Effects of Interleukin-10 Polymorphisms, Helicobacter pylori Infection, and Smoking on the Risk of Noncardia Gastric Cancer

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Abstract

Objective: Both variations in the interleukin-10 (IL10) gene and environmental factors are thought to influence inflammation and gastric carcinogenesis. Therefore, we investigated the associations between IL10 polymorphisms, Helicobacter pylori (H. pylori) infection, and smoking in noncardia gastric carcinogenesis in Koreans.

Methods: We genotyped three promoter polymorphisms (-1082A>G, -819T>C, and -592 A>C) of IL10 in a case-control study of 495 noncardia gastric cancer patients and 495 sex- and age-matched healthy controls. Multiple logistic regression models were used to detect the effects of IL10 polymorphisms, H. pylori infection, and smoking on the risk of gastric cancer, which was stratified by the histological type of gastric cancer.

Results: The IL10-819C and -592C alleles were found to have complete linkage disequilibrium, and all three IL10 polymorphisms were associated with an increased risk of intestinal-type noncardia gastric cancer. These associations were observed only in H. pylori-positive subjects and current smokers. A statistically significant interaction between the IL10-592 genotype and H. pylori infection on the risk of intestinal-type gastric cancer was observed (P for interaction = 0.047). In addition, H. pylori-positive smokers who were carriers of either the IL10-1082G (OR [95% CI] = 17.76 [6.17–51.06]) or the -592C (OR [95% CI] = 8.37 [2.79–25.16]) allele had an increased risk of intestinal-type gastric cancer compared to H. pylori-negative nonsmokers homogygous for IL10-1082A and -592A, respectively. The interaction between the IL10-1082 polymorphism and the combined effects of H. pylori infection and smoking tended towards significance (P for interaction = 0.080).

Conclusions: Inflammation-related genetic variants may interact with H. pylori infection and smoking to increase the risk of noncardia gastric cancer, particularly the intestinal-type. These findings may be helpful in identifying individuals at an increased risk for developing noncardia gastric cancer.

Introduction

Gastric cancer is the second most common cause of cancer mortality globally and the prevalence of gastric cancer is higher in Asians than in Western populations [1]. Numerous studies have supported the concept that gastric inflammation is a critical component of tumor development [2,3]. H. pylori is a strong risk factor for gastric cancer, likely due to the extensive inflammation in the stomach mucosa caused by this bacteria [4]. However, considering that only a small portion of H. pylori-infected subjects eventually develop gastric cancer, the pathway from H. pylori infection to carcinogenesis may be affected by several factors, including H. pylori strain variability, lifestyle (e.g., smoking, low vegetable/fruit consumption, high salt intake), and host genetics [5,6].

In chronic gastric inflammation, activated neutrophils and mononuclear cells produce different types of cytokines that are crucial in regulating inflammation [7,8]. Altered cytokine levels have been observed during tumor initiation and promotion in the stomach [9,10]. Interleukin-10 (IL10) is a multifunctional anti-inflammatory cytokine that downregulates cell-mediated immune responses and cytokotic inflammatory responses. Three polymorphic promoter variants, located at positions -1082 (A>G), -819 (T>C), and -592 (A>C), have been identified in the IL10 gene. It has been reported that these variants of IL10 are associated with increased IL10 production [11–13] and an elevated risk of gastric
cancer [14,15]. Furthermore, environmental factors, such as cigarette smoking, may alter cytokine expression in a manner more favorable for the development of gastric inflammation and carcinogenesis [16]. Therefore, inflammation-related polymorphisms may interact with environmental factors in the development of gastric cancer.

Although many studies have investigated the roles of IL10 promoter polymorphisms and the risk of gastric cancer, the findings remain inconclusive, possibly due to differences in study design and/or ethnic differences in the populations studied [14,15,17–19]. Furthermore, gastric cancers that differ in anatomic location and/or histological type have different clinicopathological characteristics [3,6]. H. pylori infection and chronic inflammation have been more closely linked with noncardia gastric cancer than with cardia gastric cancer and may induce the histological progression to gastric cancer [3]. In addition, the impact of genetic and environmental factors may differ by the histological type of gastric cancer [6]. Therefore, the present study aimed to investigate the effects of IL10 genetic variants, H. pylori infection, cigarette smoking, and their interactions on the risk of noncardia gastric carcinogenesis. We also examined the differential effects of these factors according to the histological type of gastric cancer.

