Influences of Chymase and Angiotensin I-Converting Enzyme Gene Polymorphisms on Gastric Cancer Risks in Japan

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Abstract

Backgrounds and Aims: **The renin-angiotensin system plays an important role in homeostasis. Angiotensin II, which is generated by chymase and angiotensin I-converting enzyme (ACE), controls blood pressure as well as angiogenesis and cell proliferation. The aim of this study was to clarify the association of the chymase gene (*CMA/B*) and *ACE* polymorphisms with susceptibility to gastric cancer and peptic ulcer.**

Methods: **We assessed *CMA/B* A/G and *ACE* insertion/deletion (I/D) polymorphisms in *H. pylori*-positive gastric cancers (n = 119), gastric ulcers (n = 127), and duodenal ulcers (n = 105), and controls (n = 294) consisting of *H. pylori*-positive gastritis alone (n = 162) and *H. pylori*-negative subjects (n = 132) by PCR methods.**

Results: **In *CMA/B* polymorphism, the age- and sex-adjusted odds ratios (OR) of A/A and A/G genotypes relative to the G/G genotype for gastric cancer risk were 7.115 (95% confidence interval, 1.818-27.845) and 1.956 (95% confidence interval, 1.137-3.366), respectively. There was an increased risk for gastric ulcer in the A/A genotype (OR, 3.450; 1.086-10.960). However, there was no association between *ACE* polymorphism and susceptibility to gastric cancer and peptic ulcer. In allele combination analysis of *CMA/B* and *ACE* polymorphisms, the A/I allele combinations (*CMA/B* G/A or A/A and *ACE* I/I genotype) significantly increased the risk of gastric cancer development (OR, 4.749, 2.050-11.001) compared with the G/I allele combinations (*CMA/B* G/G and *ACE* I/I genotype).**

Conclusions: **The *CMA/B* polymorphism was associated with an increased risk for gastric cancer and gastric ulcer development. The genotyping test of the renin-angiotensin system could be useful for the screening of individuals with higher risks of gastric cancer and gastric ulcer.** (Cancer Epidemiol Biomarkers Prev 2006;15(10):1929–34)

Introduction

Gastric cancer remains the world's second most common malignancy (1). In 1994, the WHO/IARC designated *Helicobacter pylori* as a definite biological group 1 carcinogen of gastric cancer. For the prevention of gastric cancer and peptic ulcer diseases, eradication of *H. pylori* is recommended as the first-line therapy for patients with *H. pylori* infection (2, 3). However, so many people are infected with *H. pylori*, that it is difficult to let all *H. pylori*-infected individuals undergo the eradication therapy, and therefore, a useful tool for the selection of subjects at higher indication of eradication of *H. pylori* is desirable.

The pathogenesis and progression of gastric cancer development consists of a variety of processes, which include cell proliferation, cell differentiation, angiogenesis, and degradation of the extracellular matrix. Recently, the association of the host genetics with such processes (e.g., inflammation-related cytokine polymorphisms, cytochrome P450 enzyme polymorphisms, glutathione *S*-transferase, *N*-acetyltransferase, matrix metalloproteinase, p53, and k-ras mutation) has been intensively investigated in relation to chronic *H. pylori* infection (4-9).

The renin-angiotensin (RA) system consisting of renin, angiotensinogen, angiotensin I, angiotensin II, angiotensin I-converting enzyme (ACE), and chymase plays a key role in blood pressure regulation. A local RA system is also observed in various organs and angiotensin II is locally produced in each organ. Recently, there has been increasing evidence that angiotensin II is involved in the regulation of cell proliferation, angiogenesis, inflammation, and tissue remodeling via the angiotensin II type 1 receptors (AT1R; refs. 10-13). Therefore, the angiotensin II/AT1R pathway might be related to cancer biology.

