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Original article

PRKCH gene polymorphism is associated with the risk of severe gastric atrophy

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Abstract

Background. **Individuals infected with *Helicobacter pylori* do not necessarily develop gastric atrophy (GA) and gastric cancer (GC). Several factors, including genetic polymorphism, can regulate the development of GA and GC. A *G/A* single nucleotide polymorphism (rs3783799) of the *PRKCH* gene, which encodes the η isozyme of protein kinase C (PKCη), has been reported to be a tag single nucleotide polymorphism (SNP) of the *PRKCH* gene linked to a functional 1425*G/A* SNP in exon 9 (rs2230500). To elucidate its applicability in the development of GA and GC, this study aimed to investigate the associations of the *PRKCH* polymorphism with the risks of GA and GC.**

Methods. **The subjects consisted of 583 patients (cases) from first-visit outpatients at Aichi Cancer Center Hospital, aged 27 to 80 years, who were diagnosed as having GC from 2001 to 2005, and 1742 controls, frequency-matched for age and sex. Anti-*H. pylori* IgG antibodies and pepsinogens (PGs) in serum were measured for 1638 controls.**

Results. **Of the 1638 controls, 57.3% were seropositive and 33.0% had GA (PG1 ≤ 70 ng/dl and PG1/PG2 ≤ 3). When compared to the seronegative controls without GA, the *AA* genotype was significantly associated with severe GA (PG1 ≤ 30 ng/dl and PG1/PG2 ≤ 2); odds ratio (OR), 2.37 (95% confidence interval, 1.11–5.05) relative to the *GG* genotype. The genotype was not associated with the risk of GC.**

Conclusion. **This was the first study to examine the associations of the *PRKCH* polymorphism with GA and GC, and suggested that the *AA* genotype, relative to the *G/G* genotype, may be a higher risk genotype for severe GA.**

Key words *PRKCH* · Polymorphism · *Helicobacter pylori* · Severe gastric atrophy

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Introduction

Helicobacter pylori is an established risk factor for both intestinal-type and diffuse-type gastric cancer (GC), especially for the former [1, 2]. The development of intestinal GC begins with gastric atrophy (GA) induced by *H. pylori*. According to the cascade of events described by Correa et al. [3], *H. pylori* infection causes chronic inflammation, which then progresses through the GA-metaplasia-dysplasia-carcinoma sequence [3, 4]. However, this progression is affected by many factors, including salty food consumption, low fruit intake, the immune reaction of the host to the infection [4, 5], and genetic traits measurable by genotypes. To date, we have already reported several polymorphisms possibly associated with *H. pylori* seropositivity, GA, and GC [6–9].

Protein kinase C (PKCη) is involved in oxidative stress, resulting in the induction of inducible nitric oxide synthase (iNOS) and nitric oxide (NO) production. Indeed, blood samples from patients with severe rheumatoid arthritis (RA) showed a positive relationship between the activity of the PKCη and iNOS enzymes [10]. This finding also suggests that PKCη could mediate iNOS after *H. pylori* infection, because iNOS expression in the gastric mucosa is higher in *H. pylori*-positive individuals than in *H. pylori* negative individuals [11, 12]. iNOS can produce large amounts of NO, which reacts with superoxide produced by *H. pylori* infection [13] to form peroxynitrite, which has strong oxidizing properties, leading to the development of GA. In addition, PKCη can mediate cell proliferation through the ERK/Elk-1 [14] and Akt pathways [15].

The *PRKCH* gene, which is located on chromosome 14q22–23, encodes PKCη. The association of multiple *PRKCH* gene polymorphisms with RA [16] and with cerebral infarction [17, 18] has been reported. However, there are no studies regarding the association between *PRKCH* gene polymorphisms and GA and GC.

It was reported that two single nucleotide polymorphisms (SNPs; rs3783799 and rs2203500) in the *PRKCH* gene, which were in complete linkage disequilibrium (LD), were associated with cerebral infarction; the former was a tag SNP of the *PRKCH* gene and the latter was confirmed to be functional by a series of biological assays in vitro [17, 18]. To elucidate its applicability in the development of GA and GC, we investigated the association between the *PRKCH* gene polymorphism (rs3783799) and the *H. pylori*-induced inflammation of gastric mucosa leading to GA and GC.