Materials and Methods

Study Population

This study was a hospital-based case-control study. A total of 797 patients with noncardia gastric cancer were recruited from the National Cancer Center Hospital in Goyang between 2003 and 2007. Only histologically-confirmed adenocarcinoma patients were included in this study, and other types of neoplasms found in the stomach (e.g., MALT lymphomas, other types of primary gastric lymphoma, gastrointestinal stromal tumors, carcinoid tumors, or adenomas) were excluded. Nine hundred thirty healthy controls who underwent upper endoscopy at the same institution for gastric cancer screening in the National Cancer Screening Program in Korea and were without significant gastrointestinal symptoms were recruited in 2007. All cases and controls were of Korean ancestry. For both gastric cancer cases and controls, we excluded subjects who had a history of other malignant neoplasms, had a history of previous H. pylori treatment, had not received an H. pylori evaluation, or were lacking information about their genetic background.

Cases and controls were frequency-matched by age (within 5 years) and sex. In total, 495 cases and 495 controls were used for the final analyses. Noncardia gastric cancer patients were classified into two subgroups (intestinal-type and diffuse-type) based on the histopathology according to the Lauren classification [20]. The clinical information and genetic materials were obtained under the approval of the Institutional Review Board of the National Cancer Center in Korea, and written informed consent was obtained from each subject.

Data Collection

The questionnaire data and blood samples were obtained at the initial recruitment of both cases and controls. All participants were asked to complete a self-administered questionnaire about their sociodemographic characteristics (e.g., age, family history of gastric cancer, income, and education), smoking habits, alcohol intake, and personal medical history. H. pylori infection status was determined by a rapid urease test and a histological evaluation. The rapid urease test was performed according to the manufacturer’s instructions (Pronto Dry. Medical Instruments Corporation, Solothurn, Switzerland). For the histological evaluation of H. pylori, biopsy specimens were obtained from three different areas, the gastric antrum, the corpus lesser curvature, and the corpus greater curvature, to increase the sensitivity of the procedure. Two pieces of biopsy specimens were obtained from each site, and Wright-Giemsa staining was performed on the biopsy samples to determine if H. pylori was present.

Genotyping of the IL10 promoter (-1082/-819/-592) was conducted and interpreted by a researcher blinded to case/control status. Briefly, genomic DNA was prepared from peripheral blood samples with an automatic DNA extraction system (BioRobot M48 Workstation, Qiagen, Inc., Valencia, CA) following the manufacturer’s instructions, and the purity and concentration of isolated DNA were determined using picogreen (Molecular Probes, Inc., Eugene, OR). A total of 10 ng per sample was used for genotype analysis. Genotyping for the three SNPs was performed by the iPLEX Gold assay (Sequenom, San Diego, CA), which is based on MALDI-TOF spectrometry, per the manufacturer’s protocol, and the resulting genotype data were collected by Typer v4.0 (Sequenom). To ensure quality control, subsets of the samples were run as duplicates and 5% of the wells in a 384-well plate were used as negative controls to monitor for contamination. For all results, genotype clusters were examined manually for their fitness.

Statistical Analysis

The demographic characteristics, environmental factors, and gene frequencies of IL10 in patients and controls were compared using $\chi^2$ statistics. The Hardy-Weinberg equilibrium of genotype frequencies in the controls was tested with the $\chi^2$ test. The association between IL10 genetic variants (-1082/-819/-592) and the risk of gastric cancer was estimated by multivariate logistic regression with adjustments for age, sex, H. pylori infection, smoking, alcohol consumption, income, and education and was expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Haplotype analyses were also conducted. IL10 haplotypes were constructed based on the frequencies of three SNPs (-1082/-819/-592) in this study population. These polymorphisms were found to be in strong linkage disequilibrium, and three of the eight possible haplotypes (GCC, ACC, ATA) segregate in the Korean population. We defined the more common homozygous genotype/haplotype in the control subjects as the reference genotype/haplotype. The effects of H. pylori infection and smoking on the association between genetic variants and the risk of gastric cancer were examined in a subgroup analysis by H. pylori infection status (H. pylori-positive/H. pylori-negative) or smoking status (non-smoker/past smoker/current smoker). In addition, to investigate the combined effects of H. pylori infection and smoking on the association between genetic variants and the risk of gastric cancer, subjects were grouped according to their dichotomized H. pylori infection (H. pylori-positive/H. pylori-negative) and smoking (smokers (all subjects who had ever smoked were classified as smokers)/non-smokers) status. Data were stratified by the histological type of gastric cancer (intestinal-type/diffuse-type). The significance of the interactions between genetic variants and environmental risk factors was assessed with the likelihood ratio test, which compared the model with the interaction term with the one that only contained the main effects. All statistical analyses were performed with SAS 9.1 software (SAS Institute Inc. Cary, NC). Two-sided P-values less than 0.05 were regarded as statistically significant.

