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Familial Clustering of Gastric Cancer

A Retrospective Study Based on the Number of First-Degree Relatives

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Abstract: This comprehensive cross-sectional study aimed to identify factors contributing to familial aggregation of gastric cancer (GC). A total of 1058 GC patients and 1268 controls were analyzed separately according to the presence or absence of a first-degree relative of GC (GC-relative). Logistic regression analysis adjusted for age, gender, residence during childhood, smoking, alcohol intake, monthly income, spicy food ingestion, *Helicobacter pylori* status and host cytokine polymorphisms was performed. Cytotoxin-associated gene A (*cagA*) positivity was a distinctive risk factor for GC in the family history (FH)-positive group (odds ratio [OR], 2.39; 95% confidence interval [CI], 1.42–4.00), while current/ex-smoker, moderate to strong spicy food ingestion, and non-B blood types were more closely associated with GC in the FH-negative group. Among the FH-positive group, alcohol consumption showed a synergistic carcinogenic effect in the at least 2 GC-relatives group compared to the 1 GC-relative group (1.71 vs. 9.58, P for interaction = 0.026), and this was dose-dependent. In the subjects with ≥2 GC-relatives, *TGFB1*-509T/T was a risk factor for GC (OR 23.74; 95% CI 1.37–410.91), as were rural residency in childhood, alcohol consumption, spicy food ingestion, and *cagA* positivity. These results suggest that subjects with FH may be a heterogeneous group in terms of gastric cancer susceptibility. Especially, subjects with ≥2 GC-relatives should undergo risk stratification including *TGFB1*-509T/T and alcohol consumption.

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Abbreviations: *cagA* = cytotoxin-associated gene A, CI = confidence interval, FH = family history, GC = gastric cancer, HDGC = hereditary diffuse gastric cancer, HP = *Helicobacter pylori*, IL = interleukin, IM = intestinal metaplasia, OR = odds ratio, TGF = transforming growth factor, vacA = vacuolating toxin A.

INTRODUCTION

Gastric cancer (GC) is the 5th Supplemental Content common cancer globally and the third most frequent cause of death from cancer.[1] It is believed that the risk factors for GC differ according to the histological type and location of the tumor. The best-established risk factor is *Helicobacter pylori* (HP) infection.[2] In a meta-analysis of 19 cohort or case–control studies, the summary odds ratio (OR) for GC was estimated to be 1.92 (95% confidence interval [CI], 1.32–2.78) in HP-infected subjects compared to uninfected subjects.[3]

Family history (FH) of stomach cancer is also a strong risk factor for GC,[4] but the association has been less extensively investigated than HP infection. In most studies, the familial relative risk for GC was reported to be approximately 3-fold, which is higher than those for most other adult solid cancers, with the exception of ovarian cancer.[4] In accordance with this, we demonstrated previously that having 1st-degree relatives with GC (GC-relative) increased the risk of GC by almost 3-fold (OR, 2.85; 95% CI, 1.83–4.46).[5]

Although many individuals with FH are concerned about their risk of developing GC, guidelines for the assessment of the FH of individuals with GC have not been developed, unlike other common cancers. Fundamentally, there has been a lack of attention to the definition of familial GC, characteristics of GC with FH, and molecular basis of GC in a family.

Hereditary diffuse gastric cancer (HDGC) is the most famous familial GC, which is characterized by *CDH1* deletion. However, HDGC is rare, 0.3%–3.1% in Korea and Japan,[6] and the known cancer syndromes do not account for a large portion of the familial clustering.[7]

Indeed, FH, itself is a mixture of various factors shared by family members, from exposure to the same carcinogens (i.e., nitrogen, cigarette smoke, and alcohol) to levels of hygiene, dietary habits, bacterial virulence, and genetic susceptibility. In our previous study,[8] we suggested that a comprehensive approach, which includes a larger number of subjects with a first-degree GC family member and covers HP virulence factors, genetic polymorphisms (transforming growth factor [TGF]-β1 and interleukin-1 [IL]), environmental and dietary factors simultaneously, is necessary to identify high-risk individuals for GC development in FH-positive subjects. We hypothesized that a group with 2 or more GC-relatives could be at a higher risk for GC development compared to a

group with a single GC-relative, and the underlying mechanism could be different. To assess this, the underlying factors for familial clustering were investigated by comparing variables between GC patients and control subjects according to their number of GC-relatives.

METHODS

Study Patients

Subjects were enrolled at Gastroenterology clinics, Seoul National University Bundang Hospital from March 2006 to October 2015. Among those who had undergone a standard upper gastroscopy and biopsy of the antrum and body for HP tests, a total of 1058 GC patients and 1268 control subjects were analyzed. Patients with no endoscopic evidence of GC, dysplasia, mucosa-associated lymphoid tissue lymphoma, esophageal cancer, or peptic ulcer disease at the time of the enrollment were assigned to the control group. Patients with pathologically confirmed primary gastric adenocarcinoma were allocated into the GC group. No patient had HDGC. Tumors located within 2 cm from the gastroesophageal junction were defined as cardia GC and beyond that as noncardia GC.[9]

''GC-relative'' was defined as a 1st-degree relative (parent, sibling, or offspring) diagnosed with GC and a ''positive family history'' was defined as having any 1st-degree GC-relatives. In addition, all patients who provided informed consent were asked to complete a questionnaire under the supervision of a trained interviewer. The questionnaire included questions regarding demographics (age, gender, and residence of childhood and current residence) and socioeconomic data (smoking, drinking, and income). Some clinical data, including histologic review, were collected using the electronic medical chart system. The study protocol was approved by the Ethics Committee at Seoul National University Bundang Hospital (B-0903/071-001, B-1103-123-004, and B-0602-030-001).

