NO ASSOCIATION BETWEEN ANGIOTENSIN I CONVERTING ENZYME (ACE) I/D POLYMORPHISM AND GASTRIC CANCER RISK AMONG JAPANESE

SATOSHI HIBI1, YASUYUKI GOTO2, TAKAFUMI ANDO1, KEITARO MATSUO3, KENJI WAKAI2, KAZUO TAJIMA3, HIDEKI GOTO1 and NOBUYUKI HAMAJIMA2

1Department of Gastroenterology, Nagoya University Graduate School of Medicine, Nagoya, Japan
2Department of Preventive Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan
3Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan

ABSTRACT

The angiotensin I-converting enzyme (ACE) is involved in cell proliferation, angiogenesis, inflammation, and tissue remodeling, all of which could play a role in carcinogenesis. The DD genotype of ACE I/D polymorphism with a higher ACE level than either ID or II genotypes was reported to increase the risk of several cancers. This is a case-control study examining the association between the polymorphism and gastric cancer risks among Japanese. Cases numbered 583 patients aged 27 to 80 years with gastric cancer diagnosed at the Aichi Cancer Center Hospital from 2001 to 2005. Controls were 1,742 sex and age frequency-matched cancer-free patients, who visited the same hospital during that same period. The ACE I/D polymorphism was genotyped using a polymerase chain reaction with confronting two-pair primers. The results showed that the age- and sex-adjusted ORs of gastric cancer were 0.95 (95% CI, 0.78-1.16) for ID, and 1.08 (95% CI, 0.80-1.46) for DD relative to II. Among the controls with H. pylori sero-positive or gastric atrophy (GA), the ORs of ID and DD relative to II were 1.20 (95% CI, 0.88-1.63) and 1.16 (95% CI, 0.73-1.84) for mild GA, and 1.22 (95% CI, 0.84-1.78) and 1.08 (95% CI, 0.61-1.89) for severe GA, respectively. In conclusion, there was no significant association of the ACE I/D polymorphism with the risk of gastric cancer. Among the controls, the polymorphism was not associated with the severity of GA.

Key Words: ACE, Polymorphism, Helicobacter pylori, Gastric cancer, Gastric atrophy

INTRODUCTION

Gastric cancer is the fourth most common among all cancers, and still remains the second leading cause of cancer deaths worldwide, although its incidence has been decreasing.1-4) It has been reported that the incidence of gastric cancer was affected by exogenous factors such as an increased intake of salty food, a decreased intake of fruits and vegetables, smoking, and Helicobacter pylori (H. pylori) infection, and endogenous factors such as host genetic factors.5-8) H. pylori plays a key role in the development of gastric cancer.9) H. pylori-induced gastritis can progress via a gastric atrophy (GA)-intestinal metaplasia-dysplasia sequence.5,6,9,10) However, some individuals with H. pylori can worsen to develop gastric cancer (21% of males and 4% of females among those Japanese infected).11) That fact may be partly explained by the presence

Corresponding Author: Satoshi Hibi
Department of Gastroenterology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
Phone: +81-52-744-2144, Fax: +81-52-744-2159, E-mail: s-hibi@med.nagoya-u.ac.jp
of host genetic traits. We have been examining the associations of gastric cancer and GA with genetic polymorphisms.

The angiotensin I-converting enzyme (ACE) regulates angiotensin II (AngII), the active peptide of the renin-angiotensin system (RAS) that regulates blood pressure. ACE has also been reportedly associated with cell proliferation, angiogenesis, and inflammation. The receptor of AngII, the AngII type1 receptor (AT1R) subtype, is expressed on tumor and endothelial cells, and is upregulated in many cancer tissues. Meanwhile the AngII type2 receptor (AT2R) often acts as a counter-regulator receptor. Previous studies have reported associations of a 287-base pair Alu-repetitive sequence insertion (I) /deletion (D) ACE polymorphism in intron 16 with multiple clinical features, including gastric, breast, prostate, oral and endometrial cancer. The polymorphism has also been reported for its association with the lymph node metastasis of gastric cancer. The ACE I/D polymorphism is associated with serum ACE levels which are higher for the DD genotype than for the ID/II genotype.