Results

The demographics of the study subjects are shown in Table 1. No differences between cases and controls in the distribution of
Table 1. Characteristics of the Study Subjects.

|                      | Gastric Cancer (n = 495) | Controls (n = 495) | P-value |
|----------------------|--------------------------|--------------------|---------|
| Age (years), mean ± SD | 54.9±8.4                 | 54.3±7.4           | 0.208   |
| Male                 | 337 (68.1)               | 337 (68.1)         | 1.000   |
| Family history of gastric cancer | 220 (44.4)             | 225 (45.5)         | 0.749   |
| H. pylori infection (positive) | 443 (89.9)              | 329 (66.6)         | <0.001  |
| Smoking status       |                          |                    |         |
| Non-smoker           | 183 (37.0)               | 208 (42.0)         | <0.001  |
| Past smoker          | 108 (21.8)               | 158 (31.9)         |         |
| Current smoker       | 204 (41.2)               | 129 (26.1)         |         |
| Alcohol consumption  |                          |                    |         |
| Non-drinker          | 167 (33.8)               | 147 (29.7)         | 0.027   |
| Past drinker         | 50 (10.1)                | 53 (10.7)          |         |
| Current drinker      | 277 (56.1)               | 315 (63.6)         |         |
| Income               |                          |                    |         |
| Low                  | 205 (41.4)               | 165 (33.3)         | 0.017   |
| Medium               | 242 (48.9)               | 286 (57.8)         |         |
| High                 | 48 (9.7)                 | 44 (8.9)           |         |
| Education (years)    |                          |                    |         |
| <12                  | 231 (46.7)               | 145 (29.3)         | <0.001  |
| ≥12                  | 264 (53.3)               | 350 (70.7)         |         |
| IL10-1082            |                          |                    |         |
| AA                   | 416 (84.0)               | 435 (87.9)         | 0.198   |
| AG                   | 72 (14.6)                | 56 (11.3)          |         |
| GG                   | 7 (1.4)                  | 4 (0.8)            |         |
| IL10-819             |                          |                    |         |
| TT                   | 231 (46.7)               | 248 (50.1)         | 0.325   |
| CT                   | 214 (43.2)               | 191 (38.6)         |         |
| CC                   | 50 (10.1)                | 56 (11.3)          |         |
| IL10-592             |                          |                    |         |
| AA                   | 231 (46.7)               | 248 (50.1)         | 0.325   |
| CA                   | 214 (43.2)               | 191 (38.6)         |         |
| CC                   | 50 (10.1)                | 56 (11.3)          |         |
| Histological type of gastric cancer |                  |                    |         |
| Intestinal           | 253 (51.1)               | -                  |         |
| Diffuse              | 214 (43.2)               | -                  |         |
| Mixed                | 28 (5.7)                 | -                  |         |
| Stage of gastric cancer |                      |                    |         |
| Early gastric cancer† | 279 (56.4)               | -                  |         |
| Advanced gastric cancer‡ | 216 (43.6)              | -                  |         |

*Results presented as n (%) unless otherwise indicated.
†Depth of tumor invasion limited to mucosa or submucosa.
‡Depth of tumor invasion involved the proper muscle layer or beyond.

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To determine whether genetic susceptibility to noncardia gastric cancer was present, the association between IL10 variants and the risk of noncardia gastric cancer stratified by histological type was investigated (Table 2). The results of a multivariate regression model revealed that IL10-1082G (OR [95% CI] = 1.69 [1.06–2.69]) and -592C (OR [95% CI] = 1.55 [1.10–2.10]) carriers had a higher risk of intestinal-type gastric cancer than subjects homozygous for the IL10-1092A allele and -592A allele, respectively. In addition, carriers of the GCC haplotype had an increased risk of intestinal-type gastric cancer compared to ATA haplotype carriers (OR [95% CI] = 1.64 [1.01–2.64]). We also examined the association between IL10 genetic variants and the risk of gastric cancer after stratifying noncardia gastric cancer by stage (data not shown). However, we did not find any differences in the risk of gastric cancer between the disease stages.

Next, we investigated the effects of H. pylori infection status on the association between IL10 genetic variants and the risk of noncardia gastric cancer (Table 3). H. pylori-infected patients had an increased risk of gastric cancer compared to H. pylori-negative participants (OR [95% CI] = 4.30 [3.02–6.11]). The association between H. pylori infection and the risk of gastric cancer was stronger among genetic variant carriers, particularly among intestinal-type gastric cancer patients. The interaction between the IL10-592 genetic variant and H. pylori infection on the risk of intestinal-type gastric cancer was statistically significant (P for interaction = 0.047).

