Int J Clin Exp Pathol 2017;10(9):9753-9759
www.ijcep.com /ISSN:1936-2625/IJCEP0021065

Original Article

Effect of interactions between *LEPR* polymorphisms and smoking on coronary artery disease susceptibility

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Received December 2, 2015; Accepted September 21, 2016; Epub September 1, 2017; Published September 15, 2017

Abstract: Objective: This study was designed with the purpose of analyzing the relationship between the interactions of leptin receptor (*LEPR*) rs1137101, rs1137100 polymorphisms with smoking and the susceptibility to coronary artery disease (CAD). Methods: The subjects of this study consisted of 101 CAD patients and 110 healthy people frequency-matched with the former in age and sex. The genotyping of *LEPR* polymorphisms were performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The distribution differences of genotype and allele between two groups was estimated by χ^2 test. The relative risk of CAD was showed by odds ratio (OR) with 95% confidence interval (95% CI). Crossover analysis was applied in the studying of gene-environment interactions. Results: *LEPR* rs1137101 polymorphism showed significant difference between CAD patients and healthy controls neither genotypes nor alleles in this study population (P>0.05). Fortunately, we detected that AA genotype of *LEPR* rs1137100 polymorphism had frequency difference between two study groups with a significant level, compared with the common genotype GG (P=0.03), which showed that this polymorphism had an independent association with CAD (OR=3.07, 95% CI=1.08-8.73). So was A allele of rs1137100 (OR=1.58, 95% CI=1.04-2.39). Furthermore, we observed the interaction of smoking and rs1137100 polymorphism in CAD occurrence (OR=2.69, P=0.01). Conclusions: Not only is *LEPR* rs1137100 polymorphism an independent risk factor for CAD, but it exists the interaction with smoking in the onset of CAD in this Chinese population.

Keywords: Leptin receptor, polymorphism, smoking, interaction, coronary heart disease

Introduction

At present coronary artery disease (CAD) is one of the most common cardiovascular diseases and seriously affect the human health and life quality. Recently, the occurrence of CAD trends to be younger and it is the leading cause of deaths all over the world [1]. According to the estimation of World Health Organization (WHO), by 2030, the deaths caused by ischemic heart disease will account for 13.1% of all deaths in the world [2]. Therefore, the prevention and treatment of CAD brook no delay. CAD is proved to be a complicated disease caused by the cooperation of genetic and environmental factors, and the known traditional risk factors like smoking, family history, diabetes, insulin resistance, hypertension, obesity and dyslipidemia have attracted the attention of clinical studies, and active intervention treatment of the disease are being performed.

As the in-depth research, scientists find that leptin and leptin receptor (*LEPR*) are closely associated with obesity as well as related diseases such as hypertension, CAD, diabetes and lipid metabolism disorder [3, 4]. Leptin is a circulating hormone secreted by fat cells, and can regulate the energy balance, fat stores, and some endocrine functions of the body through its combination with the *LEPR* of the central nervous system. *LEPR* is located on chromosome 1p31 in humans successfully cloned from mice by Taltaglia et al. in 1995 firstly, including 20 exons and 19 introns [5, 6]. Until now, 19 single nucleotide polymorphisms (SNPs) have been discovered in *LEPR* exons [7]. These SNPs are proved to involve in various diseases such as obesity and diabetes which are both the risk factors for CAD, so *LEPR* polymorphisms may influence the CAD susceptibility [8-11]. Furthermore, smoking has been proved by epidemiology data to have great influences on CAD.

Table 1. Primer sequences of *LEPR* rs1137101, rs1137100 polymorphisms

Locus	Primer sequence		Length
rs1137101	Forward	5'-CAAACTCAACGACACTCTCC-3'	276 bp
	Reverse	5'-CCACTCTTAATACCCCCAGT-3'	
rs1137100	Forward	5'-GCTGGACTCTCAAAGAATAC-3'	196 bp
	Reverse	5'-TTACTGTTGAAACAAATGGC-3'	

Table 2. The basic information analysis for the case and the control groups

Feature	Case	Control	*P*
Gender			>0.05
Male	60 (59.41)	63 (57.27)	
Female	41 (40.59)	47 (42.73)	
Age ($\bar{X}$±s)	51.73±11.98	53.09±10.82	>0.05
Smoking status			<0.05
-	63 (62.38)	84 (76.36)	
+	38 (37.62)	26 (23.64)	

Note: "-" represents the non-smokers, while "+" represents the smokers; and the smokers include regular smokers and casual smokers.

The morbidity and mortality of aorta atherosclerosis are 2-6 times higher among smokers than non-smokers, and are positively correlated with the daily smoking amount. About 30% of the patients with cardiovascular disease died of smoking [12].

