Genetic Polymorphisms of the E-Cadherin Promoter and Risk of Sporadic Gastric Carcinoma in Chinese Populations

Baozhen Zhang,1 Kaifeng Pan,2 Zhaojun Liu,1 Jing Zhou,1 Liankun Gu,1 Jiafu Ji,3 Junling Ma,2 Wei-cheng You,2 and Dajun Deng1

Departments of Etiology, Cancer Epidemiology, and Surgery, Peking University School of Oncology, Beijing Cancer Hospital/Institute

Abstract

Frequent mutations and loss of expression of E-cadherin have been reported in a number of cancers. E-cadherin germ line mutations lead to a high risk of familial diffused gastric carcinoma. In the present study, to evaluate the effect of genetic polymorphisms in the E-cadherin promoter on the risk of sporadic gastric carcinoma (SGC), a comprehensive study was conducted in two populations with high and low risk of SGC in China, respectively. Five hundred seventy-two SGC cases and 625 controls from low-risk area and 589 individuals enrolled in a long-term follow-up survey in high-risk area were studied. Polymorphisms of E-cadherin around transcription start site (–437 to +314) were analyzed by sequencing. Five variations of –347del>A, –160>C, –73>A>C, +178T>C, and +23413N ins>del were linked tightly. The –347del/del and its strongly linked +178T/T, +23413N ins/ins genotypes increased male SGC risk in the high-risk area significantly [odds ratio (OR), 2.22; 95% confidence intervals (95% CI), 1.10-4.46] and correlated with the severity of gastric lesions. A synergistic effect was also observed between –347del/del genotype and Helicobacter pylori infection (OR, 4.93; 95% CI, 1.65-14.71). Compared with –347del-containing haplotypes, the –347A-containing haplotype [A(–347)C(–160)A(–73)C(178)C(234)13N del(1-234)] decreased the risk of SGC among male subjects (OR, 0.61; 95% CI, 0.37-1.01). Such correlation could not be observed among subjects from the low-risk area. The present data suggest that the –347del allele of E-cadherin strongly links with the +178T and +23413N ins alleles. The –347del/del genotype may increase the susceptibility of SGC among males in the high-risk area of China. (Cancer Epidemiol Biomarkers Prev 2008;17(9):2402–8)

Introduction

Stomach cancer is still one of the leading causes of cancer death in China (1). The possible etiologic factors for sporadic gastric carcinoma (SGC) include Helicobacter pylori (H. pylori) infection, high salt intake, endogenous formation of N-nitroso compounds, and malnutrition of antioxidants (2-5). Genetic susceptibility is another kind of factor that may interact with environmental factors and determine whether a subject is sensitive or resistant to the causal factors at natural level.

E-cadherin is an epithelial cell-cell adhesive molecule (6). Inactivation of the E-cadherin by germ line mutations is observed frequently in patients with familial gastric carcinomas (7, 8). Somatic E-cadherin mutations are common in diffused-type SGC and infiltrative lobular breast carcinoma (9, 10) but rare in other sporadic carcinomas (11-13). Down-regulation of the E-cadherin is much more frequent than somatic mutation in many sporadic human carcinomas in various organs such as stomach, colon, pancreas, breast, and prostate (14-18). Methylation of CpG island of the E-cadherin and up-regulation of transcriptional repressors such as SNAIL might account for most of lost transcription of the E-cadherin in sporadic carcinomas finally (19-21).

There are a number of polymorphisms clustered around the transcription start site (TSS) of the E-cadherin gene (Fig. 1). It was reported that single nucleotide polymorphisms (SNP) –160>C>A (rs16260) and –347del>A (rs5030625) in the promoter might alter the transcriptional activity of this gene's promoter (22, 23), which attracted a lot of attentions to investigate the possible effects of these polymorphisms on the susceptibility of SGC and other cancers (24-29). However, results of these association studies were not consistent or even contradictory. In the present study, we reported a comprehensive analysis of these variations and haplotypes of the E-cadherin promoter and their correlation with the SGC risk in two populations with high and low risk of SGC in China, respectively, examining the effect of genetic factors to SGC under different environmental pressure. The transcriptional activity of the promoter with different haplotypes was also studied.

