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Interleukin-1B-31 gene polymorphism in Hakka gastric cancer patients in Guangdong, China

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ABSTRACT. The aim of this study was to examine the interleukin-1B (IL-1B) gene promoter region -31 (IL-1B-31) polymorphism distribution characteristic of Hakka gastric cancer patients in Guangdong Province and to explore its association with gastric cancer. We used the 1:1 case-control method, matrix-assisted laser desorption ionization flight time mass spectrometry, and MassARRAY-IPLEX technology to genotype IL-1B-31 (-31C> T) in 52 Hakka gastric cancer patients and 52 Hakka control subjects in Meizhou. Three genotypes - CT, TT, and CC - of IL-1B-31 were found in the Meizhou Hakka population. Their distribution frequencies in the gastric cancer group were 40.38, 40.38, and 19.23%, respectively, whereas the frequencies in control subjects were 57.69, 17.31, and 25.00%, respectively. The differences in frequency distributions of the genotypes between the 2 groups were statistically significant (chi-square = 6.78, $P < 0.05$). Subjects with the TT genotype had a higher risk of gastric cancer compared with that in subjects carrying the CT genotype (odds ratio = 2.857, 95% confidence interval = 1.114-7.328). This risk was more apparent in male subjects. IL-1B-31 locus polymorphism may be associated with gastric cancer susceptibility in this population, but additional studies with larger sample size are needed to confirm the conclusions.

Key words: Interleukin-1B; Single nucleotide polymorphism; Gastric cancer

INTRODUCTION

Gastric cancer is one of the most common malignant cancers in China, but its etiology remains controversial. Only a few individuals in the same environment develop the disease, suggesting that host genetic susceptibility is an important factor. Studies of the association of interleukin-1B gene promoter region -31 (IL-1B-31) polymorphisms with susceptibility to gastric cancer have been inconclusive. IL-1B-31 must be investigated in various populations in different countries and regions. The Hakka population has a relatively unique genetic background in the Han branch. This study was designed to discover the frequency distribution of IL-1B-31 genotypes in this population and explore the relationship between the polymorphism and the susceptibility to gastric cancer.

MATERIAL AND METHODS

Clinical data

Newly diagnosed gastric cancer patients (classified according to the ICD-151 standard) were selected between March and July 2009 at the Meizhou People's Hospital and the affiliated hospital of Jiaying University. We included 52 gastric cancer patients: 32 males and 20 females aged 35 to 80 years (average age, 57.65 ± 10.29 years). All patients underwent endoscopy or pathological examination for clear diagnosis. We also randomly selected 52 patients with non-digestive diseases and cancer-free patients of the same age (age difference, ≤5 years) and residence years from the two hospitals as a control group. The control group included 25 males and 27 females aged 19 to 79 years (average age, 53.65 ± 12.12 years). The patients in both groups had patrilineal and matrilineal Hakka ancestry and they had been living in the Meizhou municipality jurisdiction for 3 generations or more (including 3 generations). No significant age or gender difference was present in the 2 groups.

Locus selection and primer design

IL-1B-31 gene polymorphisms were selected according to an IL-1B gene target sequence and associated polymorphic loci. Polymerase chain reaction (PCR) primers were designed with the Sequenom Assay Design 3.0 software made by the Sequenom company. The primers were amplified and extended according to the multiplex PCR specificity for this locus. The specific amplified primers were 5'-ACGTTGGATGGAAATTTCTCAGCCTCCTAC-3' and 5'-ACGTTGGATGCGAAGAGGTTTGGTATCTGC-3'. The primers for the single-base extension were 5'-TTTCTCCCTCGCTGTTTTTAT-3'.

Genomic DNA extraction

A DNA extraction kit (TIANamp Genomic DNA Kit, Tiangen Biotechnology Co., Ltd., China) was used to extract DNA according to manufacturer instructions. The DNA was stored at -70°C until further analysis.

