Original Article

The association between \textit{LEP/LEPR} polymorphisms and coronary artery disease: a case-control study in an eastern Chinese Han population

Yuanmei Chen\textsuperscript{1}, Chao Liu\textsuperscript{2}, Aizhong Shao\textsuperscript{2}, Weifeng Tang\textsuperscript{2}, Yafeng Wang\textsuperscript{3}, Liangwan Chen\textsuperscript{1}

\textsuperscript{1}Department of Cardiac Surgery, Fujian Medical University Union Hospital, Fuzhou, Fujian Province, China; \textsuperscript{2}Department of Cardiothoracic Surgery, Affiliated People’s Hospital of Jiangsu University, Zhenjiang, Jiangsu Province, China; \textsuperscript{3}Department of Cardiology, The People’s Hospital of Xishuangbanna Dai Autonomous Prefecture, Jinghong, Yunnan Province, China

Received June 10, 2018; Accepted December 9, 2018; Epub May 15, 2019; Published May 30, 2019

Abstract: Obesity and being overweight are considered risk factors for coronary artery disease (CAD), but the underlying pathogenesis remains unclear. To investigate whether genetic polymorphisms in leptin (\textit{LEP}) and the leptin receptor (\textit{LEPR}) contribute to the development of CAD, we conducted a case-control study of the \textit{LEP} rs2167270 G\textgreater{}A, rs7799039 A\textgreater{}G and \textit{LEPR} rs1137100 G\textgreater{}A, rs1137101 G\textgreater{}A polymorphisms in the eastern Chinese Han population. In total, 505 CAD patients and 1,109 healthy controls were enrolled. The \textit{LEP/LEPR} polymorphisms were genotyped using the SNPscan\textsuperscript{TM} genotyping method. The results indicate that the \textit{LEP} rs2167270 G\textgreater{}A polymorphism may be a protective factor against CAD (GA vs. GG: adjusted odds ratio [OR], 0.77; 95\% confidence interval [CI], 0.61-0.98; P = 0.034), whereas \textit{LEPR} rs1137100 G\textgreater{}A increased the risk of CAD (AA vs. GG: adjusted OR, 1.98; 95\% CI, 1.01-3.92; P = 0.048 and AA vs. GG/GA: adjusted OR, 2.02; 95\% CI, 1.03-3.97; P = 0.042). In addition, we found that the \textit{LEPR} rs1137100 G\textgreater{}A polymorphism might be associated with an increased susceptibility to CAD among the subgroup of patients without a history of myocardial infarction (AA vs. GG: adjusted OR, 2.23; 95\% CI, 1.07-4.62; P = 0.032 and AA vs. GG/GA: adjusted OR, 2.31; 95\% CI, 1.11-4.78; P = 0.024). In conclusion, our results suggest that the \textit{LEP} rs2167270 G\textgreater{}A polymorphism may be considered a protective factor against CAD in the eastern Chinese Han population, while the \textit{LEPR} rs1137100 G\textgreater{}A polymorphism is likely a risk factor for CAD. Future studies considering detailed lifestyle and environmental factors are needed to confirm our findings.

Keywords: Polymorphism, \textit{LEP}, \textit{LEPR}, coronary artery disease, risk

Introduction

Coronary artery disease (CAD) is the leading cause of mortality in developed countries, and its prevalence continues to increase in China. The etiology of CAD is complex, but previous studies have shown that obesity and being overweight play important roles in the pathogenesis of CAD [1, 2]. It is well-established that adipose tissue secretes a number of adipokines, and a shift to the production of proinflammatory cytokines by these cells in obesity and overweight individuals likely results in a low-level systematic inflammation that may contribute to some chronic pathologies related to metabolic syndrome such as atherosclerosis [3].