ACE inhibitors inhibit the ACE-mediated conversion of angiotensin II from angiotensin I. A recent epidemiologic study has shown that ACE inhibitors and AT1R antagonists have inhibitory effects on tumor progression, vascularization, and metastasis, and that the stimulation of angiotensin II type 2 receptors inhibits the development of cancer (14). Therefore, the RA system has recently been focused on as the candidate target of chemopreventive therapy.

ACE in the chromosome 17q23 has six polymorphisms [e.g., *ACE*-240 A/T and the presence (insertion; I allele)/absence (deletion; D allele) of 287 bp DNA fragment in intron 16; refs. (15, 16)]. Plasma ACE levels are highest in subjects with the *ACE* D/D genotype, those with the I/D genotype come next and those with the I/I genotype are lowest of the three genotype groups (15, 16). The *ACE*-240 A allele is also associated with lower plasma ACE levels compared with *ACE*-240 T allele (17). The plasma ACE level is a critical factor in the determination of the plasma angiotensin II level, and therefore, the *ACE* I/D polymorphism has been shown to influence the risk of hypertension and other cardiac diseases (18). Recently, it has been reported that women with the low-activity *ACE* genotype are at a lower risk of the development

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of breast cancer compared to those with the high producer allele/genotype (19, 20). However, the relationships between the *ACE* I/D polymorphism and the risk of gastric cancer have not fully been elucidated (21-23).

Chymase, which is a chymotrypsin-like serine protease produced in the secretary granules of mast cells, also mainly mediates the local, not systemic, generation of angiotensin II (24). There are two polymorphisms in the chymase gene (*CMA*), *CMA/A* and *CMA/B*, localized in the chromosome 14 (25). Those two polymorphisms have been shown to correlate with activity/expression of chymase, and therefore, the *CMA* polymorphism is a potential candidate for the susceptibility to hypertension, cardiovascular diseases and neoplastic diseases (25, 26).

Although the polymorphic effects of *ACE* on the development of gastric cancer have been reported (21-23), the relationships among *ACE* and *CMA/B* polymorphisms, histologic types of gastric cancer, and clinical stage are unclear. Moreover, there is no report about the association with the RA system–related gene polymorphism and peptic ulcer development. To further determine the possible role of the RA system in the development of gastric cancer and peptic ulcer in humans, we examined whether genetic polymorphisms in *CMA/B* A/G and *ACE* I/D were associated with gastric cancer and peptic ulcer risks in Japanese patients with *H. pylori* infection.

Materials and Methods

Subjects. A total of 645 Japanese patients who agreed to participate in the present study underwent gastroduodenoscopy at the University Hospital of Hamamatsu University School of Medicine from January 2001 to December 2005. Of 645 subjects, 513 patients with *H. pylori* infection on the basis of serologic testing (HM-CAP kit, Enteric Product Inc., Stony Brook, NY), rapid urease test (Helico Check, Otsuka Co., Tokushima, Japan), and/or culture, and 132 subjects without *H. pylori* infection using the above three tests, were enrolled in this study. The 513 *H. pylori*-positive subjects consisted of the gastric cancer (n = 119), gastric ulcer (n = 127), duodenal ulcer (n = 105), and gastritis alone groups (n = 162; Table 1). Each diagnosis was proven histopathologically and endoscopically. The gastric cancer group was further pathologically classified into the two subgroups, the intestinal type group and the diffuse type group, according to the Lauren classification (Table 1; ref. 27).

The protocol was approved in advance by the Human Institutional Review Board of Hamamatsu University School of Medicine. Written informed consent was obtained from each subject.

Genotyping of *CMA/B* and *ACE*. DNA was extracted from the leukocytes of each subject, using a commercially available kit (IsoQuick, ORCA Research, Inc., Bothell, WA). The *CMA/B* A/G polymorphism was determined as described by Pfeufer et al. (25). Amplification primers for the 285-bp fragment were 5′-GGA AAT GTG AGC AGA TAG TGC AGT C-3′ and 5′-AAT CCG GAG CTG GAG AAC TCT TGT C-3′. Denaturation was done for 10 minutes at 94°C, 40 cycles of 94°C for 1 minute, 70°C for 1 minute, 72°C for 1 minute, and finally at 72°C for 7 minutes. The PCR products were digested with *Bst*XI (Takara Bio, Inc., Shiga, Japan) at 45°C for 1 hour. The genotypes were designated as follows: A/A, a single 285-bp band; A/G, three bands of 90, 195, and 285 bp; and G/G, two bands of 90 and 195 bp.