Subjects and methods

Study subjects

The study subjects were derived from the Hospital-based Epidemiological Research Program at Aichi Cancer Center (HERPACC) that was launched in 1988. Details of the study design and data collection procedures were described in previous reports [19–21]. Briefly, all first-visit outpatients at Aichi Cancer Center Hospital (ACCH) were asked to complete a self-administered questionnaire on their lifestyle, past history, and family history, including items on demographic characteristics, medical history, smoking and drinking habits, regular physical exercise, and dietary habits, before the development of current symptoms, as well as menstrual and reproductive history for women. The questionnaire was checked for unanswered items and contradictory responses, by trained interviewers. In the present study, there were 583 patients, aged 27 to 80 years, who were diagnosed with GC from 2001 to 2005 (cases), and 1742 cancer-free participants (controls) who were frequency-matched to the cases in terms of age and sex. Informed consent was obtained from all the subjects and the study protocol was approved by the Ethics Committees of Aichi Cancer Center and Nagoya University Graduate School of Medicine.

Tests for H. pylori *antibody and pepsinogens*

Anti-*H. pylori* IgG antibody in serum was measured with an enzyme immunoassay (EIA) kit (E plate "Eiken" *H. pylori* Antibody; Eiken, Tokyo, Japan). A value of 10.0 U/ml or greater was regarded as being *H. pylori* infection-positive. Concentrations of serum pepsinogens 1 and 2 (PG1 and PG2) were measured by radioimmunoassay, using a commercially available kit (Dinabot, Tokyo, Japan). PG1values of 70 ng/ml or less and a PG1/PG2 ratio of 3 or less were regarded as indicating the presence of GA. Mild GA was defined as values excluding severe GA (PG1 ≤ 30 ng/ml and PG1/PG2 ≤ 2).

Genotyping

DNA was extracted from whole blood using a Qiagen mini kit (Qiagen Group, Tokyo, Japan). A tag SNP, SNP_15 in *PRKCH* (rs3783799, IMS_JST140193), was genotyped by a TaqMan assay using an ABI PRISM 7300 sequence detection system (Applied Biosystems, Foster City, CA, USA), in accordance with the manufacturer's instructions. Thermal cycling conditions for polymerase chain reaction (PCR) were, first, denaturing at 95°C for 10 min, followed by 50 cycles of 92°C for 15 s and 58°C for 1 min.

Statistical analysis

Odds ratios (ORs) adjusted for sex and age with 95% confidence intervals (CIs) were calculated using unconditional logistic regression analysis. Hardy-Weinberg equilibrium was tested for the *PRKCH* polymorphism in controls by a χ^2 test with 1 df. These calculations were performed with the software program STATA Version 9 (STATA, College Station, TX, USA).

Results

The characteristics of the study subjects are summarized in Table 1. Male subjects accounted for the majority in both the controls and cases, at 73.8% and 73.6%, respectively. *H. pylori* antibody and PGs were not measured in the cases or in 104 controls whose serum samples were not sufficient for the measurements. The frequencies of *H. pylori* seropositivity and GA among the remaining 1638 controls were 939 (57.3%) and 541 (33.0%), respectively. DNA was not successfully extracted in 4 controls and 4 cases and therefore these patients could not be genotyped. The genotype frequency in the 1738 controls was in Hardy-Weinberg equilibrium ($\chi^2 = 0.74$; $P = 0.39$).

There were 45 seronegatives with GA, who were regarded as having been infected with *H. pylori* in the past; 17 were classified as having mild GA, and 28 as having severe GA. According to the Correa cascade [3], the controls were divided into four groups: the seronegative controls without GA, and the seropositive ones without GA, with mild GA, and with severe GA. Compared to the seronegative controls without GA, the OR of the *AA* genotype relative to the *GG* genotype was 2.37 (95% CI, 1.11–5.05) for severe GA (Table 2). However, the OR was not significant in the comparisons between the seropositives without GA and those with GA (both mild and severe), and between mild GA and severe GA (data not shown).