H pylori Testing and Histology

To determine the HP infection status, histologic evaluation with the Giemsa method, rapid urease test (CLO test, Delta West, Bentley, Australia), culture study, and anti-HP test (Genedia ELISA; Green Cross Medical Science Corp, Eumsung, Korea) was performed.[8] HP identification by any of the 1st 3 invasive methods was defined as current-infection. If the HP serology was positive, but no bacteria were found in the invasive studies, it was defined as a previous HP infection. The histological features of the gastric mucosa were recorded using the updated Sydney scoring system (i.e., 0 = none, 1 = slight, 2 = moderate, and 3 = marked).[10]

H pylori Genotypes and Cytokine Genetic Polymorphisms

Genomic DNA was obtained from homogenates of antral biopsy specimens using phenol/chloroform extraction method and ethanol precipitation.[8] Polymerase chain reaction (PCR) amplifications for cytotoxin-associated gene A (*cagA*) and vacuolating toxin A (*vacA*) were conducted as described previously.[8,11] Regarding polymorphisms, 3 cytokine genes (*IL-1B*-511, *IL-1RN*, and *TGFB1*-509) reported to be associated with GC were evaluated. Host DNA polymorphisms were evaluated by PCR-restriction fragment length polymorphism analysis using Perkin Elmer model 9600 (Perkin Elmer Co., Norwalk, CT). For *TGFB1*-509 C/T polymorphism (rs1800469), specific primer sequences were designed using NCBI's Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome). Primers for *cagA*, *vacA*, and *IL-1B*-511 have been published previously.[12] *IL-1RN* penta-allelic variable number of tandem repeats are listed[13] in Supplementary Content S1, http://links.lww.com/MD/A947. These alleles were coded as follows: allele 1, 4 repeats of the 86-bp region (410 bp); allele 2, 2 repeats (240 bp); allele 3, 5 repeats (500 bp); allele 4, 3 repeats (325 bp); and allele 5, 6 repeats (595 bp); rare alleles 3, 4, and 5 and the allele 1 were categorized into 1 group, long (L) allele as described previously.[8,13]

Genotyping of ABO Blood Type

Three loci on the ABO gene chromosome 9q34.2 – rs8176719, rs8176746, and rs8176747 – were examined. The custom probes and primers for the characterization of the 3 loci by PCR were used together with a StepOnePlus real-time PCR instrument (Applied Biosystems, Foster City, CA).

Covariates

Age (years) was taken as a continuous variable. HP infection status was categorized into 2 groups: current or past infection and none. FH of GC was categorized according to the number of 1st-degree family members with GC: 0, 1, and 2 or more. Smoking status was categorized into never- and current/ex-smoker. Amount of alcohol intake was approximated on a weekly basis based on the frequency of drinking and the number of glasses of Korea's most popular alcoholic beverage, ''Soju'' or beer each time.[14] The total standard units (1 U = 12 g of ethanol) of alcohol consumed per week were then calculated and categorized into never/rare (0–1.9 U/wk), or ex-drinkers or current drinkers who consumed 2 to 11.9 U/wk (light drinkers), or 12 U/wk (heavy drinkers), as described previously.[14] Childhood residency was categorized into urban and rural areas. Socioeconomic status was defined by dividing monthly income into 2 groups: a monthly income >US $5000 or ≤US $5000.

Preference for a salty and spicy diet was defined by how salty and spicy the subjects food usually: not, moderately and strongly and consequently into not and moderately/strongly. Spicy food was defined as dishes with chili pepper seasoning. Intake of fruit was measured by how many times per week fruit are taken: everyday, 3 times per week, and rarely. Both the genotypes and phenotypes of the *ABO* gene were used in the analyses.

Statistical Analysis

Continuous variables were analyzed by Student *t* test. The χ^2 test and Fisher exact test were used for analysis of categorical variables. The allele frequency was determined by direct counting, and deviation of genotype distribution from Hardy–Weinberg equilibrium was analyzed by χ^2 test. In addition to variables that showed a significant difference in univariate analyses between patients and controls, HP toxin and genetic factors were preferentially entered into a model to identify genetic factors. Then, multivariable analyses were performed by logistic regression with backward deletion to evaluate the best model. Differences were considered statistically significant when the *P* value was less than 0.05. All analyses were carried out using the SPSS software (version 21.0, IBM, Armonk, NY).

