Polymorphism of the salt sensitivity gene angiotensinogen and gastric cancer risk

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Abstract. A high-salt diet is a risk factor for gastric cancers other than those caused by *Helicobacter pylori*. The angiotensinogen (AGT) M235T polymorphism has been associated with salt sensitivity. The aim of the present study was to clarify the association between the AGT M235T polymorphism and gastric cancer. The AGT M235T polymorphism was genotyped using PCR-RFLP analysis in 206 gastric cancers and 210 control biopsies. A logistic-regression analysis was performed to identify an odds ratio to determine whether a correlation exists between genetic polymorphism and risk in patients with gastric cancer as compared to the control samples. Statistical significance was determined using the Mann Whitney U and Chi-square tests. The genotype distribution was found to be MM=9 (4.4%), MT=57 (27.7%), and TT=140 (67.9%) in samples from patients with gastric cancer and MM=8 (3.8%), MT=60 (28.6%) and TT=142 (67.6%) in the control samples. The odds ratio of gastric cancer of the MM genotype associated with the T carrier was 1.0 (0.4-2.7) (P=0.95). The distribution pattern of AGT M235T polymorphism in the gastric cancer cases and controls was not found to be significantly different in this study. Thus, it can be concluded that other sites of AGT polymorphism or other salt sensitivity genes may be associated with gastric cancer.

Introduction

Helicobacter pylori (H. pylori) was initially identified in 1986. Since then, this infection has been accepted as a crucial factor in the development of peptic ulcer disease and atrophic gastritis. In addition, it is involved in the development of gastric carcinoma (1-5). However, there are distinct differences in the extent of gastric mucosal inflammation and atrophy among *H. pylori*-infected patients, and only a small group of infected patients develop peptic ulcer disease and gastric cancer. Thus, as yet unknown factors, such as genetic variation, may play significant roles in the long-term outcome of *H. pylori* infection (6).

Previous studies reported that a high-salt diet is a risk factor for gastric cancers other than those caused by *H. pylori* infection (7-9). Findings of an animal study also revealed that salt did not have an initiation effect for carcinogenesis but rather promoted carcinogenesis (10). The evidence revealed that high salt intake is a risk factor for gastric cancer. However, the mechanism remains to be determined. Certain studies have indicated that the product that results from the mixing of excess sodium chloride and amino acids in the stomach, such as nitrosoamine, may play a role in carcinogenesis (11).

Angiotensinogen (AGT) is one of the most significant elements of the renin-angiotensin system (RAS). AGT and RAS are involved in vascular tone, cardiovascular remodeling, and salt and water homeostasis (12). Marked interest in the AGT M235T polymorphism and its association with hypertension and salt sensitivity has been noted (13,14). This polymorphism was previously reported to be associated with plasma AGT levels (13).

The aim of the present study was to clarify the association of AGT M235T polymorphism in a salt sensitivity gene with gastric cancer risk in a Japanese cohort.

Materials and methods

Study population. This case-control study was performed with enrolled patients who underwent upper gastrointestinal endoscopy at Fujita Health University Hospital. We studied 416 Japanese patients, who were divided into two groups for assessment of AGT polymorphism. The first group comprised 206 gastric cancer (GC) patients with a mean age of 65.3 years and a male:female (M:F) ratio of 147:59. The second group included 210 patients with no evidence of GC (mean age of 62.8 years and M:F ratio of 128:82) and served as the control group. GC was diagnosed histologically by hospital pathologists and was classified according to Lauren's classification (15). Detailed information was obtained on the tumor stage and the anatomic location.

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The Ethical Committee of Fujita Health University School of Medicine approved the study protocol, and written informed consent was obtained from each patient.

Detection of H. pylori. *H. pylori* infection status was determined by histology, urea breath test (UBT), and/or a serum titer of antibodies against *H. pylori*. An *H. pylori* infection was diagnosed if at least one of the test results was positive.