\textit{LEP} is a hormone mainly secreted by adipose cells that acts to control metabolism and energy homeostasis. In humans, the \textit{LEP} gene is located on chromosome 7, and the encoded 16-kDa \textit{LEP} protein consists of 167 amino acid residues. Several studies have indicated that leptin (\textit{LEP}) and the \textit{LEP} receptor (\textit{LEPR}) are involved in the development of becoming overweight, as well as obesity [4-6]. \textit{LEP} is known to respond specifically to adipose-derived inflammatory cytokines [7]. Moreover, research indicates that \textit{LEP} may play an important role in the regulation of the immune and inflammatory response [8-10]. Specifically, Taleb et al. reported that \textit{LEP/LEPR} influences the immune response to atherosclerosis, for which obesity is likely a predisposing factor [11]. Together,
these findings suggest that altered LEP/LEPR signaling may influence the development of CAD.

Variants located on the LEP/LEPR genes likely affect the function of LEP/LEPR signaling. Yang et al. reported that the LEP rs1137101 G>A variant is associated with obesity in the Chinese population [12]. Wang et al. suggested that the LEP rs7799039 GG homozygous variant is associated with an increased risk of extreme obesity in the Taiwanese aborigine population [13]. Recently, several case-control studies focused on the correlation between LEP variants and the risk of CAD [14-16]. An et al. reported that the LEP rs1137101 G>A polymorphism confers a significant susceptibility to coronary atherosclerosis [17]. With the aim of defining the association between LEP/LEPR variants and the risk to CAD, we conducted a case-control study to determine the correlations between the LEP rs2167270 G>A, and rs7799039 A>G and LEPR rs1137100 G>A, rs1137101 G>A single nucleotide polymorphisms (SNPs) and CAD.

Materials and methods

Study population and sample collection

Five hundred five patients diagnosed with CAD were recruited at the Department of Cardiology and Cardiac Surgery of Fujian Medical University Union Hospital, Fuzhou, China between October 2014 and May 2016. The criterion for the diagnosis of CAD was coronary stenosis ≥ 50% on a coronary angiography [18]. The main inclusion criteria for the CAD cases were: (a) CAD confirmed by coronary angiography, and (b) chest pain associated with specific ischemic electrocardiograph changes. The main exclusion criteria for the CAD cases were: (a) cardiomyopathy, (b) coagulopathy, and (c) acute intoxication (e.g., carbon monoxide or amphetamine). The control group consisted of 1109 healthy age- and sex-matched individuals recruited from the physical examination center of the hospital at the same time. The main inclusion criteria for controls were: (a) no history and symptoms of CAD, and (b) no evidence of myocardial ischemia on electrocardiography examination [18]. Accordingly, participants with a history or symptoms of CAD or who showed specific ischemic electrocardiography changes were excluded.

Each study participant provided written informed consent and completed a questionnaire. We collected the demographic data and data regarding related risk factors through interviews. Sex, age, tobacco use, alcohol consumption, height, weight, body mass index (BMI), history of type 2 diabetes mellitus (T2DM), history of hypertension, and the levels of high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), total cholesterol (TC), fasting blood glucose, and low-density lipoprotein cholesterol (LDL-C) were measured. The criterion for smoking and drinking are described in our previous report [19]. T2DM was diagnosed based on the World Health Organization (WHO) 1999 criteria. Hypertension was defined by a systolic pressure ≥ 140 mmHg and/or diastolic pressure ≥ 90 mmHg [20]. BMI ≥ 24 kg/m² was the criterion for overweight or obese status [21]. According to the Chinese Medical Association 2016 criteria, hyperlipidemia is defined as LDL ≥ 3.37 mmol/L, TG ≥ 1.7 mmol/L, TC ≥ 5.18 mmol/L, or HDL < 1.04 mmol/L. This case-control study was approved by the Ethics Committee of Fujian Medical University.

Isolation of DNA and genotyping

Genomic DNA was isolated from peripheral venous blood samples using the Promega DNA Kit (Madison, WI, USA). The LEP/LEPR polymorphisms were genotyped using the SNPsCan™ genotyping method (Genesky Biotechnologies Inc., Shanghai, China). Genotyping was performed by two technicians blinded to the group assignment. To ensure the accuracy of genotyping, a randomized controlled assay was performed to check the genotyping of 65 samples with consistent results. The genotyping success rate for these SNPs was greater than 99.00%.