Therefore, the present study investigated the correlation between rs1137101, rs1137100 polymorphisms in *LEPR* gene and CAD risk, as well as the interaction of these two polymorphisms with smoking in the development CAD.

Materials and methods

Study subjects

This study adopted a case-control design. 101 CAD patients from cardiology of Beijing Anzhen Hospital, Capital Medical University were selected as the case group, including 60 males and 41 females with a mean age of 51.73±11.98 years old. 110 healthy people including 63 males and 47 females with a mean age of 53.09±10.82 years were recruited into the control group. In order to the accuracy of this research, we randomly selected all the subjects from a Chinese Han population of Beijing region and they were no blood relationship each other. We gained the permission of the Ethics Committee of Beijing Anzhen Hospital, Capital Medical University and all participants signed an informed consent. The sample collection process strictly abode by the ethnics guidelines of National Human Genome Research Institute.

The cases all suffered from stable or unstable angina, or had a history of old myocardial infarction confirmed by electrocardiogram (ECG) and echocardiography. They had coronary angiography examinations at the cardiology department because of chest tightness and chest pain. Judkins method was used for the coronary angiography examination and the test results were checked by 2-3 diplomats experienced in cardiac catheterization. According to the CAD diagnostic criteria published by International Society of Cardiology (ISFC) and WHO, and ACC/AHA and ESC/ACC diagnostic criteria of acute coronary syndrome in 2000, the narrowing of diameter in any one of the left main coronary artery, anterior descending branch, circumflex branch and right coronary artery reached to 50% or more, it is as the stenosis lesion; otherwise, it is mild lesion [13, 14]. Patients would be excluded if they suffered from serious heart failure, severe liver and kidney dysfunction, recent acute and chronic inflammatory diseases, autoimmune diseases, disseminated intravascular coagulation and tumors together. The healthy controls whose sex and age were frequency-matched with those of the cases were selected from the physical examination center of the same hospital at the same time with the cases, and they were proved to have no chest pain or chest congestion after inquiries and all the indexes were normal through physical examinations.

The basic information of subjects were collected by unified questionnaire and recorded in the Excel form, including sex, age, smoking status and so on. People who smoked one or more than one cigarette every day and continued at least one year were as the smokers, so was the long-term smokers who smoking cessation was less than half a year.

Sample collection process

The participants experienced fasting 14 h for overnight, and 2 ml peripheral venous blood

Table 3. Genotypes and alleles distributions of rs1137101, rs1137100 polymorphisms between the case and control groups

Genotype/allele	Case, n=101 (%)	Control, n=110 (%)	χ^2 (*P*)	OR (95% CI)	P_{HWE}
rs1137101					0.72
GG	58 (57.43)	69 (62.73)	-	1.00 (Ref.)	
AG	36 (35.64)	37 (33.63)	0.25 (0.62)	1.16 (0.65-2.06)	
AA	7 (6.93)	4 (3.64)	1.31 (0.25)	2.08 (0.58-7.47)	
G	152 (75.25)	175 (79.55)	-	1.00 (Ref.)	
A	50 (24.75)	45 (20.45)	1.12 (0.29)	1.28 (0.81-2.02)	
rs1137100					0.42
GG	41 (40.59)	58 (52.73)	-	1.00 (Ref.)	
AG	47 (46.54)	46 (41.82)	1.61 (0.21)	1.45 (0.82-2.56)	
AA	13 (12.87)	6 (5.45)	4.68 (0.03)	3.07 (1.08-8.73)	
G	129 (63.86)	162 (73.64)	-	1.00 (Ref.)	
A	73 (36.14)	58 (26.36)	4.70 (0.03)	1.58 (1.04-2.39)	

was gained from every subject with empty stomach on the next morning. The blood sample was then put into ethylene diamine tetraacetic acid disodium salt dihydrate (EDTA-2Na) anticoagulated tubes and saved in the refrigerator at -80°C. Next step, genome DNA was extracted using phenol-chloroform extraction method and stored at -20°C for standby application.