RESULTS

General Characteristics

A total of 913 control subjects and 840 GC patients in the FH-negative group and 355 control subjects and 218 GC

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patients in the FH-positive group were included in the final analyses. Approximately one-half of the control subjects had dyspeptic symptoms at the time of enrollment; the others were participants in a screening program for GC. Since many healthy subjects with an FH have visited our clinic worried about an increased risk of GC, a higher proportion of subjects with FH were included in the control group than the GC group (30.0% vs. 20.6%). Patients with GC numbers 840 (47.9%) in the group with an FH and 218 (38.1%) in the group without an FH.

Stratification of study population according to FH was conducted. Subject characteristics and univariate analyses are listed in Table 1. Increased age, male gender, rural residency in childhood, current or ex-smokers, alcohol consumption and currently low income (<$5000/month), intestinal metaplasia (IM), HP infection, and *cagA* positivity were risk factors regardless of FH. With regard to genetic polymorphisms of cytokines, no genetic polymorphism was associated with an increased risk of GC (Table 1). In the FH-negative group, ingestion of moderate to strong spicy food and non-B blood type were also associated with increased GC risk.

Multivariable Analysis and Risk Factors of Gastric Cancer According to Family History

The ORs for GC determined by separate multivariable analyses according to the presence or absence of FH of GC are listed in Table 2. Increased age, rural residency in childhood, alcohol consumption, HP infection, gastric antrum IM, and a current monthly income <$5000 were independent risk factors for GC in both the FH-positive and FH-negative groups.

While smoking, ingestion of moderate to strong spicy food, gastric corpus IM, and non-B blood type were associated with an increased risk of GC only in the FH-negative group, *cagA* showed a significant association with an increased risk of GC only in FH-positive group (Table 2).

TABLE 1. Comparison of Clinicopathologic Variables With Regard to the Family History of GC

	Family History (−) (n = 1753)			**Family History (+) (n = 573)**		
Variables (Reference)	**Control (n = 913)**	**GC (n = 840)**	**OR (95% CI)**	**Control (n = 355)**	**GC (n = 218)**	**OR (95% CI)**
Age, year, median (IQR)	56 (46–66)	62 (51–70)	**1.03 (1.02–1.04)**	52 (44–61)	62 (53–69)	**1.07 (1.05–1.09)**
Male gender (women)	481 (52.7)	564 (67.1)	**1.84 (1.52–2.23)**	148 (41.8)	144 (66.1)	**2.67 (1.88–3.80)**
Rural residency in childhood (urban)	384 (44.1)	467 (58.6)	**1.81 (1.49–2.19)**	161 (48.1)	146 (69.2)	**2.42 (1.68–3.48)**
Current/ex-smoking (never)	432 (47.7)	521 (62.8)	**1.85 (1.53–2.25)**	122 (35.0)	127 (58.3)	**2.55 (1.80–3.62)**
Current/ex-alcohol consumption (never)	396 (43.4)	478 (56.9)	**1.72 (1.43–2.08)**	128 (36.1)	118 (54.1)	**2.09 (1.48–2.95)**
Monthly income <$5000 (>$5000/month)	556 (68.0)	583 (80.4)	**1.94 (1.53–2.45)**	214 (68.6)	166 (83.0)	**2.24 (1.45–3.48)**
Mod to strong salty diet (no)	669 (76.5)	636 (79.9)	1.22 (0.97–1.55)	260 (77.8)	168 (81.6)	1.26 (0.81–1.95)
Mod to strong spicy food intake (no)	634 (73.0)	625 (79.5)	**1.44 (1.14–1.81)**	240 (72.7)	166 (79.8)	1.47 (0.97–2.23)
Fruit intake >3/wk (≤3/wk)	173 (20.0)	187 (23.9)	**1.19 (1.04–1.41)**	54 (16.2)	52 (25.1)	**1.46 (1.15–1.87)**
Non-B blood type (B)	537 (70.0)	629 (76.9)	**1.43 (1.14–1.79)**	244 (73.9)	164 (76.6)	1.13 (0.76–1.69)
Antrum IM (none)	276 (30.9)	553 (67.0)	**4.51 (3.68–5.53)**	108 (31.0)	145 (67.1)	**4.67 (3.24–6.73)**
Corpus IM (none)	138 (15.5)	368 (44.3)	**4.32 (3.45–5.43)**	76 (21.8)	93 (42.9)	**2.74 (1.89–3.97)**
Current/past HP (negative)	682 (74.7)	712 (84.8)	**1.89 (1.48–2.40)**	255 (71.8)	192 (88.1)	**2.88 (1.80–4.61)**
cagA (negative)	236 (29.9)	272 (35.6)	**1.30 (1.05–1.61)**	80 (28.7)	90 (44.8)	**1.99 (1.36–2.92)**
vacA m1 (negative)	323 (36.1)	314 (37.6)	1.07 (0.88–1.30)	119 (34.0)	86 (39.8)	1.29 (0.91–1.84)
TGFB1-509						
[C/C]	226 (25.5)	184 (22.3)	1 (Referent)	94 (27.0)	65 (30.5)	1 (Referent)
C/T	448 (50.5)	459 (55.7)	1.26 (1.00–1.59)	172 (49.4)	97 (45.5)	0.81 (0.54–1.21)
T/T	213 (24.0)	181 (22.0)	1.00 (0.79–1.38)	82 (23.6)	51 (23.9)	0.90 (0.56–1.44)
*IL-1RN**2 carriers (noncarrier)	126 (14.0)	96 (11.5)	0.80 (0.60–1.06)	47 (13.4)	23 (10.6)	0.77 (0.45–1.31)
IL-1B-511 T allele (C/C)	740 (82.0)	663 (79.8)	0.87 (0.68–1.10)	287 (81.8)	169 (78.3)	0.80 (0.52–1.22)
Intestinal: diffuse	0	481:340	(–)	0	131:81*	(–)
Noncardia: cardia	0	767:74	(–)	0	193:24*	(–)
Affected family member						
Mother (no)	0	0	(–)	123 (35.0)	63 (28.8)	0.77 (0.54–1.11)
Father (no)	0	0	(–)	148 (41.8)	83 (37.9)	0.88 (0.62–1.24)
Sister (no)	0	0	(–)	52 (14.8)	33 (15.3)	1.05 (0.65–1.68)
Brother (no)	0	0	(–)	75 (21.1)	60 (27.6)	1.43 (0.96–2.11)
Offspring (no)	0	0	(–)	1 (0.3)	7 (3.2)	**11.80 (1.44–96.58)**
GC-relative = 1	0	0	(–)	308 (86.8)	188 (86.2)	(–)
GC-relative ≥2	0	0	(–)	47 (13.2)	30 (13.8)	(–)