Materials and Methods

Study Populations

Subjects from Linqu County. Linqu County is a rural area in Shandong province in northeast China and a high-risk area for stomach cancer, where the world age-adjusted incidence of stomach cancer from 1993 to 1997 was 73.2 per year per 100,000 males according to China Cancer Database4 and adult chronic atrophic gastritis
(CAG) was nearly universal (98%; ref. 30). Genomic DNA of subjects with different gastric lesions, including CAG (n = 196), intestinal metaplasia/gastric dysplasia (IM/DYS; n = 197), and SGC (n = 96), was isolated from peripheral blood samples. As shown in Table 1, information on status of pathologic changes of gastric mucosa (diagnosed by a panel of three senior pathologists of Peking University School of Oncology), H. pylori infection (measured by ELISA; ref. 31), alcohol drinking, and smoking was available for these analyzed subjects who enrolled in a 20-year gastroendoscopy follow-up survey.

**Subjects from Beijing.** Beijing is a low-risk area for stomach cancer in China, where the world age-adjusted incidence of stomach cancer from 1993 to 1997 was 19.8 per year per 100,000 males. Genomic DNA was extracted from the frozen surgical adjacent gross pathologic normal gastric tissue of SGC from cases (n = 572) diagnosed and treated surgically at Peking University School of Oncology. Clinical pathologic information was available for all 572 SGC cases. Blood DNA samples of nonmalignant controls (n = 625) were obtained from Peking University First Hospital.

The study was approved by the institutional review board of Peking University School of Oncology, and all subjects gave written informed consent.

**Amplification and Sequencing of the E-Cadherin Promoter.** The forward and reverse genotyping primers for the TSS flanking region (−437 to +314; Fig. 1) of E-cadherin (Genbank accession number gi:18057085) are 5′-CTGGGGCCAGAGGACCCGTCTGA-3′ and 5′-CTTCAGGCCGCTCCGCTCTG-3′, respectively. The PCR amplification was carried out in a final volume of 40 μL containing 50 ng genomic DNA, 0.2 mmol/L deoxynucleotide triphosphate, 0.2 μmol/L of each primer, 0.5 units plaque-forming unit DNA polymerase (Sangon Company), using the touchdown PCR protocol: 95°C for 5 min→(95°C for 30 s→70°C for 30 s, −1°C/ cycle→−72°C for 30 s)×12 cycles→(95°C for 30 s→58°C for 30 s→72°C for 30 s)×28 cycles→72°C for 10 min in PTC-200 DNA Engine (MJ Research, Inc.). The PCR product (752 bp) was directly sequenced with ABI 3730 DNA sequencer. The genotypes of representative samples were verified by cloning sequencing.

**PCR-RFLP Analysis.** As the −347del/A heterozygote may affect the resolution of +178 genotype due to the long distance between these two sites, the genotype of +178>T>C SNP was further confirmed by PCR-RFLP analysis, in which the 100 bp sequence (+124→+223) was amplified by PCR using the primers 5′-CATGGGCCC-TTGGAGCCGCCGCAGAGG-3′ and 5′-CGGAGCTTGCGGCCC-GAATG-3′ with thermal cycle condition [95°C for 5 min→(95°C for 30 s→70°C for 30 s, −1°C/cycle→−72°C for 30 s)×12 cycles→(95°C for 30 s→58°C for 30 s→72°C for 30 s)×28 cycles→72°C for 10 min] and digested withMspI (New England Biolab) followed by separating on 10% polyacrylamide gel (PAGE).

**Dual-Luciferase Reporter Assay.** To determine the effect of these variations on transcriptional activity of the E-cadherin promoter, the same fragment (−437 to +314) carrying all these variations was amplified by PCR using the reporter primers were the same as the above genotyping primers, except that each of them contained a NheI and a HindIII site introduced to the 5′ end, respectively. The PCR

---

**Figure 1.** Information of variations in the E-cadherin promoter and downstream region. The position +1 refer to TSS. Dark boxes, Sp1 binding sites; gray box, CAAT box; unfilled boxes, E-boxes. The variation rs number was from the National Center for Biotechnology Information SNP database (http://www.ncbi.nlm.nih.gov/SNP/). rs3833051 is a polymorphism of 13 bp (CTGCCCCAGCCCG) insertion (13N ins) or deletion (13N del). Six variations detected in the present study were underlined. **Arrows**, locations of two PCR primer sets used in the present study.