The comparisons between 1738 controls, including subjects without the results of laboratory tests, and 579

Table 1. Characteristics of the study subjects

Characteristics	Controls		Cases	
	Number	%	Number	%
Sex				
Male	1286	73.8	429	73.6
Female	456	26.2	154	26.4
Age in years (mean ± SD)	58.5 ± 10.6		58.8 ± 10.5	
Anti-*Helicobacter pylori* IgG antibody				
Positive	939	57.3	—	—
Negative	699	42.7	—	—
No serum	104	—	—	—
Gastric atrophy measured with pepsinogens				
Positive	541	33.0	—	—
Negative	1097	67.0	—	—
No serum	104	—	—	—
PRKCH				
G/G	1068	61.5	343	59.2
G/A	581	33.4	213	36.8
A/A	89	5.1	23	4.0
Not genotyped	4	—	4	—

Table 2. Sex- and age-adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) of *PRKCH* genotypes for sensitivity to *H. pylori* infection and gastric atrophy (GA) by the degree of severity, compared with seronegative non-GA controls[a]

	Seronegative non-GA[a] (*n* = 651)	Seropositve non-GA[b] (*n* = 443)			Mild GA[c] (*n* = 344)			Severe GA[d] (*n* = 196)		
Genotype	*n* (%)	*n* (%)	OR	95% CI	*n* (%)	OR	95% CI	*n* (%)	OR	95% CI
G/G	401 (61.6)	267 (60.3)	1	Reference	217 (63.1)	1	Reference	114 (58.2)	1	Reference
G/A	221 (34.0)	156 (35.2)	1.03	0.79–1.34	105 (30.5)	0.90	0.67–1.20	68 (34.7)	0.99	0.68–1.43
A/A	29 (4.4)	20 (4.5)	1.16	0.63–2.14	22 (6.4)	1.53	0.85–2.77	14 (7.1)	2.37	1.11–5.05
G/A+A/A	250 (38.4)	176 (39.7)	1.04	0.81–1.34	127 (36.9)	0.97	0.73–1.28	82 (41.8)	1.11	0.78–1.58

PG, pepsinogen
[a] Seronegative controls without GA (PG1 ≤70 ng/dl and PG 1/2 ≤3)
[b] Seropositive controls without GA
[c] Controls with GA except those with PG1 ≤30 ng/dl and PG1/2 ≤2
[d] Controls with PG1 ≤30 ng/dl and PG1/2 ≤2

Table 3. Sex- and age-adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for gastric cancer of *PRKCH* genotypes

Polymorphism	Controls	Cases	OR	95% CI
G/G	1068	343	1	Reference
G/A	581	213	1.14	0.94–1.39
A/A	89	23	0.81	0.50–1.29
G/A+A/A	670	236	1.10	0.90–1.33

cases showed that the *PRKCH* polymorphism was not associated with the risk for GC (Table 3).

Discussion

The present study found that the *PRKCH* minor homozygous genotype was significantly associated with severe GA (OR, 2.37) in the comparison between the seronegative controls without GA and the controls with severe GA. According to the cascade described by Correa et al. [3], three steps are included in the significant association between *H. pylori* infection and GA: (i) persistent *H. pylori* infection, (ii) GA development, and (iii) progression to severe GA. Each step was examined in the present study, but a significant OR was not detected for any steps, partly due to the limited statistical power of the study. Therefore, it is possible that the genotype could affect all steps to severe GA to a certain extent. Otherwise, considering that the OR for severe GA was significant and that the gradually elevated ORs for *H. pylori* seropositivity without GA and mild GA were assumed to be random phenomena, we could not deny the possibility that the genotype may have influenced the last step of the progression to severe GA. Actually, this progression could be possibly explained from a

