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Original Article

LEPR polymorphsims and smoking interactions associated with the risk of gastric cancer

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Abstract: Purpose: To analyze the relationship between the Leptin receptor (*LEPR*) gene polymorphisms (rs1137100 and rs1137101) and gastric cancer (GC) susceptibility. Besides, explore the interactions between smoking and *LEPR* polymorphisms (rs1137100 and rs1137101) in GC. Methods: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to analyze the polymorphisms of rs1137100 and rs1137101 in 89 GC patients and 97 healthy controls. Gene-environment interaction was evaluated by crossover analysis. What's more, Hardy-Weinberg equilibrium (HWE) was utilized to distinguish the differences of the frequencies of genotypes. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were used for assess the risk of GC. Results: Analysis of *LEPR* polymorphisms and GC risk showed that AA genotype and A allele of rs1137100 were significantly decreased the GC risk (*P*=0.036, OR=0.259, 95% CI=0.068-0.987; *P*=0.008, OR=0.493, 95% CI=0.291-0.833). No obvious correlation has existed between rs1137101 AA and GA genotypes and the risk of GC. But, rs1137101 A allele might reduce the GC risk (*P*=0.006, OR=0.446, 95% CI=0.248-0.802). Gene-environment interaction analysis denominated that rs1137100 GG and GA genotypes and smoking interaction increased the risk of GC (*P*=0.007, OR=10.947, 95% CI=1.313-91.251), and the same to GG and GA genotypes of rs1137101 (*P*=0.019, OR=9.143, 95% CI=1.027-81.385). Conclusion: A allele of rs1137100 and A allele of rs1137101 might decrease the GC risk, respectively. Interactions between smoking and *LEPR* polymorphisms (rs1137100 and rs1137101) were positive with GC risk.

Keywords: *LEPR,* polymorphism, gastric cancer, interaction

Introduction

Gastric cancer (GC), one of the top five malignant tumors, possesses a high mortality in the worldwide [1, 2]. GC is one of the tumors which with the highest incidence and mortality in digestive system in China [3, 4]. GC occurrence is caused by various factors and processes, including diet, smoking and drinking, carcinogen, genetic factors and etc [5-9]. Genetic factors include the out of control of genes, DNA methylation and single nucleotide polomorphisms (SNPs). Recent years, multiple studies focus on the pathogenesis of GC. However, up to now, the etiology of GC is not well known. Previous researches indicate that the genetic and environment factors play important roles in the GC pathogenesis. Researches also have shown multiple biomarkers, which related to the GC susceptibility, including Leptin receptor (*LEPR*) gene [10].

LEPR protein belongs to the gp130 cytokine receptors family, and firstly found in the hypothalamus [11], and expressed in human gastric mucosa [12]. *LEPR* gene is located at 1p31, include 24 exon, and play an important role in the regulation of body fat balance and energy metabolism [13-16]. Additionally, the *LEPR* gene has become a research focus since its polymorphisms were discovered. A number of studies have shown that mutations in *LEPR* might associate with obesity and some clinical diseases. *LEPR* polymorphisms can affect nervous system and different kinds of gastrointestinal hormones through regulating the functions of the digestive system.

Epidemiological studies have shown that various SNPs might correlate with the susceptibility of GC. Matsuoka N et al. indicated that 7 nucleotide variants (Lys109Arg, Gln223Arg, Ser343-Ser, Lys656Asn, Ser492Thr, Ala976Asp and

Table 1. Clinical data in the case and control groups

Clinical-features	Case n=89 (%)	Control n=97 (%)	*P* value
Gender			>0.05
male	61 (68.54)	69 (71.13)	
female	28 (31.46)	28 (28.87)	
Age ($\overline{X}$ ±s)	54.76±4.89	54.81±5.65	>0.05
Smoking			<0.01
-	35 (39.33)	77 (79.38)	
+	54 (60.67)	20 (20.62)	

-: nonsmoking; +: smoking.

Pro1019Pro) exist in *LEPR* gene [17]. Besides, multiple studies clarified that smoking might enhance the susceptibility of many diseases, including GC [18]. In China, there were a seldom researches related to the *LEPR* variants and GC risk, and the gene-environment interaction between *LEPR* SNPs (rs1137100 and rs1137101) and smoking.

Therefore, we carried out this study to probe the correlation between *LEPR* SNPs (rs1137100 and rs1137101) and GC susceptibility in Chinese Han population. At the same time, we performed a further study on the correlate of GC risk with gene-environment interaction.