The *ACE* I/D polymorphism was identified on the basis of PCR amplification of the respective fragments from intron 16 of *ACE*, as previously reported (28). Primer sequences to determine *ACE* I/D polymorphism by PCR method are 5′-GCC CTG CAG GTG TCT GCA GCA TGT-3′ and 5′-GGA TGG CTC TCC CCG CCT TGT CTC-3′. The PCR conditions were run at 94°C for 10 minutes, then 35 cycles of 94°C for 1 minute, 70°C for 1 minute, 72°C for 1 minute, and finally at 72°C for 7 minutes. The genotypes were designated as follows: I/I, a single band of 597 bp; D/I, two bands of 319 and 597 bp; and D/D, a single band of 319 bp (28). Because the D allele in heterozygous subjects is preferentially amplified, there is a tendency for misclassification of the *ACE* I/D genotype as the D/D genotype (4-5%; ref. 28). In order to avoid this misclassification, a second independent PCR was done with a primer pair that recognizes insertion-specific sequences (5′-TGG GAC CAC AGC GCC CGC CAC TAC-3′ and 5′-TCG CCA GCC CTC CCA TGC CCA TAA-3′), with identical PCR conditions. The reaction yields a 335 bp amplicon only in the presence of an I allele, and no product in *ACE* D/D genotype.

Assay of Serum Pepsinogen Levels. Gastric atrophy and inflammation are an important abnormality associated with the development of gastric ulcer and gastric cancer. Although histologic examination of the gastric mucosa is the most accurate method of assessing gastric atrophy and inflammation, it is possible to use a functional surrogate marker for this purpose. Severe corpus inflammation and atrophy are associated with a reduction in the pepsinogen (PG) I level and the PG I/PG II ratio, and both have been used as surrogate markers of gastric atrophy and inflammation (29, 30). Then, we measured serum levels of PG I and PG II levels by RIA (Abotto Japan, Tokyo, Japan), and the PG I/PG II ratio was calculated for the serologic assessment of gastric atrophy in >50-year-old patients with *H. pylori* infection.

Table 1. Demographic characteristics and frequencies of *CMA/B* A/G and *CEA* I/D polymorphisms

	H. pylori-negative (n = 132)	Gastritis alone (n = 160)	Gastric ulcer (n = 127)	Duodenal ulcer (n = 105)	Gastric cancer (n = 119)	P
Age, y (mean ± SD)	53.8 ± 1.0	51.4 ± 0.9	52.3 ± 1.1	50.0 ± 1.2	68.6 ± 9.7	<0.001
Sex (male/female, *n/n*)	83/49	109/51	105/21	87/18	94/25	<0.001
Histology						
Intestinal type					87	
Diffuse type					32	
Clinical stage						
Stage I-II					80	
Stage III-IV					29	
CMA/B polymorphism						
G/G genotype	102 (77.3%)	114 (71.3%)	83 (65.4%	75 (71.4%)	67 (56.3%)	0.003
G/A genotype	27 (20.5%)	44 (27.5%)	36 (28.3%)	26 (24.8%)	45 (37.8%)	
A/A genotype	3 (2.2%)	2 (1.2%)	8 (6.3%)	4 (3.8%)	7 (5.9%)	
ACE polymorphism						
I/I genotype	50 (37.9%)	51 (31.9%)	50 (39.4%)	41 (39.0%)	54 (45.4%)	0.129
I/D genotype	60 (45.5%)	83 (51.9%)	63 (49.6%)	54 (51.4%)	53 (44.5%)	
D/D genotype	22 (16.6%)	26 (16.2%)	14 (11.0%)	10 (9.6%)	12 (10.1%)	