We previously reported the marginally significant association between the ACE polymorphism and gastric cancer risk. Our study aimed to confirm that association using a larger sample. In addition, the associations with H. pylori sero-positive and GA were examined.

SUBJECTS AND METHODS

Study subjects
Our subjects were 583 patients with gastric cancer who were diagnosed from 2001 to 2005. They were aged 27 to 80, and composed of 1,742 sex and age frequency-matched cancer-free controls. All subjects were obtained from the Hospital-based Epidemiological Research Program at Aichi Cancer Center (HERPACC) launched in 1988. Further details of the study subjects were described in previous papers.

Tests for H. pylori antibody and pepsinogens
Anti-H. pylori IgG antibody was tested with an EIA kit “E plate ‘Eiken’ H. pylori Antibody” (Eiken Kagaku, Tokyo, Japan). A measurement of 10.0 U/ml or over was regarded as H. pylori infection-positive. Pepsinogen (PG) I and II were measured by radioimmunoassay using a commercially available kit (DINABOT, Tokyo, Japan). GA was defined as PG I<70 ng/ml and PG I /PG II ratio<3. In addition, PG I<30 ng/ml and PG I/PG II<2 were defined as severe atrophy, while otherwise subjects were defined as mild atrophy. Anti-H. pylori IgG antibody and PG were measured only for the control group because the sera of cases had been used for other analyses.

Genotyping
DNA was extracted from the buffy coat fraction with the Qiagen QIAamp DNA Blood Mini Kit (QIAGEN Inc., Valencia, CA, USA). ACE genotyping was conducted by polymerase chain reaction with confronting two-pair primers (PCR-CTPP). The primers and PCR conditions were presented in our previous paper.

Statistical analysis
The sex-age adjusted odds ratio (OR) and 95% confidence interval (CI) were estimated by unconditional logistic regression analysis. The ORs of the ACE genotype for gastric cancer were evaluated among all subjects, while those with mild GA and severe GA were evaluated by comparing them with sero-positives without GA. The genotype frequency of the ACE polymorphism
was tested with the Hardy-Weinberg equilibrium. Calculations were performed using the computer program STATA Version 9 (STATA, College Station, TX, USA).

RESULTS

Characteristics of the study subjects are presented in Table 1. Anti-*H. pylori* IgG antibody and PG were measured for the control group only. The rates of sero-positivity and GA among the controls were 57.3% and 33.0%, respectively.

The *ACE I/D* genotype of the control group was in the Hardy-Weinberg equilibrium ($\chi^2=0.073$, $p=0.79$). Two controls and one case were not successfully genotyped. As shown in Table 2, the genotype frequency of *ACE I/D* among the controls was 745 (42.8%) for *II*, 791 (45.5%) for *ID*, and 204 (11.7%) for *DD*. The ORs of *ID*, *DD* and *D* alleles relative to *II* for gastric cancer were 0.95 (95% CI, 0.78-1.16), 1.08 (95% CI, 0.80-1.46) and 0.98 (95% CI, 0.81-1.18), respectively. There was no significant association between the genotype and the risk of gastric cancer.

Among the controls, 939 (57.3%) subjects were sero-positive for *H. pylori*, while 699 (42.7%) were sero-negative. No serum was available for the remaining 104 controls. OR of the sero-positive control group was 1.10 (95% CI, 0.89-1.36) for the *ID* genotype and 1.30 (95% CI, 0.93-1.82) for the *DD* genotype relative to the *II* genotype.

Table 3 shows the OR of the *ACE* genotype for GA among those testing sero-positive or with GA. The ORs of *ID*, *DD* and *D* alleles relative to *II* were 1.20 (95% CI, 0.88-1.63), 1.16 (95% CI, 0.73-1.84), and 1.19 (95% CI, 0.89-1.59) for mild GA, and 1.22 (95% CI, 0.84-1.78), 1.08 (95% CI, 0.61-1.89), and 1.19 (95% CI, 0.83-1.70) for severe GA, respectively.