PCR amplification

The genotyping of *LEPR* rs1137101, rs1137100 polymorphisms were done by the method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The PCR primers of *LEPR* polymorphisms were designed with Primer premier 5.0 programs and were synthesized in Shanghai Sangon Biotech Co., Ltd. The primer sequences are shown in **Table 1**. The PCR amplification system was 20 μl, and included template DNA 2.0 μl, forward and reverse primers 0.5 μl each, Taq DNA polymerase 0.5 μl, $MgCl_2$ 1.0 μl, 10× Buffer solution 2.0 μl, and dNTPs 1.0 μl and added ddH_2O to 20 μl. The reaction system was put into a PCR instrument and the amplification conditions were as follows: 95°C for 5 min; then 35 cycles of 94°C for 45 s, 56°C for 30 s and 72°C for 50 s; and finally 72°C extension for 5 min. The PCR products were digested by *BstE* II (rs1137101), *Hae* III (rs1137100) enzyme, and then 2%-3% agarose gel electrophoresis (AGE) was carried out to ascertain the genotypes of each single nucleotide polymorphism (SNP).

Statistical analysis

The PLINK1.07 software was used to detect the Hardy-Weinberg equilibrium (HWE) in control group, and HWE was satisfied when $P>0.05$. We used the PASW Statistics 18 software for statistical processing. χ^2 test was used to compare differences of genotype and allele frequencies as well as the basic indexes of subjects in two study groups. The $\bar{x}\pm s$ or % was utilized to represent the data in this article. Odds ratio (OR) and 95% confidence interval (95% CI) stood for the relative risk of CAD. The gene-environment interaction was assessed by crossover analysis. $P<0.05$ was as a statistically significant difference.

Results

Clinical data comparison of study objects

The basic situation of 101 CAD cases and 110 healthy controls was exhibited in **Table 2**. In CAD patients, the number of males was more than females and accounted for near 60% of all cases. The mean age of the cases was about 52 year old. The controls had no significant difference in age and gender with the cases ($P>0.05$). But the smokers in CAD patients were more than in the controls and the difference reached to the significant level of 0.05 ($P<0.05$), so smoking may be an independent risk factor for the onset of CAD.

HWE test

The genotype distributions of *LEPR* rs1137101, rs1137100 polymorphisms in controls were

Table 4. *LEPR* rs1137101 polymorphism in interactions with smoking in CAD

rs1137101	Smoking	Case	Control	*P*	OR	95% CI
-	-	37	54	-	-	-
-	+	21	15	0.07	2.04	0.93-4.47
+	-	26	30	0.49	1.27	0.65-2.48
+	+	17	11	0.06	2.26	0.95-5.36

Note: "-" represents GG genotype, "+" represents AG+AA genotype in line 1; "-" represents non-smoking and "+" represents smoking in line 2.

Table 5. Effects of the interactions between *LEPR* rs1137100 polymorphism and smoking in CAD

rs1137100	Smoking	Case	Control	*P*	OR	95% CI
-	-	29	48	-	-	-
-	+	12	10	0.16	1.99	0.76-5.17
+	-	34	36	0.18	1.56	0.81-3.02
+	+	26	16	0.01	2.69	1.24-5.84

Note: "-" represents GG genotype, "+" represents AG+AA genotype in line 1; "-" represents non-smoking and "+" represents smoking in line 2.

consistent with HWE (P>0.05), which suggested the good representativeness of the sample population.

Correlation analysis of LEPR polymorphisms and CAD risk

The distributions of genotypes and alleles s in two SNPs of *LEPR* gene are presented in **Table 3**. The distribution of genotypes in rs1137101 polymorphism was not significantly different between the case and control groups (P>0.05). Likewise, allele distribution between two study groups was not obvious different, either. However, a high frequency of AA genotype in rs1137100 was observed in the cases and the difference with the controls had statistically significance (12.87% vs. 5.45%, P=0.03). A allele frequency was also markedly higher in cases than that in controls (P=0.03). Therefore, *LEPR* rs1137100 polymorphism was associated with the development of CAD under two models in this study (AA vs. GG: OR=3.07, 95% CI=1.08-8.37; A vs. G: OR=1.58, 95% CI=1.04-2.39).

Interactions of two SNPs with smoking on the development of CAD

The analysis result which the interaction between rs1137101 and smoking based on CAD was displayed in **Table 4**. We didn't find significant difference between any two combinations of rs1137101 with smoking status, which suggested that the interaction of this gene polymorphism and smoking was not apparent and it was not as a marker to indicate the CAD development.

We can see in the **Table 5** that the interaction of rs1137100 with smoking in development risk of CAD was analyzed. Compared with the common genotype GG with non-smoking, smokers with the genotype including A allele was the high risk to be subject to attacking of CAD (OR=2.69, 95% CI=1.24-5.84). So the interaction in both of two had an important influence on the onset of CAD and may be as an indicator of CAD to prevent the development of CAD.