Some values were absent from the pathology reports; therefore, these numbers may not be equal to the number of total number of subjects. Data are presented as n (%) unless otherwise indicated. Bold style indicates the statistical significance. *cagA* = cytotoxin-associated gene A, CI = confidence interval, GC = gastric cancer, HP = *Helicobacter pylori*, IL = interleukin, IM = intestinal metaplasia, IQR = interquartile range, OR = odds ratio, SD = standard deviation, TGF = transforming growth factor, *vacA* = vacuolating toxin A.

*Not significant between family history-positive and-negative cancer groups.

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TABLE 2. Independent Risk Factors Associated With GC According to Family History by Multivariable Analyses

Family History (−)			Family History (+)		
Variables	***P* Value***	**aOR* (95% CI)**	**Variables**	***P* Value***	**aOR* (95% CI)**
Age	0.051	1.01 (1.00–1.02)	Age	**<0.001**	1.07 (1.04–1.09)
HP infection	**<0.001**	2.27 (1.61–3.18)	HP infection	**0.017**	2.37 (1.17–4.83)
Gastric antrum IM	**<0.001**	2.38 (1.78–3.18)	Gastric antrum IM	**<0.001**	2.46 (1.50–4.04)
Gastric corpus IM	**<0.001**	2.79 (2.03–3.84)			
Rural residency in childhood	**0.002**	1.53 (1.27–2.01)	Rural residency in childhood	**0.003**	2.14 (1.31–3.51)
Income <$5000/month	**<0.001**	1.71 (1.17–2.31)	Income <$5000/month	**0.016**	2.10 (1.15–3.82)
Alcohol	**<0.001**	2.69 (1.58–4.57)	Alcohol	**0.040**	1.83 (1.03–3.25)
Smoker (current/ex)	**<0.001**	2.03 (1.42–2.91)			
Spicy food ingestion	**0.047**	1.37 (1.00–1.88)	Male gender	0.052	1.72 (1.00–2.96)
Non-B blood type	**0.021**	1.42 (1.05–1.91)	*cagA*	**0.001**	2.39 (1.42–4.00)

Bold style indicates the statistical significance. aOR = adjusted odds ratio, *cagA* = cytotoxin-associated gene A, CI = confidence interval, GC = gastric cancer, HP = *Helicobacter pylori*, IM = intestinal metaplasia.

*Logistic model including terms for age, gender, HP infection, current income, residency during childhood, smoker, alcohol consumption, alcohol consumption by smoker, diet of spicy/salty food, fruit intake, intestinal metaplasia, non-B blood type, *cagA*, *IL-1RN*2 carriers, and *TGFB1*-509 T carriers.

These results did not differ greatly if the multivariable analysis was restricted to noncardia GC (Supplementary Content S2, http://links.lww.com/MD/A947). However, in the case of cardia GC, while gastric corpus IM was associated with cardia GC regardless of FH, HP infection showed no association (FH-negative cardia cancer: gastric corpus IM: OR, 5.70, 95% CI, 3.17–10.26; rural residency in childhood: OR, 2.47, 95% CI, 1.34–4.52; and alcohol consumption: OR, 2.30, 95% CI, 1.68–5.35) (FH-positive cardia cancer: gastric corpus IM: OR, 5.19, 95% CI, 1.82–14.60 and age: OR, 1.10, 95% CI, 1.04–1.16).

Risk Factors for Gastric Cancer According to the Number of Affected Relatives

The FH-positive group was divided into 2 categories: 1 GC-relative and 2 or more GC-relatives to evaluate the familial aggregation of GC (Table 3).