Data Analysis. Hardy-Weinberg equilibrium of allele frequencies at individual loci was assessed by comparing the observed and expected genotype frequencies using the χ^2 test. Differences in the *CMA/B* A/G and *ACE* I/D genotype/allele frequencies between the control and *H. pylori* infection–related disease groups were determined by the χ^2 test. Differences in serum levels of PG I and PG I/PG II ratios between different genotype groups were assessed by Student's *t* test. The effects of genotypes/alleles of *CMA* and *ACE* polymorphisms on the risk of gastric cancer development were expressed as odds ratios (OR) with 95% confidence intervals (CI) adjusted by age and sex. All *P* values were two-sided, and $P < 0.05$ were considered statistically significant.

Results

Characteristics of Enrolled Subjects. The mean age of subjects with gastric cancer was significantly higher than those of any other group ($P < 0.001$; Table 1). Then, the ORs for the development of gastric cancers, gastric ulcers, and duodenal ulcers were adjusted by sex, as well as age, as noted above.

***CMA/B* A/G and *ACE* I/D Polymorphisms and the Development of Gastric Cancers.** The genotype frequencies of the *CMA/B* A/G and *ACE* I/D polymorphisms in the *H. pylori*-negative control group did not deviate significantly from those expected under the Hardy-Weinberg equilibrium (Table 1).

In the *H. pylori*-negative subjects and in the *H. pylori*-positive gastritis alone group, the number of *CMA/B* A/A, A/G, and G/G genotypes and *ACE* I/I, I/D, and D/D genotypes were 3/27/102 and 50/60/27 in subjects without *H. pylori* infection and 2/44/116 and 51/83/26 in patients with gastritis alone, respectively, and no significant differences in the genotype frequencies of *CMA/B* and *ACE* polymorphisms among the two subgroups. Therefore, we combined patients from the *H. pylori*-negative group and *H. pylori*-positive gastritis alone group and used them as the control group for the gastric cancer and peptic ulcer cases in the present study.

The frequencies of the *CMA/B* A/A, A/G, and G/G genotype were 1.7%, 24.3%, and 74.0% in the control group, whereas those in the gastric cancer group were 5.9%, 37.8%, and 56.3%, respectively (Table 1). The adjusted ORs for gastric cancer risk in patients with A/A or A/G genotype of the *CMA/B* significantly increased (A/A genotype, adjusted OR, 7.115; 95% CI, 1.818-27.845; A/G genotype, adjusted OR, 1.956; 95% CI, 1.137-3.366, respectively) in comparison with those with the G/G genotype (Table 2). The adjusted OR of the carriage of the A allele was 2.219 (95% CI, 1.315-3.744), which was higher than non–A allele carriers (Table 2). When the gastric cancer group was classified into the intestinal type and diffuse type, the adjusted ORs of *CMA/B* G/A and A/A genotypes for intestinal type of gastric cancer were 1.899 (95% CI, 1.029-3.503) and 8.334 (95% CI, 1.832-37.906), respectively. The adjusted ORs of A allele carriers for intestinal type of gastric cancer were 2.181 (95% CI, 1.209-3.932) and 2.250 (95% CI, 1.025-4.943; Table 3).

The frequencies of the I/I, I/D, and D/D genotypes of the *ACE* were 34.6%, 49.0% and 16.4% in the control group, whereas those in the gastric cancer group were 45.4%, 44.5% and 10.1%, respectively (Table 1). There was no statistically significant difference in the frequencies of *ACE* genotypes between the gastric cancer group and the control group (Table 2). There were also no significant differences in the adjusted ORs of *ACE* genotypes with respect to the two different pathologic classifications and clinical stage of the gastric cancer group (Table 3).