---

4 http://cancernet.cicams.ac.cn/data/epi/monitor/incidence.htm
Table 1. Information of age, sex, and selected risk factors for subjects from the high-SGC-risk area Linqu and low-SGC-risk area Beijing

<table>
<thead>
<tr>
<th>Variable</th>
<th>Linqu</th>
<th>Beijing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAG* (n = 196)</td>
<td>IM/DYS (n = 197)</td>
</tr>
<tr>
<td>Mean age ± SD</td>
<td>55.4 ± 7.3</td>
<td>55.8 ± 8.6</td>
</tr>
<tr>
<td>P</td>
<td>0.637</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>99 (50.5)</td>
<td>98 (49.7)</td>
</tr>
<tr>
<td>Female</td>
<td>97 (49.5)</td>
<td>99 (50.3)</td>
</tr>
<tr>
<td>P</td>
<td>0.880</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>H. pylori infection (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>131 (68.2)</td>
<td>155 (78.7)</td>
</tr>
<tr>
<td>No</td>
<td>61 (31.8)</td>
<td>42 (21.3)</td>
</tr>
<tr>
<td>P</td>
<td>0.020</td>
<td>0.020</td>
</tr>
<tr>
<td>Smoking status (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>83 (42.3)</td>
<td>89 (45.4)</td>
</tr>
<tr>
<td>No</td>
<td>109 (55.6)</td>
<td>107 (54.6)</td>
</tr>
<tr>
<td>P</td>
<td>0.666</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol drinking (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>88 (46.1)</td>
<td>84 (42.9)</td>
</tr>
<tr>
<td>No</td>
<td>103 (53.9)</td>
<td>112 (57.1)</td>
</tr>
<tr>
<td>P</td>
<td>0.524</td>
<td>0.003</td>
</tr>
</tbody>
</table>

* CAG and control as a reference group for SGC cases from Linqu and Beijing, respectively.

Results

Single-Locus Analysis. The frequency distribution of gender, age, H. pylori infection, cigarette smoking, and alcohol drinking for subjects from Linqu and Beijing is summarized in Table 1. Because of the differences of these factors between control and case groups, these factors were adjusted in the following statistical analysis.

Within the region (−437→+314) around the TSS (+1) of the E-cadherin, six known SNP ([−347del]A (allele A proportion, 26.5%), −163T>del (0.1%), −160C>A (20.6%), −73A>C (14.8%), +178T>C (26.3%), +234CGTCCCCAGCCC (13N) ins>del (26.5%)] were characterized among the total of 1,686 tested subjects, as underlined in Fig. 1. Because the frequency of the −163T>del was <1%, this variant was ignored in the following analysis. Linkage disequilibrium analysis showed that all variations were in strong linkage disequilibrium with each other (D’≈1), especially for −347del>A, +178T>C, and +234 13N ins>del polymorphisms (r2≈1; Supplementary Table S1). In addition, the...
genotypes of these variations in 23 cancer cell lines were also analyzed. A novel variant (+177C>G) was observed in six cell lines but not in the above tested subjects. The detailed genotypes of each cell line were listed in the Supplementary Table S2.

Among the subjects from the high-risk area Linqu, the 347del/del and its linked +178T/T and +234 13N ins/ins genotypes were found to be associated with the increased SGC risk significantly among male subjects (P = 0.026, two sides; OR, 2.22; 95% CI, 1.10-4.46) and correlated with the severity of gastric cancers (CAG 47.5%, IM/DYS 54.1%, SGC 65.8%; trend test, P = 0.053). However, such correlation could not be observed female subjects from the same area. Frequency of the 347del/del genotype among subjects with intestinal type of SGCs was slightly higher than that among subjects with diffused type of SGCs but not significant (P = 0.18; Table 2).

The risks of SGC related to 347del/del and its linked genotypes were further examined for subjects from Linqu with stratification by H. pylori infection, cigarette smoking, and alcohol drinking (Table 3). The OR of SGC for subjects carrying the 347del/del genotype or H. pylori infection alone was 2.0 (95% CI, 0.53-7.61) or 2.35 (95% CI, 0.73-7.54), respectively. However, the OR was elevated significantly for H. pylori-infected subjects carrying the del/del genotype (OR, 4.93; 95% CI, 1.65-14.71). There was an interaction between the 347del/del genotype and H. pylori infection, with the relative excess risk of interaction of 1.68 and a synergy index of 1.67.

In contrast, among subjects from the low-risk area Beijing, similar correlation was not discovered between these variations and the susceptibility of SGC. Moreover, frequencies of these SNPs were not correlated with various clinical pathologic characteristics such as histotypes, tumor locations, and lymph node/distal metastasis status either (Supplementary Table S3). Somatic mutation was not observed in the gross normal gastric tissues from SGC cases by sequencing of PCR products.