Statistical analysis

Continuous variables (e.g., TG, TC, LDL-C, HDL-C, and fasting blood glucose levels as well as height, weight, BMI, and age) are presented as the mean ± standard deviation (SD). We used Student’s t-test to assess differences in these variables between the CAD cases and the controls. Differences in categorical variables (e.g., smoking status, drinking status, BMI, age, sex, hypertension, history of T2DM, history of hyperlipidemia, and genotypes of LEP rs2167270 G>A, rs7799039 A>G and LEPR rs1137100 G>A, rs1137101 G>A polymorphisms) were determined using the x² test or Fisher’s exact test. The relationships between the LEP rs2167270 G>A rs7799039 A>G and LEPR
LEP/LEPR polymorphisms and CAD

rs1137100 G>A, rs1137101 G>A genotypes and CAD risk were evaluated based on crude/adjusted odds ratios (ORs) and their 95% confidence intervals (CIs). We performed \(^2\) tests using an online software package (online at http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) [22-25] to assess the Hardy-Weinberg equilibrium (HWE) in controls. All other data analyses were performed with SAS 9.4 software (SAS Institute, Cary, NC, USA).

### Results

#### Characteristics of the study population

The mean age of the patients in the CAD group was 64.57 ± 9.91 years (range, 30-89 years),...
and the mean age of patients in the healthy control group was 64.75 ± 10.20 years (range, 33-89 years). The two groups were matched for age and gender (P = 0.942 and P = 0.704, respectively). The frequencies of smoking, drinking, BMI ≥ 24 kg/m², T2DM, hyperlipidemia and hypertension were significantly higher in the CAD group than in the healthy control group [49.50% vs. 30.30%, P < 0.001; 21.46% vs. 12.17%, P < 0.001. 54.26% vs. 46.26%, P = 0.003, 30.09% vs. 15.42%, P < 0.001, 74.06% vs. 69.70% P = 0.61-0.98; P = 0.034; Table 1]. Among the enrolled CAD patients, 147 had a history of myocardial infarction (MI), and 358 did not.

**Characteristics of LEP rs2167270 G>A, rs7799039 A>G and LEPR rs1137100 G>A, rs1137101 G>A SNPs**

The basis characteristics of the LEP rs2167270 G>A, rs7799039 A>G and LEPR rs1137100 G>A, rs1137101 G>A SNPs are listed in Table 2. LEP rs2167270 G>A and rs1137101 G>A are coding variants. LEP rs7799039 A>G is located in the promoter. LEP rs2167270 G>A is a 5' untranslated region (UTR) SNP. In the controls, the genotype distributions of the LEP rs2167270 G>A, rs7799039 A>G and LEPR rs1137100 G>A, rs1137101 G>A variants were in HWE (Table 2).

**Associations between LEP rs2167270 G>A, rs7799039 A>G and LEPR rs1137100 G>A, rs1137101 G>A SNPs and CAD risk**

The genotype frequencies of LEP rs2167270 G>A, rs7799039 A>G and LEPR rs1137100 G>A, rs1137101 G>A polymorphisms in CAD patients and controls are summarized in Table 3. We found no association between the LEP rs7799039 A>G and LEPR rs1137101 G>A polymorphisms and the risk of CAD (Table 4).

The frequencies of the LEP rs2167270 GG, GA and AA genotypes were 63.73%, 31.46% and 4.81% in CAD patients and 58.77%, 36.53%, and 4.70% in controls, respectively. When the frequency of the LEP rs2167270 GG genotype was applied as a reference, the frequency of the LEP rs2167270 GA genotype differed significantly between the CAD group and the control group (crude OR = 0.78, 95% CI: 0.62-0.98, P = 0.035). This difference was still observed after adjustment for age, sex, hypertension, T2DM, smoking, drinking, hyperlipidemia and BMI, (GA vs. GG: adjusted OR, 0.77; 95% CI, 0.61-0.98; P = 0.034; Table 4).

