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/A 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.

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...
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 secretory 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/B, 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 \textit{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 \textit{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 \textit{H. pylori} infection using the above three tests, were enrolled in this study. The 513 \textit{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'-CGA 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 BstXI (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 \textit{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 CCT 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'-TGG CAC GTG ACG GGC CCC AAC TAC-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 

| 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 | Diffuse type | Clinical stage | Stage I-II | Stage III-IV | 80 | 29 |
| CMA/B polymorphism | G/G genotype | G/A genotype | A/A genotype | 102 (77.3%) | 27 (20.5%) | 3 (2.2%) | 102 (77.3%) | 27 (20.5%) | 3 (2.2%) | 0.003 |
| ACE polymorphism | I/I genotype | I/D genotype | D/D genotype | 50 (37.9%) | 60 (45.5%) | 22 (16.6%) | 51 (31.9%) | 83 (51.9%) | 26 (16.2%) | 0.129 |

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Data Analysis. Hardy-Weinberg equilibrium of allele frequencies at individual loci was assessed by comparing the observed and expected genotype frequencies using the $\chi^2$ 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 $\chi^2$ 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 A allele carriers for intestinal type of gastric cancer were 2.219 (95% CI, 1.315-3.744) and 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/G 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).

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/B I/I genotype), G/D (CMA/B G/G and CMA/B I/D or D/D genotypes), A/I (CMA/B A/A or A/G and CMA/B I/I genotype), and A/D (CMA/B A/A or A/G and CMA/B 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 carries of ACE polymorphism ($P = 0.6142$; Fig. 1B).
The present study was designed to test the hypothesis that chymase is associated with the local overexpression of several components of the RA system in carcinogenesis, such as neutrophils, macrophages, and T lymphocytes (37). In this study, we found no significant association between the CMA/B A 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/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 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.
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. Furthermore 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 (37). Moreover, we observed that the expression of angiotensin II stimulates angiogenesis in the chorio-allantoic membrane of the chick embryo. Eur J Pharmacol 1991;195:305–6.


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).


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