Materials and methods

Research objects

We adopted the design of case-control and chose 89 patients with GC in Renmin Hospital of Wuhan University. Meanwhile, 97 healthy individuals were enrolled in the control group. Additionally, age and gender in the control group were corresponding with the case group. GC patients had no medical history of other malignant tumors, autoimmune diseases, co-occurring malignant tumors in endoscopic morphology and histopathology, and did not receive radiotherapy and chemotherapy before blood sampling. People in the control group were chosen among the same period people who had received health check-up in the hospital and did not had any digestive system disease. Clinical features were surveyed and shown in **Table 1**. People who smoke a cigarette every day at least, and last for six months or more was defined as smokers. This study was approved by the ethics committee of the hospital. Sample collection process was conducted on the basis of the principles of research ethics. Moreover, all of the participants signed the informed consent before the study. All participates were unrelated Chinese Han population.

DNA extraction

2 mL peripheral venous blood was collected from every participates who has a 12 h fast, and processed with ethylene diamine tetraacetic acid (EDTA). Stored at -80°C for spare. DNA was extracted with the phenol-chloroform method, and reserved at -20°C.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

PCR primers of *LEPR* polymorphisms (rs1137100 and rs1137101) were designed by Primer 5.0, and synthesized by Shanghai GeneCore Bio Technologies Co., Ltd. The primer sequences are as follows: forward 5'-ACTTTTCTAACTTATCCAA-3' and reverse 5'-ATAAGTTAGAAAAGTGAGTA-3' were for rs1137100; rs1137101 primers were forward 5'-AGGCAGTTTTCAGATGGTTC-3' and reverse 5'-GTGAACCATCTGAAAACTGC-3'.

The method of PCR-RFLP was adopted to analyze *LEPR* polymorphisms. The volume of PCR reaction system was 25 μl, including 5.0 μL template DNA, 0.4 μL forward primer, 0.4 μL reverse primer, 0.3 μL Tag DNA polymerase, 2.5 μL $MgCl_2$, 2.5 μL 10× buffer solution, 0.5 μL 4× dNTPs and 13.4 μL sterile water. PCR amplification was initially performed with 5min predenaturation at 95°C, followed by 30 cycles of 40 s degeneration at 95°C, 30 s annealing at 56°C, 50 s extension at 70°C; then 10 min finally extension at 72°C. The PCR products were digested by MspI enzyme. At the end, genotypes of each polymorphism were determined by agarose gel electrophoresis.

Statistical analysis

The χ^2 test was conducted with PASW Statistics software and was used to compare the distribution differences of genotypes and alleles of *LEPR* polymorphisms in the case and control groups ($P<0.05$ indicates the difference with statistical significance). Hardy-Weinberg equilibrium (HWE) examination was conducted with PLINK1.07 software in the case and control

Table 2. Distributions of genotypes and alleles of rs1137100, rs1137101 and rs1805094

Genotype/ Allele	Case group n=89 (%)	Control group n=97 (%)	χ^2	*P* value	OR (95% CI)
rs1137100					
GG	66	57	-	-	1
GA	20	30	2.653	0.103	0.576 (0.295-1.122)
AA	3	10	4.399	0.036	0.259 (0.068-0.987)
G	152 (85.39)	144 (74.23)	-	-	1
A	26 (14.61)	50 (25.77)	7.12	0.008	0.493 (0.291-0.833)
rs1137101					
GG	71	62	-	-	1
GA	17	29	3.690	0.055	0.512 (0.257-1.019)
AA	1	6	4.069	0.044	0.146 (0.017-1.242)
G	159 (89.33)	153 (78.87)	-	-	1
A	19 (10.67)	41 (21.13)	7.508	0.006	0.446 (0.248-0.802)

groups (*P*>0.05 indicates the equilibrium). Crossover analysis was adopted to analyze the interaction between *LEPR* SNPs and smoking. Besides, the relative risk of GC was denoted by odds ratios (ORs) with 95% confidence intervals (95% CIs).

Results

HWE test

In the 89 GC patients and 97 healthy individuals, the genotypes distributions of rs1137100 and rs1137101 of the *LEPR* gene were conformed to HWE (*P*>0.05). Specifically, goodness of fit of HWE in every polymorphism showed that the participants were in a good balance and the samples were representative.

Alleles and genotypes of LEPR polymorphisms associated with GC

Genotype distributions of rs1137100 and rs1137101 have shown in **Table 2**. Compared with the control group, genotype frequencies of AA and AA of rs1137100 and rs1137101 were statistically significant lower in the case group, respectively (*P*<0.05). Additionally, A alleles of rs1137100 and rs1137101 polymorphisms existed a significant association with GC susceptibility (*P*<0.05), and might reduce the risk of GC (OR=0.493, 95% CI=0.291-0.833; OR=0.446, 95% CI=0.248-0.802), respectively. What's more, AA genotype of rs1137100 was significantly decr-eased the susceptibility of GC (OR=0.259, 95% CI=0.068-0.987). But, there were no significant correlations of the GA and AA genotypes of rs11-37101 with GC susceptibility.