Gene analysis

After extraction and purification, genomic DNA samples were diluted quantitatively

and placed on a 384-well plate with the designed sequence. The PCR amplification system was added to a final concentration of reactants as follows: 0.1 U Taq polymerase, 5 ng genomic DNA, 2.5 pmol PCR primers, and 2.5 deoxyribonucleotide triphosphate (dNTP). PCR amplification conditions were 95°C for 15 min, then 45 cycles of 95°C for 20 s, 56°C for 30 s, and 72°C for 30 s. The remaining dNTP was removed by adding 0.3 U alkaline phosphatase. Then, 5.4 pmol of each extension primer was added to complete the single-base extension process. The primers included 50 µmol dNTP/dideoxyribonucleotide triphosphate mixture and 0.5 U Thermosequenase DNA polymerase. The reaction conditions were 94°C for 2 min, then 40 cycles of 94°C for 5 s, 50°C for 5 s, and 72°C for 5 s. After resin desalination, the reaction products were spotted into SpectroCHIP chips (Sequenom) with an automatic spotting instrument. The chips were detected using matrix-assisted laser desorption ionization flight time mass spectrometry (SpectroREADER, Sequenom).

Statistical analysis

The SPSS 17.0 statistical software was used for analysis. After testing for Hardy-Weinberg equilibrium, we analyzed the genotype and allele frequency of the patient and control groups using the chi-square test. A binary logistic regression analysis was used to evaluate the association between the polymorphic loci and gastric cancer susceptibility with the odds ratio (OR) and 95% confidence interval (95%CI) as the relative risk. A P value of <0.05 was considered to be significant difference.

RESULTS

Characteristic mass spectra of 3 genotypes of IL-1B-31

Mass spectra of the Il-1B-31 genotypes are shown in Figure 1, where Figure 1A shows the CC genotype, Figure 1B shows the CT genotype, and Figure 1C shows the TT genotype.

Genotype frequency distribution of IL-1B-31 in patients and controls

As shown in Table 1, the CT, TT, and CC polymorphisms were present in the IL-1B-31 loci in the Meizhou Hakka population. The total distribution frequencies were 49.04, 28.85, 22.11%. The distribution frequencies in the gastric cancer group were 40.38, 40.38, and 19.23%, respectively, whereas those in control subjects were 57.69, 17.31, and 25.00%, respectively. The differences in the 3 genotype frequencies were statistically significant between the 2 groups (chi-square = 6.78, $P < 0.05$). The C and T allele frequencies in the gastric cancer group were 39.42 and 60.58%, respectively, whereas those in the control group were 53.85 and 46.15%, respectively. The differences in allele frequencies between the 2 groups were statistically significant (chi-square = 4.35, $P < 0.05$). Both allele frequencies of the polymorphisms fit Hardy-Weinberg genetic equilibrium (chi-square = 1.54, $P = 0.22$). The genetic equilibrium of each allele was reached, which meant that the samples represent the total population. Binary logistic regression analysis using the CT genotype as the dumb amount showed that the risk of developing gastric cancer for subjects carrying the TT genotype was higher than that for subjects carrying the CT genotype (OR = 2.857, 95%CI = 1.114-7.328).

