gastric cancer.[37–39] Notably, Tahara *et al.*[40] reported that individuals who were carriers of the T allele of rs2294008 had highly methylated gastric mucosa, which is a common characteristic of enlarged folds. Carriers of rs2294008T have been known to have

**Table 1.** Association between the *PSCA* SNP, patients' baseline characteristics and Kyoto classification

	Total	CC	CT + TT	*p* value
No.	323	52	271	
Age, mean (SD), years	50.1 (12.3)	49.6 (11.2)	50.2 (12.5)	0.719
Male sex, %	50.8	55.8	49.8	0.432
Body mass index, mean (SD), kg/m^2	22.3 (3.1)	22.2 (3.1)	22.4 (3.1)	0.689
Smoking, %	8.0	5.8	8.5	0.703
Drinking, %	25.4	28.8	24.7	0.532
Family history of gastric cancer, %	17.0	13.5	17.7	0.455
Proton pump inhibitor use, %	5.0	1.9	5.5	0.294
NSAID use, %	2.2	1.9	2.2	0.895
Positive *H. pylori* status, %	88.2	80.8	89.7	0.068
Kyoto classification score, mean (SD)	4.56 (1.88)	3.87 (2.18)	4.70 (1.79)	0.005
Atrophy, mean (SD)	1.35 (0.69)	1.02 (0.73)	1.41 (0.67)	<0.001
Intestinal metaplasia, mean (SD)	0.62 (0.88)	0.65 (0.90)	0.62 (0.88)	0.784
Enlarged folds, mean (SD)	0.47 (0.50)	0.35 (0.48)	0.50 (0.50)	0.045
Nodularity, mean (SD)	0.41 (0.49)	0.31 (0.47)	0.42 (0.50)	0.117
Diffuse redness, mean (SD)	1.72 (0.63)	1.54 (0.75)	1.75 (0.61)	0.016

P values between the CC genotype and T carriers were calculated with the chi-square test using categorical variables and with the Mann-Whitney *U* test using continuous variables. NSAID, non-steroidal anti-inflammatory drug.

a pathological pathway that results in the development of diffuse gastric cancer via enlarged folds. Second, diffuse redness was considered to represent increased blood flow in the mucosa and to be related to chronic inflammation with *H. pylori* infection.[41,42] Nomura *et al.*[43] indicated that diffuse redness and lack of RAC were related to pathological chronic inflammation and neutrophil activity, which were associated with diffuse gastric cancer. The interaction between *H. pylori*, chronic active inflammation, and the host genetic background may promote diffuse gastric cancer.[44]

The implication of genetic polymorphisms in *PSCA* on gastric cancer pathogenesis has already been reported. A long form of the cell surface PSCA protein encoded by the T allele of rs2294008 promotes cell proliferation.[45,46] and may increase the risk of *H. pylori*-related gastric cancer. In contrast, short cytosolic PSCA proteins associated with the C allele are rapidly degraded by the ubiquitin proteasomal pathway, which might lead to reduced risk of gastric cancer.[47] Genotypes related with loss of functional PSCA (C allele of rs2294008 and A allele of rs138377917) are associated with a reduced risk of gastric cancer.[11] Further, *PSCA* expression in the gastric mucosa correlates with the severity of gastritis and is regulated by *H. pylori* infection and *PSCA* SNP.[13]

Both *PSCA* and *MUC1* polymorphisms were reportedly to be linked with high risk of atrophic gastritis, defined as pan-active
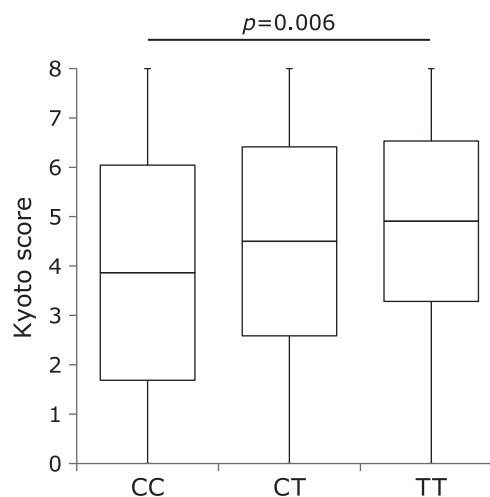
Fig. 1. Kyoto classification score according to the genotype of the *PSCA* SNP. Box-plots depicting the average Kyoto score. Kyoto scores of the CC genotype of the *PSCA* SNP (*n* = 52), CT genotype (*n* = 140), and TT genotype (*n* = 131). *P* value was calculated by the Kruskal-Wallis test.

Table 2. Multivariate analysis of the effect of the *PSCA* SNP on patients' baseline characteristics and Kyoto classification

	Odds ratio	95% confidence interval	*p* value
Age	0.99	0.96–1.02	0.530
Male sex	0.68	0.35–1.32	0.259
Body mass index	1.06	0.95–1.18	0.300
Smoking	2.35	0.62–8.93	0.209
Drinking	0.79	0.39–1.61	0.514
Family history of gastric cancer	1.25	0.51–3.02	0.625
NSAID use	1.16	0.11–11.9	0.900
Positive *H. pylori* status	1.07	0.38–3.00	0.902
Kyoto classification score	1.30	1.06–1.59	0.012

The odds ratios were calculated by considering the CC genotype as a reference. *P* values were calculated using a binominal logistic regression analysis. NSAID, non-steroidal anti-inflammatory drug.

gastritis, corpus-predominant active gastritis, or presence of intestinal metaplasia.[48] *PSCA* polymorphism was associated with *H. pylori*-related promoter DNA methylation in the gastric mucosa, while *MUC1* was not associated with the methylation status. Therefore, the expression of *PSCA* and *MUC1* may be different in endoscopic findings of gastritis.[40]

Our study has some limitations. First, the number patients included in the study was small and the data were from a single center. Second, endoscopic findings involved some bias due to interobserver variability,[49] and we did not assess any endoscopic findings except for those associated with the Kyoto classification (e.g., gastroesophageal reflux disease, Barrett's esophagus, and fundic gland polyp). Third, the accuracy of the updated Sydney system, was not verified. Further investigation of clinicopathological findings will be needed for multi-institutional studies with a large number of cases. Finally, a higher (but not significant) rate of positive *H. pylori* status in T carriers might lead to a higher Kyoto score. In future studies, the analysis of this score based on *H. pylori* status is warranted.

In conclusion, the Kyoto classification of gastritis was associated with the *PSCA* SNP. This study offers genetic evidence supporting the Kyoto classification.

Author Contributions

OT designed the study, recruited patients, analyzed data, and wrote the manuscript. TN and KM designed the study. KS, TM, RK, HS, and CT critically revised the manuscript. HW performed histological diagnoses. KK supervised the study.

Acknowledgments

Abbreviations

EGD	esophagogastroduodenoscopy
HE	hematoxylin-eosin
IM	intestinal metaplasia
MUC1	Mucin 1
OR	odds ratio
PSCA	Prostate Stem Cell Antigen
RAC	regular arrangements of collecting venules
SNP	single nucleotide polymorphism

Funding

Ethics Approval

This study was approved by the institutional review board at the Institute of Medical Science, University of Tokyo on September 21, 2013 (approval no. 25-34-0921).

Informed Consent Statement

Written informed consents were obtained from the participants.

Conflict of Interest

No potential conflicts of interest were disclosed.

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