Haplotype Analysis. As all variations were in strong linkage disequilibrium with each other, there were only four common haplotypes (H1-4) with frequency higher than 3.0% among all of the tested cases and controls. Haplotype H4 is the only haplotype that carries -347A and its linked +178C and +234 13N del alleles (the protective alleles for the risk of SGC as shown in Table 2). Borderline significant difference of the frequency of the haplotype H4 [A(-347A)C(+160)A(-73A)C(+178A)13N del(+234A)] was observed between SGC cases and controls among male subjects from Linqu (P = 0.053), which decreased the SGC risk compared with haplotypes carrying 347del, +178T, and +234 13N ins alleles (OR, 0.61; 95% CI, 0.37-1.01; Table 4). Such association could not be observed among females from Linqu and subjects from Beijing (Supplementary Table S4).

Effect of the -347 Alleles on Promoter Activity. To examine the effect of the -347del and -347A alleles with their linked alleles on transcription of the E-cadherin gene, we analyzed the promoter activity of the H4 and H1 haplotypes (which were different in -347, +178, and +234 sites) using the Dual Luciferase Reporter Assay System in MCF7, MKN74, and HeLa cells. The luciferase activity of the haplotype H4 was lower than that of H1 only in MKN74 cell line but not in MCF7 and HeLa cell lines (Fig. 2).

Discussion

The E-cadherin gene covers an ~100 kb sequence including 16 exons. A number of polymorphisms and somatic mutations have also been identified within the E-cadherin gene, especially in the coding regions. Several studies showed that genetic variations such as -347del-A and -160C-A of the E-cadherin promoter might affect the susceptibility of SGCs (24-29). Lindstrom et al. (33) once reported a comprehensive study of E-cadherin genetic variants and prostate cancer risk, but only -160C-A polymorphism in the promoter was analyzed. However, the haplotypes and genotypes of the E-cadherin promoter were not studied well yet. In the present study, we analyzed the relationship between the SGC susceptibility and the genotypes and haplotypes of all these variants around the TSS region of E-cadherin.

Table 2. Allele and genotype frequency (%) of the E-cadherin promoter polymorphisms in subjects with various gastric lesions from Linqu, a high-risk area for SGC

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>Histotype of SGCs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAG</td>
<td>IM/DYS</td>
<td>SGC</td>
<td>CAG</td>
</tr>
<tr>
<td>-347del&gt;A</td>
<td>(n = 196)</td>
<td>(n = 197)</td>
<td>(n = 96)</td>
<td>(n = 99)</td>
</tr>
<tr>
<td>del/del</td>
<td>103 (52.6)</td>
<td>103 (53.3)</td>
<td>60 (62.5)</td>
<td>47 (47.5)</td>
</tr>
<tr>
<td>del/A</td>
<td>78 (39.8)</td>
<td>78 (39.6)</td>
<td>30 (31.2)</td>
<td>47 (47.5)</td>
</tr>
<tr>
<td>A/A</td>
<td>15 (7.7)</td>
<td>14 (7.1)</td>
<td>6 (6.3)</td>
<td>5 (5.1)</td>
</tr>
<tr>
<td>-160C&gt;A</td>
<td>C/C</td>
<td>123 (62.8)</td>
<td>128 (65.0)</td>
<td>53 (55.2)</td>
</tr>
<tr>
<td>C/A</td>
<td>65 (33.2)</td>
<td>61 (31.0)</td>
<td>38 (39.6)</td>
<td>33 (33.3)</td>
</tr>
<tr>
<td>A/A</td>
<td>8 (4.1)</td>
<td>8 (4.1)</td>
<td>5 (5.2)</td>
<td>5 (5.1)</td>
</tr>
</tbody>
</table>

SGC versus CAG; P = 0.026; OR, 2.22; 95% CI, 1.10 to 4.46; calculated by logistic regression; adjusted for age, H. pylori infection, smoke, and drink; trend test P = 0.053.

Genotype frequencies of the -73A/C SNP were not in Hardy-Weinberg equilibrium in IM/DYS samples (P < 0.05).
with the largest number of SGC cases investigated thus far from populations with high- and low-SGC risk, respectively.

It was reported that the frequency of the −347A allele was associated with the high risk of familial gastric carcinomas (28 cases and 142 controls) in Korea (23). In contrast, we observed that the −347A allele (and its linked +178T and +234 13N ins alleles) was a protective allele for SGC in Chinese populations because the −347del/del genotype might increase the SGC susceptibility and *H. pylori* infection elevated this susceptibility for male subjects from the high-risk area Linqu in China (Tables 3 and 4). However, only 76 of the male SGC cases were included in the present study. More investigation is needed to confirm the exact role of these genotypes in the SGC susceptibility.