***CMA/B* A/G and *ACE* I/D Polymorphisms and the Development of Peptic Ulcers.** The frequencies of the *CMA/B* A/A, A/G, and G/G genotype in the gastric ulcer and duodenal ulcer group were 6.3%, 28.3%, and 65.4%, and 3.8%, 24.8%, and 71.4%, respectively (Table 1). The adjusted ORs for gastric ulcer development in patients with the *CMA/B* A/A genotype significantly increased (adjusted OR, 3.450; 95% CI, 1.086-10.960) in comparison with those with the G/G genotype (Table 2). There was no association between duodenal ulcer development and *CMA/B* polymorphism. There was no statistically significant difference in the frequencies of *ACE* genotypes between the peptic ulcer group and the control group (Table 2).

Table 2. Influences of *CMA/B* and *ACE* genotypes on gastric cancer, gastric ulcer, and duodenal ulcer risks

	Gene		Adjusted OR (95% CI)	*P*
Gastric cancer	*CMA/B*	G/G	1.000 (ref)	
		G/A	1.956 (1.137-3.366)	0.015
		A/A	7.115 (1.818-27.845)	0.005
		A allele carriage	2.219 (1.315-3.744)	0.003
	ACE	I/I	1.000 (ref)	
		I/D	0.655 (0.384-1.117)	0.120
		D/D	0.439 (0.191-1.008)	0.052
		D allele carriage	0.625 (0.376-1.039)	0.070
Gastric ulcer	*CMA/B*	G/G	1.000 (ref)	
		G/A	1.283 (0.794-2.074)	0.309
		A/A	3.450 (1.086-10.960)	0.036
		A allele carriage	1.436 (0.911-2.266)	0.119
	ACE	I/I	1.000 (ref)	
		I/D	0.790 (0.497-1.255)	0.318
		D/D	0.592 (0.295-1.187)	0.140
		D allele carriage	0.739 (0.475-1.150)	0.180
Duodenal ulcer	*CMA/B*	G/G	1.000 (ref)	
		G/A	1.020 (0.601-1.730)	0.942
		A/A	1.784 (0.461-6.907)	0.402
		A allele carriage	1.070 (0.645-1.774)	0.794
	ACE	I/I	1.000 (ref)	
		I/D	0.795 (0.485-1.302)	0.362
		D/D	0.492 (0.255-1.079)	0.071
		D allele carriage	0.720 (0.449-1.155)	0.173

NOTE: ORs were adjusted by age and sex.

Combination of Allele Carriage of *CMA/B* and *ACE* Polymorphisms. The combination of allele carriage of *CMA/B* and *ACE* polymorphisms was classified into the four subgroups: G/I (combination of *CMA/B* G/G and *CMA* I/I genotype), G/D (*CMA/B* G/G and *CMA* I/D or D/D genotypes), A/I (*CMA/B* G/A or A/A and *CMA* I/I genotypes), and A/D (*CMA/B* G/A or A/A and *CMA* I/D or D/D genotypes). The adjusted ORs of the A/I combination group relative to the G/I combination group in gastric cancer patients was 4.749 (95% CI, 2.050-11.001; Table 4). However, G/D and A/D combinations of *CMA/B* and *ACE* polymorphisms had no significant effect on gastric cancer development (Table 4).

Characteristics of the Gastric Cancer Group by PG Assay in Relation to *CMA/B* and *ACE* Polymorphisms. In gastric cancer patients >50-years-old, the mean serum PG I level was 39.2 ± 4.1 ng/mL, which was significantly lower than those in the controls (70.2 ± 2.9 ng/mL, $P < 0.0001$). In all patients >50 years old, however, there were no significant differences in the mean serum PG I levels among different genotype groups of *CMA/B* and *ACE* polymorphisms ($P = 0.898$ and 0.554, respectively; Fig. 1A).

In gastric cancer patients >50-years-old, the mean serum PG I/PG II ratio was 2.6 ± 0.3, which was significantly lower than those in the controls (2.9 ± 0.1, $P = 0.011$). The mean serum PG I/PG II ratio in >50-year-old patients with the *CMA/B* G/G genotype was 4.1 ± 0.2, which significantly differed from those with the *CMA/B* A/G or A/A genotypes (3.5 ± 0.2, $P = 0.0388$; Fig. 1B). However, there were no significant differences in the mean serum PG I/PG II ratios among different allele carriages of *ACE* polymorphism ($P = 0.6142$; Fig. 1B).