We also examined the effects of smoking on the association between IL10 genetic variants and the risk of noncardia gastric cancer (Table 4). The association between current smoking and an increased risk of gastric cancer was stronger among IL10-1082G carriers and IL10-592C carriers. Current smokers that carried the IL10-1082G (OR [95% CI] = 8.72 [3.64–20.91]) and -592C (OR [95% CI] = 5.40 [2.69–10.84]) alleles had an increased risk of intestinal-type gastric cancer compared to nonsmokers homozygous for IL10-1082A and -592A, respectively. However, no statistically significant interactions between IL10 genetic variants and cigarette smoking on the risk of gastric cancer were observed.

To evaluate the interactions between environmental factors and genetic susceptibility, the combined effects of H. pylori infection and smoking on the association between IL10 variants and the risk of noncardia gastric cancer were investigated (Figure 1). Smoking seemed to have no effect in the absence of H. pylori infection, but when the data were stratified by genetic variants and histological type, H. pylori-positive smokers that carried either the IL10-1082G (OR [95% CI] = 8.72 [3.64–20.91] and -592C (OR [95% CI] = 5.40 [2.69–10.84]) alleles had an increased risk of intestinal-type gastric cancer compared to non-H. pylori-negative smokers homozygous for IL10-1082A and -592A, respectively. However, no statistically significant interactions between IL10 genetic variants and cigarette smoking on the risk of gastric cancer were observed.

Discussion

A number of studies have reported an association between IL10 promoter polymorphisms (-1082G/-819C/-592C) and the risk of gastric cancer, but these findings have been inconsistent [14,15,17–19]. In accord with our findings, recent meta-analyses suggested that Asian carriers of the promoter polymorphisms of IL10 may be associated with an increased risk of gastric cancer [21,22]. However, some studies, mostly studies conducted in

age, sex, and family history of gastric cancer were found. However, cases were more likely to have H. pylori infection (P<0.001), more likely to smoke (P<0.001), and less likely to drink alcoholic beverages (P=0.027) than controls. In addition, cases also had a lower mean income (P=0.017) and less education (P<0.001) than controls. With regard to genetic frequencies, no significant differences between cases and controls in the frequencies of the IL10-1082G/-819C/-592C polymorphisms were present. IL10-819 and IL10-592 were found to be in complete linkage disequilibrium.
Western populations, have reported a different association [14,23].
Differences in the number of patients enrolled, study design, patient age at diagnosis, and genotyping methods may contribute to the differences in the results of such studies [9]. In addition, racial differences in the distribution of the \textit{IL10} genotypes may also affect the findings of the studies. Compared to other populations, the frequency of the \textit{IL10} polymorphisms is significantly lower in Asians [24]. This difference may be clinically relevant for susceptibility to gastric cancer and may, in part, explain the different incidences of gastric cancer in Caucasians and Asians [24]. \textit{IL10} genetic variants have been proposed to be associated with higher \textit{IL10} production [11–13]. \textit{IL10} levels are elevated in gastric mucosa infected with \textit{H. pylori} and are higher in patients that have severe chronic inflammation [25]. Furthermore, \textit{IL10} mRNA expression and serum levels are elevated in gastric carcinogenesis, particularly in the advanced stage [10,12,26,27]. The role that \textit{IL10} plays in carcinogenesis may be due to its ability to act not

| Table 2. The Association between \textit{IL10} Genetic Variants and the Risk of Gastric Cancer, Stratified by Histological Type of Noncardia Gastric Cancer. |
|-----------------|-----------------|-------------------|-------------------|
|                | All type        | Intestinal type (n = 253) | Diffuse type (n = 214) |
|                | No. of Controls (%) | No. of Cases (%) | Crude OR (95% CI) | Multivariate OR (95% CI) | No. of Controls (%) | No. of Cases (%) | Crude OR (95% CI) | Multivariate OR (95% CI) |
| \textit{IL10}-1082 |                 |                    |                  |                       |                    |                    |                  |                       |
| AA             | 435 (87.9)       | 416 (84.0)         | 1.0              | 1.0                    | 209 (82.6)          | 1.0              | 1.0              | 182 (85.1)          | 1.0                    |
| G carrier      | 60 (12.1)        | 79 (16.0)          | 1.37 (0.92, 1.99) | 1.42 (0.96, 2.10)      | 44 (17.4)           | 1.59 (1.01, 2.51) | 1.69 (1.06, 2.69) | 32 (14.9)           | 1.30 (0.80, 2.03) |
| \textit{IL10}-592 |                 |                    |                  |                       |                    |                    |                  |                       |
| AA             | 248 (50.1)       | 231 (46.7)         | 1.0 (Ref)        | 1.0                    | 107 (42.29)         | 1.0              | 1.0              | 102 (47.7)          | 1.0                    |
| C carrier      | 247 (49.9)       | 264 (53.3)         | 1.15 (0.89, 1.47) | 1.20 (0.91, 1.57)      | 146 (57.71)         | 1.37 (1.01, 1.86) | 1.55 (1.10, 2.18) | 112 (52.3)          | 1.10 (0.80, 1.52) |