Residence in a rural area in childhood, current or ex-smoking, and alcohol intake were risk factor for GC in groups with single or ≥2 GC-relatives (Table 3). Moreover, there was a synergistic interaction between alcohol consumption and GC risk in the group with ≥2 GC-relatives. That is, the OR for drinking in this group was 5-fold higher than that in the group with 1 GC-relative (9.58 vs. 1.71, *P* for interaction = 0.026). When the amount of alcohol was stratified, heavy drinker (more than 144 g ethanol/wk) showed the highest synergistic effect compared to none (≥6 g ethanol/wk), light (6–144 g ethanol/wk), and ex-alcohol user (Table 4). In contrast, HP infection was more closely associated with GC patients in the group with a single GC-relative than the group with at least 2 GC-relatives (3.70 vs. 1.05, *P* for interaction = 0.035) (Table 3).

When multivariable analyses were performed in the 1 GC-relative and 2 or more GC-relative groups, respectively, the significant risk factors for GC in subjects with a single GC-relative were almost identical to those in the total FH-positive subjects (Table 5). Only alcohol consumption was excluded from the risk factors. However, when the subjects were restricted to those with 2 or more GC-relatives, having *TGFB1*-509T/T was a risk factor for GC, together with rural residency in childhood, alcohol consumption, moderate to strong spicy food ingestion, and *cagA* positivity (Table 5).

Characteristics of Gastric Cancer Patients According to Affected Family Member

To evaluate the characteristics of GC patients according to the affected relative, univariate analyses were performed (Table 6).

The GC group with a maternal history had an overall larger number of affected relatives than the group with an affected father or affected siblings or offspring. When multivariable logistic analysis adjusted for age, gender, HP infection, and alcohol consumption was performed in GC patients with FH, positive maternal history was a risk factor for having 2 or more GC-relatives.

Moreover, patients in the GC group with an affected father were younger than those in the group with an affected mother and with siblings affected only. In the regression analysis adjusted for gender, HP infection, and *cagA* and alcohol consumption, paternal history was independent risk factors for early diagnosis compared to GC with maternal history ($\beta = -4.074$, $P = 0.026$).

However, when the 30 GC patients with 2 or more affected GC-relatives were analyzed, there were no significant differences according to the number of affected family members, gender, Lauren histologic types, diet, rural residency, and any polymorphism according to each combination of family members) (Supplementary Content S3, http://links.lww.com/MD/A947).

DISCUSSION

We set out to estimate the risks of genetic, bacterial, and environmental factors for the development of GC in subjects with an FH. Rural residency and *cagA* positivity were consistent risk factors for general FH-positive GC. Alcohol consumption had a synergistic effect on developing GC with an increasing number of affected relatives. Carrying *TGFB1*-509T/T was a risk factor for GC in the multivariable analysis among subjects with ≥2 GC-relatives.

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TABLE 3. Comparison of Clinicopathologic Variables With Regard to the Number of Affected Relatives of GC

	Group With 1 GC-Relative			Group With ≥2 GC-Relatives			
Variables	**Control (n = 308)**	**GC (n = 188)**	**OR (95% CI)**	**Control (n = 47)**	**GC (n = 30)**	**OR (95% CI)**	P_{Int}*
Age, year, median (IQR)	51 (43–60)	62 (52–69)	**1.07 (1.05–1.09)**	57 (49–65)	63 (55.0–72.3)	**1.08 (1.02–1.13)**	0.807
Male gender	134 (43.5)	122 (64.9)	**2.38 (1.64–3.46)**	14 (30.4)	22 (73.3)	**5.87 (2.13–16.19)**	0.102
Rural residency	141 (48.1)	120 (66.7)	**2.14 (1.46–3.15)**	22 (52.4)	26 (89.7)	**7.54 (1.98–28.69)**	0.076
Smoker (current/ex)	108 (35.6)	105 (55.9)	**2.23 (1.54–3.23)**	14 (30.4)	22 (73.3)	**6.48 (2.33–18.02)**	0.054
Alcohol	116 (37.7)	95 (50.8)	**1.71 (1.18–2.47)**	12 (25.5)	23 (76.7)	**9.58 (3.29–27.95)**	**0.026**
Income <$5000/month	189 (68.2)	144 (83.7)	**2.42 (1.50–3.90)**	25 (71.4)	22 (78.6)	1.41 (0.44–4.50)	0.584
Spicy food	208 (72.0)	138 (77.1)	1.31 (0.85–2.02)	32 (78.0)	28 (96.6)	7.64 (0.91–64.03)	0.111
Fruit intake >3/wk	41 (14.0)	44 (24.7)	**1.50 (1.15–1.96)**	13 (31.7)	8 (27.6)	1.26 (0.68–2.33)	0.155
Non-B blood	210 (73.2)	143 (76.9)	1.20 (0.78–1.84)	34 (79.1)	21 (75.0)	0.77 (0.25–2.38)	0.475
Antral IM	91 (29.9)	127 (67.9)	**5.18 (3.49–7.70)**	17 (38.6)	18 (62.1)	2.46 (0.94–6.40)	0.158
Corpus IM	67 (22.0)	78 (41.7)	**2.62 (1.76–3.90)**	9 (20.0)	15 (50.0)	**3.60 (1.32–9.80)**	0.562
HP infection	221 (71.8)	170 (90.4)	**3.70 (2.14–6.38)**	34 (72.3)	22 (73.3)	1.05 (0.37–2.95)	**0.035**
cag A	71 (29.0)	76 (44.4)	**1.94 (1.28–2.91)**	9 (26.5)	14 (46.7)	2.43 (0.85–6.92)	0.691
TGFB1-509 C/C	78 (25.7)	57 (31.1)	1 (Reference)	16 (35.6)	8 (26.7)	1 (Reference)	
TGFB1-509 C/T	150 (49.5)	82 (44.8)	0.74 (0.48–1.14)	22 (48.9)	15 (50.0)	1.36 (0.47–3.99)	0.330
TGFB1-509 T/T	75 (24.8)	44 (24.0)	0.80 (0.48–1.33)	7 (15.6)	7 (23.3)	2.00 (0.50–7.70)	0.214
*IL-1RN**2 carriers	42 (13.9)	19 (10.2)	0.71 (0.40–1.27)	5 (10.6)	4 (13.3)	1.29 (0.32–5.26)	0.440
IL-1B-511 T allele	247 (81.3)	145 (77.9)	0.81 (0.52–1.27)	40 (85.1)	24 (80.0)	0.70 (0.21–2.33)	
Affected family member							
Mother	98 (31.8)	46 (24.6)	0.70 (0.46–1.05)	22 (46.8)	13 (43.3)	0.77 (0.46–2.89)	0.333
Father	128 (41.6)	72 (38.5)	0.89 (0.62–1.29)	20 (42.6)	11 (36.7)	0.78 (0.31–2.00)	0.797
Sister	36 (11.7)	21 (11.2)	0.96 (0.54–1.69)	16 (34.0)	12 (40.0)	1.29 (0.50–3.33)	0.594
Brother	45 (14.6)	41 (21.9)	**1.64 (1.03–2.62)**	30 (63.8)	19 (63.3)	0.98 (0.38–2.54)	0.340
Offspring	1 (0.3)	7 (3.7)	11.94 (1.46–97.82)	0	0	(–)	
Intestinal: diffuse 0	112:70	(–)	0	19:11	(–)	0.851†	
Noncardia: cardia 0	167:20	(–)	0	26:4	(–)	0.753†	