DNA extraction. Biopsy specimens were obtained during endoscopy from non-cancerous mucosa on the greater curvature of the antrum. Samples were immediately frozen and maintained at -80˚C. Genomic DNA was later extracted from these samples by digestion with proteinase K and phenol (16).

Genotyping of AGT gene polymorphism. The polymorphism of AGT M235T was characterized by a PshAI digestion site. The AGT M235T polymorphism was investigated using PCR-based restriction fragment length polymorphism (PCR-RFLP) analysis. PCR amplification was performed using the following primers for AGT M235T: 5'-CCGTTTGTGCAGGGCCTGGCTCTCT-3' and 5'-GCCAG GGTGCTGTCCACACTGACTCCC-3'. PCR was performed in a reaction volume of 20 μl with 0.1 μg of genomic DNA.

Table I. Patient characteristics.

	GC group	Control group	P-value
No.	206	210	
Males/females	147/59	128/82	N.S.[a]
Average age (± SD)	65.3±10.7	62.8±12.7	N.S.[b]
HP-positive rate (%)	93.2	67.6	<0.05[a]

GC, gastric cancer; HP, *Helicobacter pylori*. [a]GC vs. control group, Chi-square test. [b]GC vs. control group, Mann-Whitney U test.

Table II. AGT polymorphism and GC risk.

Genotypes	GC group no. (%)	Control group no. (%)	OR (95% CI) vs. MM	P-value
MM	9 (4.4)	8 (3.8)	Reference	
MT	57 (27.7)	60 (28.6)	1.02 (0.34-3.04)	0.975
TT	140 (67.9)	142 (67.6)	0.96 (0.35-2.64)	0.932
T carrier	197 (95.6)	202 (96.2)	0.97 (0.35-2.67)	0.952

GC, gastric cancer; CI, confidence interval.

Table III. Association between AGT polymorphism and tumor location, staging and Lauren's classification.

Variables (no.)	Genotype			TT vs. MM	
	MM	MT	TT	OR (95% CI)	P-value
Patients without GC (210)	8	60	142	Reference	
Tumor location					
Cardia (6)	0	2	4	ND	
Non-cardia (200)	9	55	136	1.07 (0.39-2.95)	0.901
Upper third (20)	1	4	15	1.05 (0.12-9.39)	0.968
Middle third (102)	4	35	63	0.93 (0.26-3.34)	0.914
Lower third (78)	4	16	58	1.11 (0.31-4.00)	0.876
Staging					
Early (114)	4	30	80	0.77 (0.22-2.71)	0.683
Advanced (92)	5	27	60	1.39 (0.42-4.65)	0.592
Lauren's classification					
Intestinal type (117)	5	30	82	1.02 (0.31-3.37)	0.975
Diffuse type (83)	4	24	55	1.05 (0.29-3.73)	0.944
Mixed (6)	0	3	3	ND	

All data are adjusted for gender, age, and *H. pylori* infection status. GC, gastric cancer; ND, not determined.

An initial denaturing step at 94˚C for 5 min was followed by 35 cycles of 94˚C for 20 sec, 53˚C for 20 sec, and 72˚C for 40 sec with a final extension step at 72˚C for 7 min. The reaction was carried out using the Ex Taq enzyme (Takara Bio Inc., Shiga, Japan). The amplified PCR products were digested overnight at 37˚C with 5 units of PshAI, and the digested products were subjected to electrophoresis on 3% agarose gel. The gels were stained with ethidium bromide (0.5 μg/ml). The genotypes and alleles were determined by analysis of the bands as previously described (17).

Statistical analysis. Data were analyzed to assess statistical significance using the Mann-Whitney U and Chi-square tests. Logistic regression analysis was performed to calculate the odds ratios (OR) (18) and 95% confidence intervals (CI) for AGT polymorphism by comparing the GC and control groups with an adjustment for *H. pylori* infection status. $P<0.05$ was considered to be statistically significant.