Abbreviations: OR, odds ratio; CI, confidence interval.
*Adjusted for age, sex, \textit{H. pylori} infection, smoking status, alcohol consumption, education, and income.
\textit{IL10}-819 and \textit{IL10}-592 were in complete linkage disequilibrium.
\textit{IL10}-819 and \textit{IL10}-592 were in complete linkage disequilibrium.

Table 3. The Effects of Helicobacter Infection Status on the Association between \textit{IL10} Genetic Variants and the Risk of Noncardia Gastric Cancer, Stratified by Histological Type*.

|                | All type | Intestinal type | Diffuse type |
|----------------|----------|-----------------|-------------|
|                | H. Pylori (−) | H. Pylori (+) | H. Pylori (−) | H. Pylori (+) | H. Pylori (−) | H. Pylori (+) |
| All genotypes  | 165/50 | 329/443 | 165/27 | 329/225 | 165/20 | 329/194 |
| OR (95% CI)*   | 1.0 (Ref) | 4.30 (3.02, 6.11) | 1.0 (Ref) | 3.91 (2.49, 6.16) | 1.0 (Ref) | 4.84 (2.92, 8.03) |
| \textit{IL10}-1082 |         |                 |             |                       |                    |                    |
| AA             | 147/42 | 287/372 | 147/23 | 287/185 | 147/16 | 287/166 |
| OR (95% CI)*   | 1.0 (Ref) | 4.21 (2.89, 6.14) | 1.0 (Ref) | 3.64 (2.24, 5.91) | 1.0 (Ref) | 5.42 (3.09, 9.50) |
| G carrier      | 18/8 | 42/71 | 18/4 | 42/40 | 18/4 | 42/28 |
| OR (95% CI)*   | 1.46 (0.59, 3.64) | 5.59 (3.33, 9.41) | 1.25 (0.38, 4.11) | 6.02 (3.18, 11.42) | 2.13 (0.62, 7.27) | 5.54 (2.69, 11.41) |
| \textit{P} for interaction = 0.786 |         |                 |             |                       |                    |                    |
| \textit{IL10}-592 |         |                 |             |                       |                    |                    |
| AA             | 75/25 | 172/206 | 75/15 | 172/92 | 75/7 | 172/95 |
| OR (95% CI)*   | 1.0 (Ref) | 3.21 (2.03, 5.44) | 1.0 (Ref) | 2.28 (1.22, 4.24) | 1.0 (Ref) | 6.48 (2.84, 14.81) |
| C carrier      | 90/25 | 157/237 | 90/12 | 157/133 | 90/13 | 157/99 |
| OR (95% CI)*   | 0.82 (0.43, 1.54) | 4.25 (2.59, 6.95) | 0.66 (0.29, 1.51) | 4.04 (2.19, 7.46) | 1.80 (0.67, 4.81) | 7.23 (3.17, 16.52) |
| \textit{P} for interaction = 0.306 |         |                 |             |                       |                    |                    |

*Adjusted for age, sex, smoking status, alcohol consumption, education, and income.
\textit{IL10}-819 and \textit{IL10}-592 were in complete linkage disequilibrium.

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only as an anti-inflammatory cytokine but also as an immunosuppressant [28]. *H. pylori*-induced IL10 production, while having beneficial effects in terms of limiting the tissue damage caused by inflammation, may also render mucosal immune cells unable to adequately defend against malignant cells [25]. Therefore, the higher levels of IL10 found in the *IL10* genetic variants carriers

### Table 4. The Effects of Smoking Status on the Association between *IL10* Genetic Variants and the Risk of Noncardia Gastric Cancer, Stratified by Histological Type*.