**Table 1 Characteristics of subjects**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls N (%)</th>
<th>Cases N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>1,742 (100)</td>
<td>583 (100)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1,286 (73.8)</td>
<td>429 (73.6)</td>
</tr>
<tr>
<td>Female</td>
<td>456 (26.2)</td>
<td>154 (26.4)</td>
</tr>
<tr>
<td>Age (mean±standard deviation)</td>
<td>58.5±10.6</td>
<td>58.8±10.5</td>
</tr>
<tr>
<td>Anti-<em>H. pylori</em> IgG antibody</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>939 (57.3)</td>
<td>-</td>
</tr>
<tr>
<td>Negative</td>
<td>699 (42.7)</td>
<td>-</td>
</tr>
<tr>
<td>No serum</td>
<td>104</td>
<td>-</td>
</tr>
<tr>
<td>Gastric atrophy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>541 (33.0)</td>
<td>-</td>
</tr>
<tr>
<td>Negative</td>
<td>1,097 (67.0)</td>
<td>-</td>
</tr>
<tr>
<td>No serum</td>
<td>104</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2  Sex-age-adjusted odds ratio (OR) and 95% confidence interval (CI) of angiotensin converting enzyme genotype for gastric cancer

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>Cases</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1,740 (100)</td>
<td>582 (100)</td>
<td>1</td>
<td>Reference</td>
</tr>
<tr>
<td>II</td>
<td>745 (42.8)</td>
<td>252 (43.3)</td>
<td>0.95</td>
<td>0.78-1.16</td>
</tr>
<tr>
<td>ID</td>
<td>791 (45.5)</td>
<td>255 (43.8)</td>
<td>1.08</td>
<td>0.80-1.46</td>
</tr>
<tr>
<td>DD</td>
<td>204 (11.7)</td>
<td>75 (12.9)</td>
<td>0.98</td>
<td>0.81-1.18</td>
</tr>
<tr>
<td>ID+DD</td>
<td>995 (57.2)</td>
<td>330 (56.7)</td>
<td>0.98</td>
<td>0.81-1.18</td>
</tr>
</tbody>
</table>

*One case and two controls were not successfully genotyped.

Table 3  Sex-age-adjusted odds ratios (OR) and 95% confidence intervals (CI) of mild gastric atrophy (GA) and severe GA compared with sero-positive controls without GA for angiotensin converting enzyme genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sero-positive non-GAa)</th>
<th>Mild GAb)</th>
<th>Severe GAc)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N=443)</td>
<td>(N=343)</td>
<td>(N=196)</td>
</tr>
<tr>
<td></td>
<td>N (%)</td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>II</td>
<td>197 (44.5)</td>
<td>138 (40.2)</td>
<td>1</td>
</tr>
<tr>
<td>ID</td>
<td>193 (43.5)</td>
<td>161 (47.0)</td>
<td>1.20</td>
</tr>
<tr>
<td>DD</td>
<td>53 (12.0)</td>
<td>44 (12.8)</td>
<td>1.16</td>
</tr>
<tr>
<td>ID+DD</td>
<td>246 (55.5)</td>
<td>205 (59.8)</td>
<td>1.19</td>
</tr>
</tbody>
</table>

a) Sero-positive controls without GA (PG1<70 ng/dl and PG1/2<3)
b) Controls with GA except those with PG1<30 ng/dl and PG1/2<2
c) Controls with PG1<30 ng/dl and PG1/2<2

DISCUSSION

Our previous study demonstrated that the ACE D allele increased the risk of gastric cancer with a marginal significance (OR=1.48, 95% CI 0.98-2.26) among H. pylori sero-positive subjects with atrophy, and of OR=1.35 (95% CI 0.93-1.96) among all subjects. Although this study could not compare the genotype frequency among H. pylori sero-positive subjects with atrophy because of no serum available for cases, the analysis for all subjects with a larger sample size did not support the previously observed association. In addition, our study showed no associations with H. pylori infection or with the severity of GA.

In our previous study, the controls were sampled from health checkup examinees in the same area, while the present study used hospital non-cancer patients as the controls. According to a previous survey, 44.3% were disease-free, 13.1% were benign tumors or non-neoplastic polyps, 3.4% were cystic diseases, and 39.2% were miscellaneous benign diseases. The difference in the source of the control group might have influenced the results, though the size of that influence could not be evaluated.