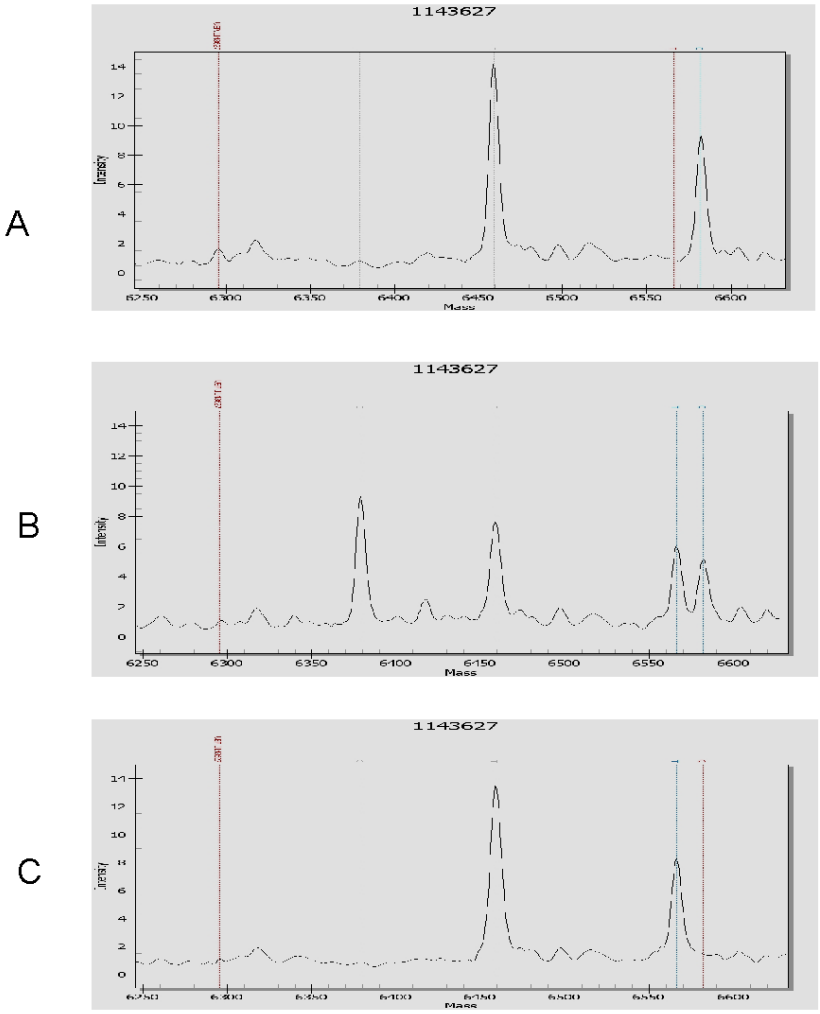

Figure 1. Schematic of the CC (**A**), CT (**B**), and TT (**C**) polymorphism of IL-1B-31.

Table 1. Distribution of the IL-1B-31 polymorphism in gastric cancer and control groups.

Genotypes	Patients [N (%)]	Control [N (%)]	OR	95%CI	P value
CT	21 (40.38)	30 (57.69)	1.000	-	-
TT	21 (40.38)	9 (17.31)	2.857	1.114-7.328	0.029
CC	10 (19.23)	13 (25.00)	1.099	0.406-2.973	0.853
C allele	41 (39.42)	56 (53.85)	-	-	0.03
T allele	63 (60.58)	48 (46.15)	-	-	-

IL-1B-31 genotype stratification analysis with the age and gender

Table 2 shows the results of the stratified analysis of age and gender in patients and

controls. Males with the -31C/C genotype were 10.50 times more likely to develop gastric cancer than those with the -31T/C genotype (95%CI = 1.620-68.072).

Table 2. Logistic analysis of the IL-1B-31 polymorphism in gastric cancer and control groups.

Variables	Patients			Control			OR (95%CI)	
	CT	TT	CC	CT	TT	CC	TT *vs* CT	CC *vs* CT
Gender								
Male	12 (23.08)	15 (28.85)	5 (9.62)	16 (30.77)	2 (3.85)	7 (13.46)	-	10.500 (1.620-68.072)
Female	9 (17.31)	5 (9.62)	5 (9.62)	14 (26.92)	8 (15.38)	6 (11.54)	-	-
Age (years)								
>60	7 (13.46)	10 (19.23)	7 (13.46)	12 (23.08)	1 (1.92)	4 (7.69)	-	-
<60	14 (26.92)	10 (19.23)	3 (5.77)	18 (34.62)	9 (17.31)	9 (17.31)	-	-

DISCUSSION

Gastric cancer is a long-term, multi-factor, multi-step malignant cancer with serious consequences for human health. Currently, its pathogenesis has not been elucidated, but the widely recognized pyloric bacillus infection is an important causal factor. However, because not all individuals develop gastric cancer under the same environmental conditions, host genetic background may be an important factor in the etiology. Since 2000, the association of the IL-1 gene polymorphism and gastric cancer has attracted the attention of many researchers. The IL-1 gene is located on the 430-kb region of chromosome 2q and consists mainly of 3 members: IL-1A, IL-1B, and IL-1ra. IL-1B is an inflammatory cytokine generated in the process of absorbing antigen-antibody complexes and presenting antigens to monocytes, macrophages, and other cells. IL-1B also initiates a series of cellular biological effects after binding with the corresponding receptors to enhance host response to *in vitro* and *in vivo* stimuli. IL-1β is the most powerful antacid known: 1 mol IL-1β has 100 times the antisecretory effect of 1 mol proton pump inhibitors. This strong acid suppression function can lead to gastric atrophy, which increases the risk of gastric cancer (El-Omar, 2001).