|                      | All type            | Non-smoker | Past smoker | Current smoker |
|----------------------|---------------------|------------|-------------|---------------|
| **All genotypes**    | No. of controls/cases | 208/138    | 158/108     | 129/204       |
| **IL10-1082**        | OR (95% CI)*        | 1.0 (ref)  | 1.05 (0.66, 1.66) | 2.45 (1.58, 3.80) |
| AA                   | No. of controls/cases | 180/155    | 140/191     | 115/170       |
| OR (95% CI)*         | 1.0 (ref)           | 1.00 (0.62, 1.61) | 2.23 (1.41, 3.50) |
| G carrier            | No. of controls/cases | 28/28      | 18/17       | 14/34         |
| OR (95% CI)*         | 1.05 (0.57, 1.93)   | 1.41 (0.64, 3.12) | 4.76 (2.20, 10.30) |

*P for interaction = 0.323

| **IL10-592**\(^\d\) | No. of controls/cases | 105/90      | 72/46       | 71/95         |
|----------------------|-----------------------|-------------|-------------|---------------|
| AA                   | OR (95% CI)*          | 1.0 (ref)   | 0.89 (0.49, 1.61) | 2.06 (1.20, 3.55) |
| C carrier            | No. of controls/cases | 103/93      | 86/62       | 58/109        |
| OR (95% CI)*         | 1.00 (0.65, 1.53)     | 1.16 (0.67, 2.03) | 2.88 (1.67, 4.95) |

*P for interaction = 0.541

| **Intestinal type**  | All genotypes         | No. of controls/cases | 208/64     | 158/71       | 129/118       |
|----------------------|-----------------------|-----------------------|------------|-------------|---------------|
| **IL10-1082**        | OR (95% CI)*          | 1.0 (ref)             | 1.54 (0.87, 2.71) | 3.66 (2.12, 6.36) |
| AA                   | No. of controls/cases | 180/55     | 140/59      | 115/95       |
| OR (95% CI)*         | 1.43 (0.79, 2.59)     | 3.18 (1.80, 5.61)    |
| G carrier            | No. of controls/cases | 28/9       | 18/12       | 14/23        |
| OR (95% CI)*         | 1.01 (0.42, 2.41)     | 2.25 (0.89, 5.66)    | 8.72 (3.64, 20.91) |

*P for interaction = 0.223

| **IL10-592**\(^\d\) | No. of controls/cases | 105/26      | 72/26       | 71/55         |
|----------------------|-----------------------|-------------|-------------|---------------|
| AA                   | OR (95% CI)*          | 1.0 (ref)   | 1.29 (0.60, 2.77) | 3.42 (1.69, 6.94) |
| C carrier            | No. of controls/cases | 103/38     | 86/45       | 58/63         |
| OR (95% CI)*         | 1.37 (0.75, 2.51)     | 2.23 (1.10, 4.51) | 5.40 (2.69, 10.84) |

*P for interaction = 0.867

| **Diffuse type**     | All genotypes         | No. of controls/cases | 208/108    | 158/29       | 129/77        |
|----------------------|-----------------------|-----------------------|------------|-------------|---------------|
| **IL10-1082**        | OR (95% CI)*          | 1.0 (ref)             | 0.64 (0.35, 1.19) | 1.82 (1.06, 3.13) |
| AA                   | No. of controls/cases | 180/91    | 140/24      | 115/67       |
| OR (95% CI)*         | 0.59 (0.31, 1.12)     | 1.73 (0.99, 3.02)    |
| G carrier            | No. of controls/cases | 28/17      | 18/5        | 14/10        |
| OR (95% CI)*         | 0.91 (0.45, 1.85)     | 1.00 (0.32, 3.13)    | 2.56 (0.94, 7.02) |

*P for interaction = 0.577

| **IL10-592**\(^\d\) | No. of controls/cases | 105/55      | 72/13       | 71/34         |
|----------------------|-----------------------|-------------|-------------|---------------|
| AA                   | OR (95% CI)*          | 1.0 (ref)   | 0.50 (0.22, 1.12) | 1.44 (0.73, 2.83) |
| C carrier            | No. of controls/cases | 103/53     | 86/16       | 58/43         |
| OR (95% CI)*         | 0.93 (0.56, 1.54)     | 0.73 (0.34, 1.56) | 2.10 (1.09, 4.03) |

*P for interaction = 0.459

*The numbers given in the first line for each group are the numbers of controls/cases while the numbers on the second line are the adjusted odds ratios with the 95% confidence intervals in parenthesis. Adjustments were made for age, sex, and *H. pylori* infection status, alcohol consumption, education, and income.

\(^\d\)IL10-819 and IL10-592 were in complete linkage disequilibrium.