biological point of view. The relationship between PKCη, encoded by *PRKCH*, and iNOS has been reported [10]. Furthermore, iNOS has been shown to be expressed in the gastric mucosa of patients with GA [11, 12], and it can produce large amounts of NO [22]. Reactive nitrogen oxide species formed from the reaction of NO either with oxygen or superoxide can cause tissue damage, leading to the development of GA [13, 23, 24]. Accordingly, one plausible biological mechanism operating in the present study was that subjects with the *AA* genotype had higher PKCη activity and produced large amounts of NO through iNOS, which could be enhanced through *H. pylori* infection [11], resulting in facilitating the progression from mild GA to severe GA. In fact, a 1425*G/A* SNP (leading to V371I) within an ATP-binding site of PKCη in the *PRKCH* (rs2230500) gene was confirmed to be functional by a series of biological assays in vitro [17]. PKCη-374I had 1.6 times higher activity than PKCη-374V. This SNP was in complete LD with the rs3783799 we adopted in the present study [17, 18].

Multiple SNPs have been located in the *PRKCH* gene. Among them, rs3783799 and rs2230500 have been reported to be associated with cerebral infarction [17, 18]. Other SNPs in the *PRKCH* gene, found in intron 2, 5, or 9, were reported to be associated with RA [16]. We used rs3783799, not rs2230500, in the present study, because this genetic polymorphism is a tag SNP of the *PRKCH* gene and was linked to a functional 1425*G/A* in exon 9 (rs2230500) [17, 18]. We could not genotype rs2230500 with TaqMan. Serizawa et al. [18] reported that they genotyped rs2230500 using the Mass ARRAY system, instead of TaqMan. Moreover, considering that the rs3783799 SNP is completely linked to rs2230500 [17, 18], we thought it redundant to evaluate the associations of rs2230500 with GA and GC. Regarding rs3783799, there was no remarkable difference in the minor allele frequency between the controls in the present study and the HapMap database for Japanese; the frequency in the former was 0.218 and in the latter was 0.239. The minor allelic frequencies in other populations were reported in the HapMap database as 0.178 in Han Chinese in Beijing, 0.008 in CEPH samples (Utah residents with ancestry from northern and western Europe), and 0.000 in Yoruba from Ibadan, Nigeria. These data suggest that the rs3783799 SNP is likely to be limited to Asian populations.

We could not find any association of the *PRKCH* gene polymorphism with the development of GC (Table 3). This result might reflect the fact that the effect of PKCη on proliferation depends on the cell type. PKCη can enhance the proliferation of glioblastoma cells [15] and epithelial breast adenocarcinoma [25]. However, other cell types, such as keratinocytes, inhibit the effect of PKCη on cell-cycle progression [26]. PKCη probably has no effect on the proliferation of GC cells.

In the present study, there were 45 seronegative controls with GA, of whom 17 had mild GA. It is well known that *H. pylori* is spontaneously eliminated through intestinal metaplasia replacement, resulting in the loss of *H. pylori* serological markers [27, 28]. Hence, we supposed that the above 17 controls had lost their seropositivity not due to the severity of GA but probably due to *H. pylori* eradication therapy, because the spontaneous regression or clearance of *H. pylori* is rare under normal circumstances [29–31], and extensive mucosal atrophy and intestinal metaplasia were found to be irreversible even after *H. pylori* eradication [32]. Therefore, we regarded the above 17 seronegative controls as having mild GA, not severe GA, for the purpose of assessing the effect of this genetic polymorphism on the development of mild GA.

There was a limitation in this study. For the cases, we did not have information on the status of *H. pylori* infection, including the titer of *H. pylori* antibody and levels of PGs. Because resources were limited for carrying out serological studies, the status of *H. pylori* infection and levels of PGs were not examined for the GC cases. We have already planned other studies with a higher priority than this study.

In conclusion, this was the first study to show that a *PRKCH* gene polymorphism could affect the development of severe GA, and it may provide a clue to finding a new mechanism related to the development of GA and GC. Replication studies in other independent populations are required to confirm these results.

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