Discussion

CAD is a kind of cardiovascular disease with the highest morbidity at present. For the past few years, the morbidity and mortality of CAD in China have been gradually increasing, and the disease has become the second leading cause of Chinese deaths. Coronary artery stenosis, and plaque rupture and bleeding caused by the arteriosclerosis of coronary artery lead to thrombosis, which induces CAD. Smoking, obesity and other risk factors lead to incomplete vascular endothelial structure and functions, which weakens the inhibition of leukocyte and platelet adhesion and leads to thrombosis, vascular narrowing and even blocking. On the other hand, it doesn't effectively inhibit the proliferation of vascular smooth muscle cells (VSMCs) so as to accelerate arteriosclerosis. CAD occurrence results from a combination of heredity and environmental factors, and clinical data indicate that adverse cardiac events can be reduced and even prevented by regulating the known risk factors CAD [15].

Leptin is a new independent risk factor of CAD occurrence [16, 17]. High level of leptin not only promotes platelet aggregation, increases blood viscosity and causes thrombogenesis, but also stimulates the proliferation of VSMCs, induces oxidative stress and accelerates arteriosclerosis, which finally leads to the occurrence of CAD

[18, 19]. Considine et al. firstly reported the genetic variant in exon 6 of *LEPR* gene, namely Gln223Arg (rs1137101) in 1996 [20]. And rs1137100 (Lys109Arg) is a mutation in exon 4 of *LEPR* gene. A study carried out by Rosmond et al. shows that *LEPR* Lys109Arg, Gln223Arg polymorphisms can influence the BMI and blood pressure of people with obesity [21]. Sun et al. discovered that *LEPR* Gln223Arg polymorphism affected the development process of CAD via facilitating the deposition of high density lipoprotein cholesterol (HDL-C) on blood vessel walls [22]. In this study, the allele frequencies of rs1137101 were similar to those of women in Shanghai reported by Zheng et al. and those of Japanese and Koreans reported by some other literature [23], but not in Caucasians, which reflects racial diversity in the allele distribution of rs1137101 polymorphism. However, in present study, we didn't find significant distribution difference in both of genotypes and alleles in rs1137101. On the contrary, AA genotype and A allele were high frequencies in CAD patients and had obvious difference with healthy controls in statistically. Therefore, *LEPR* rs1137100 may be a candidate susceptibility SNP to CAD, but not rs1137101 in our study population.

It is known to all that smoking not only induces disease for smokers but also threatens the health of people around them. It has been demonstrated that both active and passive smoking can increase the onset risk of CAD [24]. Long-term smoking can induce many serious diseases like tumors, cardiovascular and cerebrovascular diseases and chronic obstructive pulmonary diseases and is the second leading cause of global deaths after hypertension [25]. Excessive smoking can lead to coronary artery spasm, weaken the oxygen-carrying function of hemoglobins and eventually cause myocardial anoxia [26]. Firstly, the nicotine in cigarettes can directly damage vascular intima and prompt atherogenesis and CO; thiocyanate can induce coronary artery spasm, increase blood viscosity, interfere with the lipid metabolism and promote cholesterol deposition. Secondly, long-term smoking reduces coronary endothelium-dependent vasodilation function, increases platelet aggregation and the level of fibrinogen, and results in the increasing of von Willerbrand factor levels, thus causing and even aggravating the formation of coronary atherosclerotic plaque [27]. In addition, smoking changes the composition of blood fats by regulating the levels HDL-C and low density lipoprotein cholesterol (LDL-C). Consequently, the serum antioxidant effects are weakened, which promotes the occurrence and development of arteriosclerosis and CAD. As an independent high risk factor of CAD, it is a linear relationship with the severity of CAD [28]. Compared with persistent smokers with cardiovascular diseases, the relative risk of death in ex-smokers with such disease decreases 36% [29]. The vast majority of cardiovascular disease risk related to smoking is removed in smokers 12 to 18 months later after they quit smoking, and such risk in ever-smokers 3 to 5 years after they cease smoking is equivalent to that in never-smokers [30]. In this article, rs1137101 genotypes and smoking in the onset of CAD didn't been found the marked interaction, but smokers with genotype carrying A allele of rs1137100 had a significant higher risk to suffer from CAD than those non-smoker with the common GG genotype. Therefore, maybe this polymorphism and smoking regulate the CAD occurrence and development together. Furthermore, further studies should be conducted to verify the interaction association in CAD with well-design in the future.

CAD is a disease highly relevant to lifestyles, so controlling the risk factors of CAD like blood glucose and blood pressure, smoking cessation, exercises and diet management are particularly important in the prevention of disease. Hence, early detection and timely prevention of susceptible population is an effective means, certainly, the popularization of smoking harm for health and advocacy of smoking cessation are also not avoided.

Disclosure of conflict of interest

None.

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