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A. Association by IL10-1082 Genotype

| All type   | No. of Controls/Cases | Odds Ratio (95% CI) |
|------------|-----------------------|---------------------|
|            |                       |                     |
|            |                       | 0.1 (Ref)           |
|            |                       | 1.81 (0.47-7.08)    |
|            |                       | 1.28 (0.60-2.74)    |
|            |                       | 1.05 (0.52-2.10)    |
|            |                       | 3.79 (2.12-6.79)    |
|            |                       | 3.47 (1.56-7.73)    |
|            |                       | 6.15 (3.22-11.74)   |
|            |                       | 11.04 (4.94-24.69)  |

**Intestinal type**

|            |                       |                     |
|            |                       | 2.00 (0.33-12.13)   |
|            |                       | 1.84 (0.65-5.20)    |
|            |                       | 2.10 (0.36-12.38)   |
|            |                       | 3.24 (1.36-7.75)    |
|            |                       | 2.67 (0.81-8.83)    |
|            |                       | 7.60 (3.01-19.20)   |
|            |                       | 17.76 (6.17-51.06)  |

**Diffuse type**

|            |                       |                     |
|            |                       | 1.98 (0.33-11.73)   |
|            |                       | 0.76 (0.24-2.42)    |
|            |                       | 2.16 (0.39-12.45)   |
|            |                       | 4.56 (2.16-9.82)    |
|            |                       | 3.73 (1.42-9.90)    |
|            |                       | 5.61 (2.42-13.01)   |
|            |                       | 7.20 (2.46-21.10)   |

P for interaction=0.340

B. Association by IL10-592 Genotype

| All type   | No. of Controls/Cases | Odds Ratio (95% CI) |
|------------|-----------------------|---------------------|
|            |                       | 0.94 (Ref)          |
|            |                       | 1.40 (0.54-3.69)    |
|            |                       | 0.97 (0.37-2.53)    |
|            |                       | 3.26 (1.55-6.93)    |
|            |                       | 3.24 (1.53-6.86)    |
|            |                       | 4.73 (2.12-10.56)   |
|            |                       | 6.84 (3.09-15.14)   |

**Intestinal type**

|            |                       |                     |
|            |                       | 0.75 (0.18-3.12)    |
|            |                       | 1.83 (0.52-6.47)    |
|            |                       | 1.02 (0.28-3.78)    |
|            |                       | 1.97 (0.67-5.84)    |
|            |                       | 2.91 (1.01-8.40)    |
|            |                       | 4.64 (1.52-14.19)   |
|            |                       | 8.37 (2.79-25.16)   |

P for interaction=0.366

**Diffuse type**

|            |                       |                     |
|            |                       | 1.0 (Ref)           |
|            |                       | 1.39 (0.39-4.96)    |
|            |                       | 0.55 (0.09-3.23)    |
|            |                       | 1.30 (0.34-5.04)    |
|            |                       | 5.13 (1.84-14.30)   |
|            |                       | 4.51 (1.61-12.67)   |
|            |                       | 5.29 (1.74-16.09)   |
|            |                       | 7.17 (2.40-21.45)   |

P for interaction=0.212

P for interaction=0.530
may partly explain the attenuated immune responses observed during chronic infection. Similarly, the local immunosuppressive cytokine production in chronic gastritis may predispose individuals to the development of gastric carcinogenesis [25]. However, the roles of IL10 and its polymorphisms in the pathogenesis of gastric cancer require further investigation.

Environmental factors may also play an important role in gastric carcinogenesis [29]. In the present study, the effects of smoking seemed to be confined to H. pylori-infected participants, and a synergistic effect between H. pylori infection and smoking in terms of increasing the risk of noncardia gastric cancer was observed. This result is in agreement with the findings of several previous studies [30,31]. Excessive inflammation in response to H. pylori infection is associated with an increased vulnerability to gastric carcinogenesis [5]. The synergistic effect of smoking on the risk of developing H. pylori-associated cancer may result from various mechanisms that enhance the deleterious state induced by the infection. Smokers have elevated levels of circulating inflammatory mediators, which may exacerbate the detrimental effects of H. pylori-associated gastric inflammation [8,16,32]. It is also plausible that the gastric mucosal damage caused by H. pylori-infection is exacerbated by tobacco carcinogens [33]. The male predominance of gastric cancer, which is related to a 10- to 15-year delay in the development of intestinal-type gastric cancer in females compared to males, may be attributed to the protective role of estrogens against gastric carcinogenesis [34]. However, in light of our findings, the higher rates of H. pylori infection and smoking in adult Korean men compared to women may also partly explain the male predominance of gastric cancer in the Korean population [35].