Three polymorphic loci occur in IL-1B, and they are located at the transcription initiation end of -511 bp, -31 bp, and +3945 bp. All of these loci have the C-T mutation. The regulatory sequences of the IL-1B gene have polymorphisms that affect protein expression. IL-1B has become a candidate gene in the study of host genetic factors in gastric cancer. El-Omar et al. (2000) were the first to study the IL-1B gene polymorphism and gastric cancer susceptibility. Their results have shown that at least 20 polymorphisms occur in the IL-1B gene. The IL-1B-31 gene polymorphism (rs1143627) is located in the TATA box. C/T transition can significantly affect the efficiency of IL-1B gene transcription and expression. Association studies of the IL-1B gene polymorphism and gastric cancer have since become a hot topic. Rollinson et al. (2003) have confirmed that the frequencies of the 3 genotypes in the healthy population of United Kingdom are as follows: C/C, 14.2%; C/T, 40.1%; and T/T, 45.7%. The C allele frequency is 54.3%. Hu and Zeng (2004) have measured the distribution of the IL-1B-31 gene polymorphism in the healthy population in Guangdong and Shaanxi (which has a high incidence of gastric cancer). The results showed that the distribution frequencies of the C/C, C/T, and T/T genotypes in Guangdong were 81.3, 18.2, and 5%, respectively, whereas those in Shaanxi were 75.7, 20.1, and 42, respectively. The C allele frequencies were 99.5 and 95.8, respectively (Zeng et al., 2003; Hu and Zeng, 2004). Zhang et al. (2007a) have found that

the frequencies of IL-1B-31 in Qingdao were C/C, 22.42%; C/T, 47.20%; and T/T, 35.05%. The results of the present study revealed CT, TT, and CC polymorphisms on IL-1B-31 in the Meizhou Hakka population. The total distribution frequencies were 49.04, 28.85, and 22.11%, respectively. The frequencies of distribution among gastric patients were 40.38, 40.38, and 19.23%, whereas those in the controls were 57.69, 17.31, and 25.00%, respectively. The 3 genotypes displayed statistically significant differences between the patient and control groups (chi-square = 6.78, $P < 0.05$). Differences in distribution frequency were also found between our study and other domestic and international studies.

The association of the IL-1B-31 gene polymorphism and gastric cancer shows some differences locally and abroad. Garza-González et al. (2005) have found that IL-1B-31 C+ is associated with gastric cancer susceptibility in the Mexican population. He et al. (2004) have found that TT homozygosity may be a predisposing factor for gastric cancer in Shenyang city of China. Lee et al. (2003) have found no evidence that IL-1B-31 polymorphism is associated with gastric cancer in the Korean population. Similarly, a study of white Italians by Zambon et al. (2004) and a study of 91 patients by Murphy et al. (2009) uncovered no association. Zhang et al. (2007b) also found no association between IL-1B-31 gene polymorphisms and gastric cancer in 101 gastric cancer patients and 113 healthy subjects. Matsukura et al. (2003) have found that the association between the IL-1B-31 polymorphism and gastric cancer susceptibility had racial differences in 4 Asian ethnic groups. These studies suggest that allele distribution frequency may have racial links.

We studied the Hakka population in this study. The Hakkas are a branch of the Chinese Han population who speak Hakka. This ethic group numbers 120 million around the world, with more than 58 million living in China. Hakka is one of the 3 residence systems in the Guangdong Province. Academically, the origin of the Hakka is controversial. Luo (1989) has concluded that the Hakka are descendants of the most pure Han in central China. However, other scholars have analyzed the principal components of the paternal Y chromosome single nucleotide polymorphism and concluded that the Hakka are most like the Han but also display features of the She nationality who speak the Miao language in China, and are different from southern Han who speak the DongTai language (Li et al., 2003). A gene polymorphism study (e.g., human glutathione S-transferase gene) has also suggested that the Hakka are different from the other residence systems in the Chinese Han population (Pan et al, 2005). These studies suggest that the Hakka have a relatively unique genetic background.

In this study, statistically significant differences were found in the CC, CT, and TT genotype distributions of IL-1B-31 between the case and control groups. Subjects with the TT genotype had a higher risk of developing gastric cancer compared with those carrying the CT genotype (OR = 2.857, 95%CI = 1.114-7.328). These results are similar to the findings of El-Omar et al. (2000). In conclusion, this study shows that the IL-1B-31 polymorphism may be associated with gastric cancer susceptibility in the Hakka population in Guangdong Province, but further studies are needed to expand the sample size to confirm the association.

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