Some values were absent from the pathology reports; therefore, these numbers may not be equal to the number of total number of subjects. Data are presented as n (%) unless otherwise indicated. Bold style indicates the statistical significance. GC = gastric cancer, HP = *Helicobacter pylori*, IL = interleukin, IM = intestinal metaplasia, Int = interaction, IQR = interquartile range, SD = standard deviation, TGF = transforming growth factor.

*This can assess the size or direction of effect of each variable on developing GC in the group with ≥2 GC-relatives compared with that in the group with single GC-relative.

†Compared between the group with 1 GC-relative and the group with ≥2 GC-relatives.

Although several studies reported that having a 1st-degree GC-relative was a consistent risk factor for GC,[15–17] the molecular basis responsible for the familial aggregation of GC remains unknown. First, HP infection or virulence factors can be transferred within families. FH had a synergistic effect on developing GC with HP infection.[5] Infection with *cagA*-positive HP strains and a positive FH appear to be strong independent risk factors for GC.[18] In the present study, *cagA*

TABLE 4. OR for the GC With Regard to the Amount of Alcohol Consumption and Number of Affected Relatives of GC

	Group With 1 GC-Relative			Group With ≥2 GC-Relatives			
Alcohol*	**Control (n = 308)**	**GC (n = 188)**	**OR (95% CI)**	**Control (n = 47)**	**GC (n = 30)**	**OR (95% CI)**	P_{Int}†
Never/rare	192 (62.3)	92 (49.2)	1 (Referent)	35 (74.5)	7 (23.3)	1 (Referent)	
Light	60 (19.5)	35 (18.7)	1.22 (0.75–1.98)	10 (21.3)	9 (30.0)	**4.50 (1.34–15.12)**	0.392
Heavy	31 (10.1)	29 (15.5)	**1.95 (1.11–3.43)**	1 (2.1)	11 (36.7)	**55.0 (6.08–497.42)**	**0.022**
Ex	25 (8.1)	31 (16.6)	**2.59 (1.45–4.63)**	1 (2.1)	3 (10.0)	**15.0 (1.36–166.05)**	0.456

Bold style indicates the statistical significance. CI = confidence interval, GC = gastric cancer, OR = odds ratio.

*Never/rare drinker refers to a nondrinker or those who drink ≤0.5 U/wk; 0.5 U/wk < light drinker < 12 U/wk; heavy drinker ≥12 U/wk (1 U = 12 g of ethanol).

†This can assess the size or direction of effect of each variable on developing GC in the group with ≥2 GC-relatives compared with that in the group with single GC-relative.