In addition, all analyzed subjects from Linqu have been enrolled in a >10-year-long-term follow-up by gastroendoscopic examination (34). Thus, the important dynamic information of pathologic status of gastric mucosa is available for each subject from Linqu in the present study. We found that the severity of gastric lesions (CAG, IM/DYS, SGC) in males was correlated with the frequency of the −347del/del and its linked genotypes too (Table 2), which suggest that the −347del/del and its linked genotypes may play a role in gastric carcinogenesis through promoting progression of gastric precancerous lesions. However, among females from the same area and subjects from the low-risk area Beijing, such association could not be observed. It is well known that genetic inactivation of *E-cadherin* by germ line mutations lead to familial diffused gastric carcinomas. However, the frequency of the −347del/del genotype in patients with the intestinal type of SGCs was slightly higher than that in patients with the diffused type of SGCs (68.6% versus 55.6%, not significant). This result was consistent with the possibility that the −347del/del genotype might promote progression of gastric IM/DYS lesions.

Generally, as an “environmental sensor”, a genetically susceptible factor can easily display a role in the development of cancer among subjects under a high pressure of environmental risks. Although the causes for the elevated SGC risk for the residents in Linqu are not clear, a series of studies have identified a number of environmental factors there, including *H. pylori* infection, high intake of sour pan cakes, salted food, N-nitroso compounds, and low consumption of fresh fruits and vegetables (3, 4, 35). The *H. pylori* infection was a prevalent event there and an important SGC causal factor for subjects in Linqu (36), which would contribute to a high environmental pressure and enhance the effect of risk factor of −347del/del and its linked genotypes, as we observed in the present study (Table 3). These might account for the fact that the significant risk effect of the −347del allele on SGC could only be observed among subjects from the high-risk area Linqu. Moreover, the incidence of SGC in males is 2-fold of that in females. This might also account for the fact that the role of −347del and its linked alleles could be observed only in males from the high-risk area Linqu, but not in females from the same area. Because of the limited number of female SGC cases (n = 20) diagnosed during the long-term follow-up survey, an additional 92 female SGC cases from Linqu County Hospital were further analyzed to evaluate effect of the −347del/del genotype and female SGC susceptibility (data not shown). Again, no signifi-

### Table 3. The −347 genotype related SGC risk for male subjects from Linqu was associated with *H. pylori* infection, smoke, and drink status

<table>
<thead>
<tr>
<th>−347del/A and A/A genotype</th>
<th>−347del/del genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. (SGC/CAG)</strong></td>
<td><strong>OR (95% CI)</strong></td>
</tr>
<tr>
<td><strong>H. pylori infection</strong></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>6/18</td>
</tr>
<tr>
<td>Positive</td>
<td>20/33</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1/9</td>
</tr>
<tr>
<td>Yes</td>
<td>25/42</td>
</tr>
<tr>
<td>Alcohol drinking</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>4/10</td>
</tr>
<tr>
<td>Yes</td>
<td>22/41</td>
</tr>
</tbody>
</table>

*OR and 95% CI values were calculated by logistic regression and adjusted for age.

### Table 4. Estimated haplotype frequencies of *E-cadherin* among subjects from Linqu

<table>
<thead>
<tr>
<th>Five-marker haplotypes*</th>
<th>Frequencies (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAG</td>
<td>SGC</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1 (del-C-A-T-13N ins)</td>
<td>75 (37.9)</td>
<td>60 (39.5)</td>
</tr>
<tr>
<td>H2 (del-A-A-T-13N ins)</td>
<td>43 (21.7)</td>
<td>37 (24.3)</td>
</tr>
<tr>
<td>H3 (del-C-C-T-13N ins)</td>
<td>23 (11.6)</td>
<td>25 (16.4)</td>
</tr>
<tr>
<td>H4 (A-C-A-C-13N del)</td>
<td>57 (28.8)</td>
<td>30 (19.7)</td>
</tr>
<tr>
<td>All</td>
<td>198 (100.0)</td>
<td>152 (100.0)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1 (del-C-A-T-13N ins)</td>
<td>75 (38.7)</td>
<td>13 (32.5)</td>
</tr>
<tr>
<td>H2 (del-A-A-T-13N ins)</td>
<td>38 (19.6)</td>
<td>11 (27.5)</td>
</tr>
<tr>
<td>H3 (del-C-C-T-13N ins)</td>
<td>30 (15.5)</td>
<td>4 (10.0)</td>
</tr>
<tr>
<td>H4 (A-C-A-C-13N del)</td>
<td>51 (26.3)</td>
<td>12 (30.0)</td>
</tr>
<tr>
<td>All</td>
<td>194 (100.0)</td>
<td>40 (100.0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1 (del-C-A-T-13N ins)</td>
<td>150 (38.3)</td>
<td>73 (38.0)</td>
</tr>
<tr>
<td>H2 (del-A-A-T-13N ins)</td>
<td>81 (20.7)</td>
<td>48 (25.0)</td>
</tr>
<tr>
<td>H3 (del-C-C-T-13N ins)</td>
<td>53 (13.5)</td>
<td>29 (15.1)</td>
</tr>
<tr>
<td>H4 (A-C-A-C-13N del)</td>
<td>108 (27.6)</td>
<td>42 (21.9)</td>
</tr>
<tr>
<td>All</td>
<td>392 (100.0)</td>
<td>192 (100.0)</td>
</tr>
</tbody>
</table>