Results

Study population. A total of 206 GC patients and 210 control subjects without evidence of GC participated in this study. Their characteristics are shown in Table I. No significant differences were observed between the two groups with regards to age or gender distributions, indicating that the observed effects were not due to these two variables. The *H. pylori* infection rate was found to be higher in the GC patients ($p<0.05$).

Distribution of AGT genotypes. Table II shows AGT M235T genotype frequencies in the GC and control groups. The AGT M235T polymorphism was genotyped in all 416 subjects. In the 206 GC patients, the genotype distribution was found to be MM=9 (4.4%), MT=57 (27.7%) and TT=140 (67.9%). In the control patients, the genotype distribution was MM=8 (3.8%), MT=60 (28.6%) and TT=142 (67.6%). The genotype distribution was not significantly different between the GC and control patients (Table II).

To perform a more detailed investigation, the associations between AGT polymorphism and various clinicopathological characateristics of GC (tumor location, stage and Lauren's histological classification) were assessed by stratified analysis. No significant differences were observed between GC cases and the controls (Table III).

Discussion

A large number of epidemiologic studies have reported a correlation between a high level of dietary salt intake and an increased risk of gastric cancer (19-21). Findings of studies using animal models have shown that the presence of *H. pylori* and a high-salt diet have a synergistic effect on gastric carcinogenesis (10,22). These associations have been detected in both prospective and case-control studies (8,23,24). These studies reported that *H. pylori*-infected subjects who consumed a high-salt diet had an increased risk of gastric cancer when compared to *H. pylori*-infected subjects who consumed lower levels of salt. The mechanism by which a high-salt diet increases the risk of gastric cancer in humans has yet to be clarified. One possibility is that salt may have direct effects on the gastric mucosa that lower the threshold for malignant transformation (25). Another proposed explanation is that salt damages the gastric mucosa, thereby allowing increased entry of carcinogens into gastric tissue (26).

Cardiac investigators are engaged in finding a genetic association between salt sensitivity and hypertension. AGT is a salt sensitivity gene involved in this process and is a significant gene in the RAS system. As previously mentioned, the AGT M235T polymorphism was reported to be associated with the plasma AGT levels (13). The T235 allele varies widely in frequency, occurring in 35-45% of subjects of Caucasian descent, 75-80% of Asians and African Americans, and 90% of subjects of African descent (27,28). These distribution differences led to a hypothesis that the T235 allele, which is associated with a higher AGT expression and greater sodium reabsorption, was adaptive in the tropical, sodium-poor environment of sub-Saharan Africa. However, this hypothesis was rejected due to demographic relocation by these population groups.

The T235 polymorphisms may predispose populations to hypertension and other disorders when resources are prevalent and overconsumed (29). It was proposed that this polymorphism leads to a susceptibility to gastric mucosal damage in the presence of high concentrations of salt due to AGT concentration levels. For these reasons, we focused on the M235T polymorphism of a salt sensitivity gene, AGT. This is the first investigation into a possible correlation between a salt sensitivity gene and gastric carcinogenesis. Previously, the relationship between genes in the renin-angiotensin system and the risk of gastric cancer was investigated (30-34). One study reported a correlation between the A-20C AGT polymorphism and an increased risk of gastric cancer (34). However, results of our study revealed no association between the AGT M235T polymorphism and gastric cancer risk. One reason for this inconsistency is the different linkage disequilibrium between M235T and other polymorphism sites in the gene. The AGT M235T polymorphism exhibits a strong linkage disequilibrium with the AGT G-6A and T68C polymorphisms, but only a weak linkage disequilibrium with the AGT A-20C polymorphism (35). Further studies are required to investigate other sites of AGT gene polymorphisms and gastric cancer susceptibility to confirm this theory.

In conclusion, the distribution pattern of the AGT M235T polymorphism in cases of GC and controls was not found to be significantly different in this study. No association was found between the AGT M235T polymorphism and patient susceptibility to gastric cancer. Therefore, other polymorphism sites in the AGT gene or other genes related to salt sensitivity may be associated with gastric cancer development.

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