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TABLE 5. Risk Factors for Family History–Positive GC According to the Number of Affected 1st-Degree Relative by Multivariable Analyses

	1 GC-Relative*		≥2 GC-Relatives†		
Variables	***P* Value**	**aOR‡(95% CI)**	**Variables**	***P* Value**	**aOR‡(95% CI)**
Male gender	**0.002**	2.24 (1.33–3.77)			
Age	**<0.001**	1.06 (1.034–1.09)			
Antrum IM	**<0.001**	2.80 (1.65–4.75)			
Current income (<$5000/month)	**0.017**	2.19 (1.15–4.16)			
HP infection	**0.024**	2.50 (1.13–5.55)			
cagA	**0.006**	2.17 (1.25–3.77)	*cagA*	**0.038**	9.06 (1.12–72.97)
Rural residency in childhood	**0.002**	1.85 (1.10–3.12)	Rural residency in childhood	**0.004**	26.49 (2.90–241.99)
			Alcohol consumption	**0.002**	27.83 (3.37–230.16)
			≥Moderate spicy food ingestion	**0.004**	65.74 (3.91–105.99)
			TGFB1-509 C/T	0.267	3.42 (0.39–30.05)
			TGFB1-509 T/T	**0.029**	23.74 (1.37–410.91)

cagA = cytotoxin-associated gene A, GC = gastric cancer, aOR = adjusted odds ratio, CI = confidence interval, HP = *Helicobacter pylori*, IM = intestinal metaplasia, TGF = transforming growth factor.

*For 308 control subjects and 188 patients with gastric cancer.

†For 47 control subjects and 30 patients with gastric cancer.

‡Logistic model including terms for age, gender, HP infection, HP infection by gender, current income, residency during childhood, smoker, alcohol consumption, alcohol consumption by smoker, diet of salty/spicy food, fruit intake, intestinal metaplasia, non-B blood type, *cagA*, *IL-1RN**2 carriers, and *TGFB1*-C509T polymorphism (reference *TGFB1*-509C/C).

positivity was a significant risk factor for GC in the FH-positive group (OR, 2.39; 95% CI, 1.42–4.00). This association was more prominent when the subjects were restricted to those with more than one GC-relative (*cagA* positivity: OR, 9.06; 95% CI, 1.12–72.97). The frequency of *cagA* positivity detected using a culture-based method is ~90% in our research team.[19] In the present study, gastric mucosa from all participants (including HP-negative subjects) were analyzed for *cagA* due to the time and cost of the culture-based method. This may decrease *cagA* positivity to lower than the expected value.

Although familial risk suggests a hallmark of genetic susceptibility, the genetic abnormalities in GC seem to be

TABLE 6. Characteristics of Patients With GC According to Different Parental History of GC

	Affected Family Member				
Variables	**Mother (n = 59)**	**Father (n = 79)**	**Only Sibling/ Offspring (n = 75)**	***P* Value***	***P* Value†**
Male gender, n, %	41 (69.5)	53 (67.1)	47 (62.7)	0.694	0.764
Age at diagnosis, mean (SD)	62.2 (9.2)	57.0 (11.4)	64.5 (9.8)	**<0.001**	**0.012**
GC-relative ≥2, n, %	13 (22.0)	7 (8.9)	6 (8.0)	**0.025**	**0.030**
No of affected family member, mean (SE)	1.36 (0.98)	1.10 (0.34)	1.08 (0.27)	**0.012**	**0.034**
HP infection, n, %	64 (85.3)	72 (91.1)	52 (88.1)	0.694	0.563
cagA, n, %	17 (30.9)	33 (46.5)	37 (52.9)	**0.045**	0.076
Rural residency, n, %	39 (68.4)	48 (65.8)	53 (71.6)	0.745	0.748
Alcohol consumption, n, %	29 (49.2)	39 (49.4)	30 (40.5)	0.477	0.980
Spicy food ingestion, n, %	44 (77.2)	60 (83.3)	58 (77.3)	0.592	0.381
Intestinal-type, n, %	37 (63.8)	48 (62.3)	46 (63.0)	0.985	0.862
Cardiac cancer, n, %	5 (8.5)	5 (6.3)	14 (18.7)	**0.039**	0.631
TGFB-509 T allele, n, %	43 (74.1)	55 (71.4)	46 (63.0)	0.340	0.727
*IN-1RN**2 carrier, n, %	7 (12.1)	5 (6.3)	10 (13.5)	0.310	0.240
IL-1B 511 T allele, n, %	45 (77.6)	60 (75.9)	60 (81.1)	0.738	0.823

Bold style indicates the statistical significance. *cagA* = cytotoxin-associated gene A, HP = *Helicobacter pylori*, IL = interleukin, No = number, SD = standard deviation, TGF = transforming growth factor.

*Compared among the 3 groups (maternal, paternal, and sibling/offspring).

†Compared between maternal and paternal.