*OR and 95% CI values were calculated by logistic regression and adjusted for age.

*P = 0.053.
The ethnic diversity of these cell lines might account for the decreased linkage. The exact biological meanings of this linkage need to be studied further. The protective effect of the \(-347A\) containing haplotypes H4 among subjects from Linqu suggests that the strong linkage between these alleles might contribute to the risk of SGCs.

The proximal E-cadherin promoter contains multiple characterized cis-acting elements, such as E-boxes, CAAT box, and GC-rich element (Sp1-binding site), as shown in Fig. 1. It was reported that deletion or mutation of these elements might be detrimental to the activity of the E-cadherin promoter, but deletion of upstream sequences had no effect on promoter activity (39, 40). Shin et al. (23) reported that the \(-347\) del allele increased the transcriptional efficiency of E-cadherin in several cell lines (AGS, CV-1, HeLa, KATO-III, and Sun-719) compared with the \(-347\) A allele. However, Nakamura et al. (41) reported that variants \(-347\) del and \(-347\) A displayed the same promoter activity in the CV-1 cell line. The promoter fragment \((-647+147) or (-410+125)\) they used did not cover the \(+178\) and \(+234\) alleles, which we found linked with the \(-347\) allele almost completely. To elucidate whether the \(-347\) variants affect the promoter activity in their related haplotype patterns, we analyzed the transcription activity of the fragment \((-437+314)\) covering all these alleles, including the linked \(+178\) and \(+234\) SNPs around the E-cadherin TSS. We did not observe such difference between the most frequent \(-347\) del related haplotype H1 [\(\text{A}(\text{C}-\text{C}-\text{C}-\text{A}-13\text{N} \ \text{ins})\)] and the low-risk \(-347\) A-related haplotype H4 [\(\text{A}(\text{C}-\text{C}-\text{C}-\text{A}-13\text{N} \ \text{del})\)] in the MCF7 and HeLa cell lines by the Dual Luciferase Reporter Assay. In contrast, the promoter activity of the \(-347\) A haplotype H4 was even significantly lower than that of the H1 in MKN74 cell line (Fig. 2). More study is necessary to illustrate whether different length and localization of tested promoter fragments and transfected cell lines might result in different transcriptional changes in the luciferase promoter activity assay. Alterations of transcriptional activity of promoters with various SNPs by the assay might not always correlate with their contribution to disease susceptibility.

In conclusion, the present study shows that \(-347\) del allele is strongly linked with \(+178\) and \(+234\) alleles almost completely and that the \(-347\) del/del and its linked genotypes of E-cadherin may increase the susceptibility of SGC among males in the high-risk area of China.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**References**

Polymorphisms of E-Cadherin and Gastric Carcinoma


Genetic Polymorphisms of the *E-Cadherin* Promoter and Risk of Sporadic Gastric Carcinoma in Chinese Populations

Baozhen Zhang, Kaifeng Pan, Zhaojun Liu, et al.


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/17/9/2402

Supplementary Material
Access the most recent supplemental material at:
http://cebp.aacrjournals.org/content/suppl/2009/04/07/17.9.2402.DC1

Cited articles
This article cites 41 articles, 11 of which you can access for free at:
http://cebp.aacrjournals.org/content/17/9/2402.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://cebp.aacrjournals.org/content/17/9/2402.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cebp.aacrjournals.org/content/17/9/2402.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.