Table 3. Influences of the *CMA/B* and *ACE* genotype status on the development of two different histologic types of gastric cancer

Histologic type	Gene		Adjusted OR (95% CI)	*P*
Intestinal type (n = 87)	*CMA/B*	G/G	1.000	
		G/A	1.899 (1.029-3.503)	0.040
		A/A	8.334 (1.832-37.906)	0.006
		A allele	2.181 (1.209-3.932)	0.010
	ACE	I/I	1.000	
		I/D	0.738 (0.467-1.341)	0.319
		D/D	0.434 (0.166-1.131)	0.088
		D allele	0.701 (0.396-1.240)	0.222
Diffuse type (n = 32)	*CMA/B*	G/G	1.000	
		G/A	1.983 (0.874-4.503)	0.102
		A/A	7.398 (1.203-45.491)	0.031
		A allele	2.250 (1.025-4.943)	0.043
	ACE	I/I	1.000	
		I/D	0.583 (0.254-1.339)	0.203
		D/D	0.544 (0.160-1.847)	0.329
		D allele	0.572 (0.263-1.246)	0.160

NOTE: ORs were adjusted by age and sex.

Discussion

The present study was designed to test the hypothesis that polymorphisms of enzymes involved in the local RA system, such as chymase and ACE, are associated with gastric carcinogenesis and peptic ulcer development. We showed that a significant association between the *CMA/B* A/G polymorphism and susceptibility to gastric cancer and gastric ulcer in Japanese patients with *H. pylori* infection. The A allele carriage of *CMA/B* (i.e., A/A and A/G) significantly increased the risk of gastric cancer development. Although the plasma ACE level of individuals with the *ACE* I/I genotype has been reported to be lower than those with the D/D genotype (15, 16), the *ACE* I/D polymorphism is not associated with susceptibility to, pathologic classification, and clinical stage of gastric cancer. However, we observed that the *ACE* I allele increased the gastric cancer risk in patients having the *CMA/B* A allele.

In carcinogenesis, angiotensin II/angiotensin II receptors signaling pathways are associated with cell proliferation, angiogenesis, and inflammation. First, AT1R induces cell proliferation in cancer cells through various intracellular protein cascades associated with growth factor stimulations, of which, the epidermal growth factor receptor–related kinase and protein kinase C are major mediators in cells (31, 32). Second, AT1R induces vascular endothelial growth factor, vascular endothelial growth factor-2 receptor, and angiopoietin II, resulting in the angiogenesis of cancer tissues (33, 34). Third, the activation of AT1Rs enhances the transcription of several proinflammatory cytokines (e.g., interleukin-1 and tumor necrosis factor-α) and chemokines via signaling pathways involving nuclear factor κB and activator protein-1 (10).

Recent studies have shown the local overexpression of chymase and ACE in various cancer cells and tissues (e.g., lung, pancreas, breast, prostate, skin, and cervix carcinoma), suggesting that local overexpression of several components of the RA system is associated with carcinogenesis, such as cell proliferation, angiogenesis, and inflammation (21, 35, 36). For example, the positivities and expressions of chymase are significantly higher in *H. pylori*-associated chronic gastritis and gastric cancer cells than the normal gastric mucosa without *H. pylori* infection (21, 36, 37). The overexpression of chymase observed in mucosa infected with *H. pylori* has been reported to be closely related to an infiltration of inflammation cells, such as neutrophils, macrophages, and T lymphocytes (37). Therefore, chymase is assumed to be associated with the pathogenesis of *H. pylori*-related disorders.