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related to a number of low-penetrant alleles acting in combinations, rather than 1 highly penetrant dominant cancer gene. Single nucleotide polymorphisms related to cytokines have been investigated as they may affect chronic gastritis, which predisposes to the GC. El-Omar et al[20] reported that *IL-1B*-511 T allele and *IL-1RN**2/*2 were associated with an increased risk of GC among Caucasians. Although we included a larger number of GC-relatives in the present study to enable analysis of *IL-1B*-C511T allele and *IL-1RN*, no significant associations were found. Actually, the genotype frequencies in the present study were significantly different from those reported by El-Omar et al[20] and by Machado et al.[21] That is, the frequency of *IL1B*-511T/T of both control and GC patients in our study was ~30%, far greater than the 10%–20% they reported.[20,21] This may be a partial explanation for why the result of this polymorphism differed from the West. Moreover, Kato et al[12] reported that having the *IL-1B*-511C allele was closely related to an increased level of gastric mucosal IL-1β and an increased risk of gastric mucosal atrophy in the Japanese population, suggesting differences in genetic background among ethnicities. Similarly, the frequency of *IL-1RN**2/*2 was very low among Koreans and Japanese (<5%).[22] In a recent meta-analysis, the *IL-1RN**2 variant was associated with an increased risk of GC only in Caucasians.[23] Finally, TGF-β1 has dual roles, inhibiting and promoting carcinogenesis. Although association studies of the *TGFB1*-509C/T polymorphism and the risk of developing GC have been performed, the results are not uniform. One study reported overexpression of TGF-β1 in the gastric mucosa of patients with GC and their 1st-degree relatives.[24]

Since specific genetic factors were not detected in FH-positive GC, FH was divided into having a single GC-relative and having at least 2 GC-relatives to maximize the characteristics of familial aggregation in the present study. After the stratification, alcohol consumption was associated with a more marked increase in GC risk in the latter than in the former. Moreover, this synergistic effect was more prominent among heavy drinkers (Supplementary Content S3, http://links.lww.com/MD/A947). Although the role of alcohol consumption in the development of GC has been investigated less extensively than smoking, a recent meta-analysis showed that heavy drinking was significantly associated with noncardia GC.[25] However, how alcohol promotes carcinogenesis in the population with multiple GC-relatives has not been evaluated. Our group reported that among heavy drinkers, *aldehyde dehydrogenase* (*ALDH2*) *1/*2 heterozygotes had an increased risk of GC compared with *1/*1 homozygotes.[14] The proportion of *ALDH*2 polymorphism was not significantly different between the 2 FH subgroups in the present study. Molecular epidemiology investigations based on GWAS may facilitate identification of the genetic factors responsible for this phenomenon.

GC patients with an affected father were younger than those with affected mother or siblings. GC patients with both affected mother and father were younger than those with 1 affected parent in the present study, suggesting an earlier age distribution of familial cases. The larger number of affected GC relatives in GC patients with an affected mother is consistent with a previous report of a maternal inheritance pattern.[26] Because of the small number of subjects with FH in this study, this finding should be interpreted cautiously.

There is a lack of awareness of the extent to which GC is familial. The present study also suggests ''familial GC'' to be a heterogeneous group that requires characterization and stratification. Indeed, the GC group with at least 2 GC-relatives may be different from that with 1 GC-relative. In the subjects with at least 2 GC-relatives, carrying *TGFB*1-509T/T was a risk factor for GC in a multivariable model in the present study. There has been a report regarding TGF-β1 overexpression in the gastric mucosa of patients with GC and their 1st-degree relatives.[27] In addition, expression of mucosal or blood TGF-β1 in subjects with *TGFB1*-509 T hetero or homozygotes is increased.[28,29] Therefore, the *TGFB1*-C509T polymorphism should be reevaluated as a potential mediator for familial clustering, since the association between this polymorphism and the risk of GC remains inconclusive.[30]

The most accessible prediction model for GC risk is the Disease Risk Index run by Harvard School of Public Health.[31] Colditz et al,[32] who contributed to construction of the web-based personalized calculation system, have recognized low socioeconomic status, blood group A, first-degree relative with GC, salt intake, and smoking as risk factors for GC. We developed a stratified scoring system for the risk of GC according to presence or absence of FH using the method of a previous study.[33] (Supplementary Content S4, http://links.lww.com/MD/A947). However, validation cohorts that provide the same information as ours were not available, and so we plan a further prospective study to refine and validate this scoring system. When using our prediction model, AUCs of the ROC curves of predictive probability in each formula were 0.78 (95% CI 0.75–0.80) for subjects without FH, 0.82 (95% CI 0.78–0.86) for subjects with FH, 0.82 (95% CI 0.77–0.86) for subjects with 1 1st-degree GC relative, and 0.94 (95% CI 0.88–0.99) for subjects with 2 or more 1st-degree GC relatives (Supplementary Content S5, http://links.lww.com/MD/A947).

This study has several limitations. Its hospital-based and retrospective nature results in the findings being subjects to several biases. Since healthy individuals with FH more willingly participated in this study, the control group consisted of a higher proportion of FH-positive subjects than the cancer group. Therefore, stratification according to FH was performed instead of using FH as a common independent variable. Nevertheless, this study suggests the necessity of characterization or stratification of GC with FH by evaluating to what extent genetic and environmental factors contribute to familial aggregation of GC.

In summary, while rural residency in childhood and *cagA*-positive HP were distinct risk factors for FH-positive GC, *TGFB1*-509T/T was selected as a risk factor of GC in subjects with at least 2 GC-relatives. Alcohol consumption had a greater carcinogenic effect in subjects with at least 2 GC-relatives than those with a single GC-relative, and this synergistic effect was dose-dependent. Individuals with 2 or more GC-relatives should undergo risk stratification including *TGFB1*-509T/T and alcohol consumption. Further study is required to identify markers for identifying individuals at high risk of GC among subjects with FH.

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