Rocken et al. have reported that ACE was expressed locally in gastric cancer and that the ACE D allele was associated with the number of nodal metastases but not the prevalence of
ACE I/D POLYMORPHISM AND GASTRIC CANCER RISK

gastric cancer.\textsuperscript{25,26} In the present study, there was no significant association between the \textit{ACE} \textit{D} allele and the risk of gastric cancer, although we could not evaluate the association with nodal metastasis because no information was available. There might be an association with the progression but not with the occurrence of gastric cancer. In contrast to our hypothesis, Sugimoto et al. have reported that the OR of the \textit{ACE DD} relative to \textit{II} for gastric cancer was 0.439 (95\% CI, 0.191-1.008).\textsuperscript{36} Our results did not support theirs because its OR was 1.08 (95\% CI, 0.80-1.46) in this study. A previous meta-analysis reported an association between the \textit{ACE I/D} polymorphism and gastric cancer in Caucasians, but not Asians.\textsuperscript{37} The result of their meta-analysis was consistent with ours.

As for other cancers, including those of the breast, prostate, oral and endometrium, the associations with the \textit{ACE I/D} polymorphism have been examined in case-control studies.\textsuperscript{29,30,38-41} As for breast cancer, Alves Correa et al. reported that \textit{ID} might be more protective against cancer than \textit{DD} and \textit{II},\textsuperscript{38} while Koh et al. have shown that \textit{I} allele reduced the risk in Chinese,\textsuperscript{39} and Haiman et al. have discovered that \textit{II} had a marginally significant risk in African-Americans.\textsuperscript{40} The \textit{ACE DD} posed a significantly higher risk genotype for prostate cancer,\textsuperscript{41} whereas for oral cancer, the \textit{I} allele was the significantly higher risk allele.\textsuperscript{29} In endometrial cancer, \textit{I} allele carriers were at significantly higher risk among normotensive women aged 63 years or younger.\textsuperscript{30}

The involvement of \textit{ACE} in the development of cancer might vary according to the types of cancers. \textit{ACE} regulates AngII that mainly binds AT1R.\textsuperscript{21} AngII binds two receptors, AT1R and AT2R, and AT2R often functions as a counter-regulatory receptor for AT1R.\textsuperscript{21} Additionally, AngII is locally regulated by chymase.\textsuperscript{21} Local expressions of RAS components were reportedly different in various cancer tissues.\textsuperscript{21} \textit{ACE} also plays a role in degrading bradykinin (BK) which is the vasoactive peptide.\textsuperscript{21} In breast cancer cells, BK exhibits mitogenic effects.\textsuperscript{42} Vairaktaris et al. have suggested that the oncogenesis in oral cancer might be driven via a BK pathway and not through AngII.\textsuperscript{29} The development of gastric cancer could be affected by AngII mainly through chymase rather than \textit{ACE}.\textsuperscript{36}

The frequency of the \textit{ACE I/D} polymorphism displays ethnic variations; the \textit{I} allele was 40\% in African-Americans, 52\% in Latinos, and 46\% in Caucasians.\textsuperscript{40} In the present study, it was 66\%, which was close to that in our previous study (67\%), and another Japanese group (66\%).\textsuperscript{31,36}

One limitation of this study was its size. Although this was a medium-size study, its statistical power might not have been enough: power=0.232 for OR=1.2, and power=0.449 for OR=1.3, and power=0.663 for OR=1.4 in 582 cases and 1,742 controls with a 0.117 of \textit{DD} genotype frequency. The second limitation involved the definition of GA, for which we used only serum PGs without a pathological evaluation, although its use was common in epidemiologic studies. The third limitation was in measuring the anti-\textit{H. pylori} IgG antibody and PG only among the control group.

In conclusion, the present study showed no associations between the \textit{ACE I/D} polymorphism with gastric cancer risk and the severity of GA. Although our previous study indicated a potential association with gastric cancer risk, such an association seemed limited if it existed at all.

ACKNOWLEDGMENTS

This study was supported in part by a Grant-in-Aid for Scientific Research on Special Priority Areas of Cancer from the Japanese Ministry of Education, Culture, Sports, Science and Technology. We are deeply grateful to Ms. Yoko Mitsuda and Ms. Keiko Shibata for their valuable technical assistance.
REFERENCES

ACE I/D POLYMORPHISM AND GASTRIC CANCER RISK