Angiotensin II is an important mediator of gastric vasoconstriction and contributes to physiologic maintenance (38). The systemic generation of angiotensin II from angiotensin I is mainly mediated by ACE. However, 60% to 80% of local, not systemic, generation of angiotensin II is mediated by chymase, and the remainder is mediated by the ACE pathway (24, 39). In a hamster-sponge model, both angiotensin I and angiotensin II injected directly into the sponge enhanced angiogenesis (40). The angiogenesis by angiotensin I was inhibited by chymase inhibitors, but that by angiotensin II was not (40). These findings suggest the importance of chymase-dependent angiotensin II formation in angiogenesis. With regard to the RA system, therefore, the *CMA/B* polymorphism, one of the determinant factors of local CMA levels, may become an important factor of interindividual differences in the susceptibility to cardiovascular diseases and neoplastic diseases, compared with the *ACE* polymorphism (41). In fact, Pfeufer et al. (41) reported that patients with *CMA/B* A/A and A/G genotypes had a 3.2-fold higher risk of hypertrophic cardiomyopathy. However, the role of the *CMA/B* polymorphism in tumor growth and angiogenesis in carcinogenesis has not been directly addressed in previous studies. Although the expressions of chymase in chronic gastritis and gastric cancer cells are significantly increased (21, 36, 37), the role of the differences in local chymase generation associated with *CMA/B* polymorphisms in the stomach has not been directly shown in previous studies. In the present study, we first showed that carriage of the A allele of *CMA/B* polymorphism and the *CMA/B* A/A and A/G genotypes significantly increased the risks of gastric cancer development compared with the carriage of the G allele and the G/G genotype. Therefore, *H. pylori* infection–induced local up-regulation of chymase might be related to gastric carcinogenesis.

Recent reports showed that the *ACE* polymorphism also has a strong association with risk of development of several cancers, such as breast cancer and prostate cancer (19, 20, 22, 42). In gastric carcinogenesis, Ebert et al. (22) reported that the risks for early gastric cancer development was significantly lower in patients with *ACE* I/I and I/D genotypes than those with D/D genotype (ORs, 0.20 and 0.55, respectively). Goto et al. (23) reported that the *ACE* I/D polymorphism was associated with the incidence of gastric cancer and *H. pylori*-positive patients with atrophic gastritis. However, Rocken et al. (21) reported that although the *ACE* polymorphisms for patients with gastric cancer correlated with the number of lymph node metastasis and clinical stage, the distribution of the *ACE* genotype status did not differ significantly from the non–gastric cancer group. On the other hand, many authors have shown that the *ACE* I/D polymorphism was not likely to be a strong predictor of cancer risk (43), and that ACE inhibitors had no preventive effects on tumor growth and angiogenesis in cancer cells (44). In this study, we found no significant association between the *ACE* I/D polymorphism and susceptibility to gastric cancer in Japan. Although the number of chymase-positive mast cells was significantly

Table 4. Influences of the combination of allele carriage of *CMA/B* and *ACE* on the development of gastric cancer

Allele combination		Control/Cancer, n	Adjusted OR (95% CI)	*P*
CMA/B	*ACE*			
G	I	78/26	1.000	
G	D	103/32	1.073 (0.539-2.134)	0.841
A	I	23/27	4.749 (2.050-11.001)	<0.001
A	D	88/34	1.120 (0.539-2.250)	0.750

NOTE: High-producer allele combination of *CMA/B* and *ACE* polymorphisms were shown as follows: GI, GG/II; GD, GG/ID or DD; AI, GD or AA/II; and AD, GA, AA/ID, or DD. ORs were adjusted by age and sex.