Interactions between LEPR polymorphisms (rs1137100 and rs11-37101) and smoking in GC

Smoking existed significant difference between case and control groups (*P*<0.01, **Table 1**). We adopted a further crossover analysis on the interactions between smoking and *LEPR* polymorphisms (rs1137100 and rs1137101) with GC risk. Then we got the results that the interactions between *LEPR* polymorphisms and smoking have existed (**Tables 3**, **4**). Patients with GG and AG genotypes in rs1137100 SNP and smoking might increase the GC risk (*P*=0.007, OR=10.947, 95% CI=1.313-91.251). Smokers with rs1137101 GG and AG genotypes compared with AA genotype had 9.143 times risk of GC (*P*=0.019, OR=9.143, 95% CI=1.027-81.385), respectively.

Discussion

GC is the most common malignant tumor of the digestive system, and its mortality is one of the top five among all the malignant tumors annually [19]. GC mainly refers to the advanced GC and its morbidity and mortality is very high in China. Recently, GC trends to occur in younger populations, which brings heavy burden to the family and society. Therefore, how to improve the comprehensive prevention level of GC has attracted great attention. Researches indicated that the occurrence of GC was a complex, multiple factors associative effects process, involving genetic factors and environment factors. SNP is the third generation biomarker, and extensive researches were committed on the correlation between SNP and the disease etiology. Hence, a lot of SNPs were found to be related with the etiology of disease, such as *LEPR* polymorphisms. *LEPR* gene is about 5.1 kb in length, and can encode 1165 amino acids [17]. So far, different kinds of polymorphisms of *LEPR* gene have been discovered to correlate with many diseases [20-30]. Rs1137100 (Lys109Arg, locate at exon-2) and rs1137101 (Gln223Arg, locate at exon-4) are the common

Table 3. The interaction between rs1137100 and smoking in GC

Genotype	Smoking	Case	Control	*P* value	OR (95% CI)
AA	-	1	8	-	-
AA	+	2	2	0.125	8.000 (0.459-139.290)
AG+GG	-	34	49	0.080	5.551 (0.663-46.450)
AG+GG	+	52	38	0.007	10.947 (1.313-91.251)

Table 4. The interaction between rs1137101 and smoking in GC

Genotype	Smoking	Case	Control	*P* value	OR (95% CI)
AA	-	1	5	-	-
AA	+	0	1	0.659	1.200 (0.839-1.716)
AG+GG	-	24	56	0.488	2.143 (0.238-19.332)
AG+GG	+	64	35	0.019	9.143 (1.027-81.385)

LEPR polymorphisms in previous studies. They all related to many diseases [16, 31], including GC [10].

This case-control study analyzed the correlation between *LEPR* polymorphisms and the risk of GC, and then further explored the interactions between SNPs and smoking in GC. Through the careful analysis, we discovered that AA genotype of rs1137100 was distinctly lower in the case group compared with the control group, denominating that the AA genotype increased the GC risk. No significant correlation was found between AG genotype and GC risk. A allele of rs1137100 SNP was reduced the GC risk at 0.493 rebate. These results were different from previous study, which proved that the GA genotype was increased the GC risk with 2.926 times compared with the AA genotype [10]. However, another research showed that the G allele increase the myelomeningocele risk [32], that accorded with our result. Although AA genotype of rs1137101 was notable lower in case group, but there was no significant statistic correlation with GC risk. Result indicated that rs1137101 A allele might increase the risk of GC (*P*=0.006). That was different from the research which indicated that rs1137101 didn't associated with the risk of GC [10].

Additionally, environment factors affect the occurrence of diseases. Recent studies illustrated some external factors associate with various cancers, such as smoking and drinking, unhealthy diet, carcinogen and etc [5-9, 33]. As we all know, tobacco contain various hazardous substances which could damage body health, induce multiple diseases. So smoking might become a danger factor to human health. But not all smokers suffer from diseases, there existed Individual difference, prompting that disease occurrence was affected by gene-environment interactions. In this study, we performed the gene-environment interaction analysis and found that the SNPs and smoking existed a positive interaction with GC risk. GG genotype of rs1137100 SNP was the susceptibility genotype, and the smoking enhanced the risk of GC. GG and GA genotypes smokers had higher GC risk compared with AA genotype nonsmoking individuals, the odd ratio was 10.947. Single GG or AG genotypes of rs1137101 polymorphism has no significant association with GC risk, but AG and GG genotypes smokers had high GC risk. The results revealed that there exists an interaction between rs137101 and smoking, and the interaction was positive with the susceptibility of GC.

In summary, rs1137100, rs1137101 and smoking were all significantly associated with GC risk. We should pay attention on the gene-environment interaction, then find its effects in the occurrence of GC, and improve the methods for prevention, diagnosis and treatment of GC. So, further researches with a larger sample and investigations including gene-gene and gene-environment interactions might provide more explicit evidence.

Disclosure of conflict of interest

None.

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