Genetic susceptibility may modify the propensity for gastric carcinogenesis through an alteration of the inflammatory state and may also interact with other risk factors. In the present study, the elevated risk of noncardia gastric cancer in the IL10 variant carriers was more significant in H. pylori-positive subjects who smoked, and these results are similar to the findings of a case-control study in the Taiwanese Chinese population [18]. Another case-control study conducted in Korea reported that a genotype that yields high IL10 production was associated with an increased risk of gastric cancer only within a low soybean intake group, suggesting that the anti-inflammatory effects of soy may counterbalance the pro-carcinogenic effects of the IL10 genetic variants [36]. Taken together, these findings suggest that H. pylori-positive smokers with genetic susceptibility to gastric cancer may be an important group to target for prevention and early detection.

Gastric cancer may have different clinicopathological characteristics based on the anatomic site and histological type [6,37,38]. In the present study, we investigated only noncardia gastric cancer patients because H. pylori is a strong risk factor for noncardia, but not cardia, gastric cancer [39,40]. We found that IL10 variant carriers with high levels of inflammation (H. pylori infection and smoking) had an increased risk of intestinal-type gastric cancer. Several studies have reported a differential association between genetic variants and the histological types of gastric cancer, but the lack of consistent evidence has hindered the ability to draw any definitive conclusions [9,25]. Environment and lifestyle are known to affect the development of intestinal-type cancer more than the diffuse-type [41]. It is generally accepted that well-differentiated, intestinal-type gastric cancer develops mostly in older patients through a multistep process that include chronic inflammation, atrophy, and intestinal metaplasia [42]. This notion is supported by our supplemental data that demonstrated that the effects of environmental factors (H. pylori infection and smoking) and genetic variants on the risk of advanced atrophy and intestinal metaplasia were similar to those on intestinal-type gastric cancer (Table S1). Therefore, improvements in diet and declines in cigarette smoking and H. pylori infections may have contributed to the decrease in intestinal/noncardia type gastric cancer in recent years [38,41].

This study contains several limitations that should be considered. First, the prevalence of H. pylori infection could be underestimated because histological changes in the stomach caused by H. pylori might lead to the spontaneous clearance of the bacteria [43]. Additionally, we could not consider H. pylori strain variability, which is important because the strains vary in their carcinogenic potential [44]. However, these limitations are likely not considerable because previous data have suggested that the sensitivity and specificity for the H. pylori detection method used in the present study were both more than 95% in gastric cancer patients [45] and, in contrast to what is found in Western populations, CagA seroprevalence has been reported to be greater than 95% in infected Koreans [46]. Second, this study is a case-control study. Thus, selection and recall biases may affect the results. Third, the sample size was relatively small, especially for the examination of gene-environment interactions in stratified analyses [47]. Therefore, the near significant interactions between gene and environmental factors or even the null results should be interpreted carefully because of our limited power. Finally, several residual confounding factors may be important to consider. Other genes and environmental factors (e.g., dietary habits) may act either alone or in concert with those studied here in the pathogenesis of gastric cancer. Therefore, studies using a larger number of subjects from different populations are needed to elucidate additional gene-gene and gene-environment interactions in gastric cancer susceptibility [18].

In conclusion, our study found that IL10 related host susceptibilities may play a role in gastric carcinogenesis at noncardia locations via an interaction with H. pylori infection and cigarette smoking. These results support the notion that gastric carcinogenesis is induced in multiple steps that involve both genetic and environmental factors. These findings may be helpful in identifying individuals at an increased risk for developing noncardia gastric cancer.

**Supporting Information**

**Table S1 Combined Effects of H. pylori Infection and Smoking on the Association between IL10 Genetic Variants and the Risk of Advanced Atrophy and Intestinal Metaplasia in the Antrum and Body of the Stomach.** We evaluated the associations between either intestinal metaplasia or atrophy [advanced stage (grade 2 + grade 3) vs. early stage (grade 0 + grade 1)] and IL10 SNPs, and the combined effect of H. pylori infection and smoking on these associations. The
numbers presented on the first line for each group represent the numbers of controls/cases while the numbers on the second line are the adjusted odds ratios with the 95% confidence intervals in parentheses. Adjustments were made for age, sex, alcohol consumption, education, and income. IL10-819 and IL10-592 were in complete linkage disequilibrium.

Author Contributions
Conceived and designed the experiments: JJC JK YAC. Performed the experiments: YSL S-YK M-CK. Analyzed the data: JK YAC. Wrote the paper: JK YAC JJC. Contributed to the acquisition of data and critical revision of the manuscript: AS S-JC M-CK JHN KWR JHL Y-WK.

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