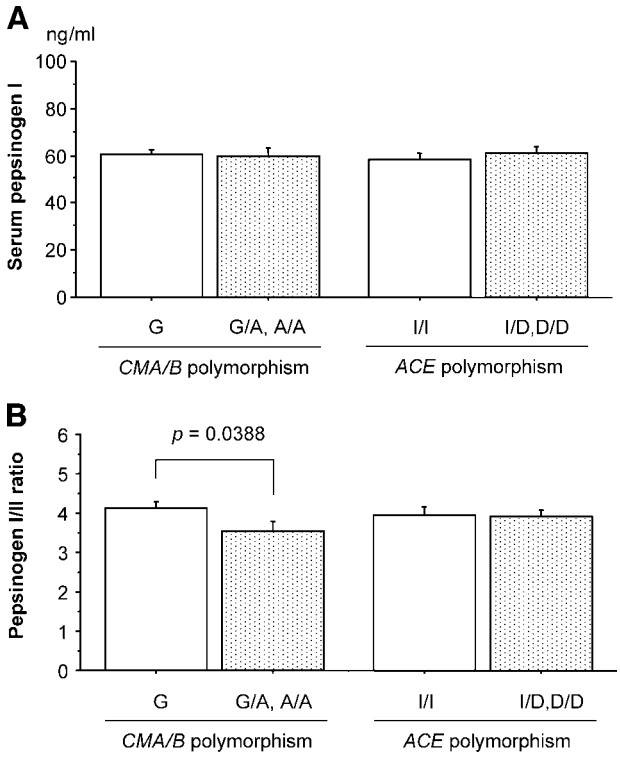

Figure 1. Mean serum PG I levels (**A**) and PG I/PG II ratios (**B**) in *CMA/B* and *ACE* polymorphisms of >50-year-old patients with *H. pylori*-infection. The mean serum PG I levels did not depend on different *CMA/B* and *ACE* genotypes (**A**). The mean PG I/PG II ratios of subjects with *CMA/B* A/G and A/A genotypes was significantly lower compared with those with the *CMA/B* G/G genotype, whereas no significant difference was observed when subjects were classified based on the ACE polymorphism (**B**).

higher in chronic gastritis with *H. pylori* infection than in normal stomach without *H. pylori* infection, the expression of ACE was not up-regulated in *H. pylori*-associated chronic gastritis (37). Moreover, we observed that the *ACE* I allele had the tendency to increase the risk of gastric cancer in subjects with the *CMA/B* A allele. Because the local generation of angiotensin II is mainly mediated by chymase (24, 39), we thought that the role of the *ACE* polymorphism in gastric carcinogenesis seems to be *CMA/B* polymorphism-dependent. Further studies are, however, required to determine the exact role of the *ACE* polymorphism in the pathogenesis of gastric cancer.

Serum PG levels are well known as a surrogate biomarker of gastric atrophy and inflammation induced by *H. pylori* infection (29, 30). The serum PG I level and the low PG I/PG II ratio are decreased with the progression of atrophic gastritis (29, 30), which is considered to be one of major risk factors of gastric cancer. In the present study, we reconfirmed that serum PG I levels and PG I/PG II ratios in the gastric cancer group were significantly decreased compared with those in the control group, and that gastric mucosal atrophy determined by PG methods differed among different *CMA/B* and *ACE* genotypes. The mean serum PG I/PG II ratio in the *CMA/B* A allele carriage group was significantly lower than those from the non-carriage group, which is consistent with our result that the *CMA/B* A allele carriage was at a higher risk for the development of intestinal types of gastric cancer. Therefore, we anticipate that the increased local angiotensin II generation mediated by chymase in subjects with the *CMA/B* A allele carriage enhances gastric inflammation, which leads to or accelerates the severe gastric atrophy and intestinal metaplasia, and finally, to the development of the intestinal type of gastric cancer.

In conclusion, we showed that *CMA/B* A/G polymorphisms in the RA system were associated with an increased risk of the development of gastric cancer and gastric ulcer. We also found the possible association of the *ACE* I/D polymorphisms with *CMA/B* A/G polymorphisms in this process. Therefore, genotyping tests of RA system–related genes (i.e., *CMA/B* A/G polymorphisms and *ACE* I/D polymorphisms) seem to be useful in screening individuals at a higher risk for gastric cancer development. Based on this genotyping test, individuals at a higher risk for gastric cancer seem to be good candidates for chemoprevention with chymase inhibitor and AT1R antagonist or eradication of *H. pylori*. However, the clinical usefulness of this genotyping test must be evaluated in future studies under the appropriate study design.

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