The frequencies of the LEPR rs1137100 GG, GA and AA genotypes were 68.00%, 28.20% and 3.80% in CAD patients and 70.34%, 27.76%, and 1.90% in controls, respectively. When the frequency of the LEPR rs1137100 GG genotype was applied as a reference, the frequency of the LEPR rs1137100 AA genotype differed significantly between the CAD patients and controls (crude OR = 2.05, 95% CI: 1.09-3.86, P = 0.027). When the frequency of LEPR rs1137100 GG/AA genotype was applied as the reference, the frequency of the LEPR rs1137100 AA genotype differed significantly between the CAD patients and controls (crude OR = 2.04, 95% CI: 1.09-3.83, P = 0.026). These differences were still observed after adjustments for age, sex, hypertension, T2DM, smoking, drinking, hyperlipidemia and BMI (AA vs. GG: adjusted OR, 1.98; 95% CI, 1.01-3.92; P = 0.048 and AA vs. GG/GA: adjusted OR, 2.02; 95% CI, 1.03-3.97; P = 0.042; Table 4).

**Association of LEP rs2167270 G>A, rs7799039 A>G and LEPR rs1137100 G>A, rs1137101 G>A polymorphisms with risk of different CAD types**

The genotype frequencies of the LEP rs2167270 G>A, rs7799039 A>G and LEPR rs1137100 G>A, rs1137101 G>A polymorphisms in different subgroups are listed in Table 3. We carried out a stratified analysis to assess whe-
ther the role of the LEP rs2167270 G>A, rs7799039 A>G and LEPR rs1137100 G>A, rs1137101 G>A variants differed according to the type of CAD. The associations between the LEPR rs1137100 G>A polymorphisms and the increased risk of CAD were also found in the non-MI subgroup (AA vs. GG: adjusted OR, 2.23; 95% CI, 1.07-4.62; P = 0.032 and AA vs. GG/GA: adjusted OR, 2.31; 95% CI, 1.11-4.78; P = 0.024; Table 4).

Discussion

A number of case-control studies have focused on the relationship between LEP/LEPR variants and the risk of T2DM. However, the potential associations of LEP/LEPR SNPs with the development of CAD remained unclear. In the present study, the LEP rs2167270 G>A polymorphism was found to be a protective factor against CAD, but the LEPR rs1137100 G>A increased the risk of CAD in our study population. In addition, we found that the LEPR rs1137100 G>A polymorphism was associated with an increased risk of CAD among patients without a history of MI.

The LEP rs2167270 G>A (+19 A>G) polymorphism is located on the 5'-UTR of the gene, and it may alter the expression of LEP gene. It also
# LEP/LEPR polymorphisms and CAD

**Table 4.** Overall and stratified analyses of LEP rs2167270 G>A, rs7799039 A>G and LEPR rs1137100 G>A, rs1137101 G>A polymorphisms with CAD

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Overall CAD cases (n = 505) vs. Controls (n = 1,109)</th>
<th>Non-MI cases (n = 358) vs. Controls (n = 1,109)</th>
<th>MI cases (n = 147) vs. Controls (n = 1,109)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude OR (95% CI)</td>
<td>Adjusted OR* (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>LEP rs7799039 A&gt;G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additive model</td>
<td>0.89 (0.71-1.11)</td>
<td>0.289</td>
<td>0.86 (0.68-1.09)</td>
</tr>
<tr>
<td>Homozygote model</td>
<td>1.01 (0.67-1.52)</td>
<td>0.967</td>
<td>0.97 (0.63-1.50)</td>
</tr>
<tr>
<td>Dominant model</td>
<td>0.92 (0.74-1.13)</td>
<td>0.422</td>
<td>0.89 (0.72-1.12)</td>
</tr>
<tr>
<td>Recessive model</td>
<td>1.07 (0.72-1.60)</td>
<td>0.744</td>
<td>1.04 (0.68-1.59)</td>
</tr>
<tr>
<td>LEP rs2167270 G&gt;A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additive model</td>
<td>0.78 (0.62-0.98)</td>
<td>0.035</td>
<td>0.77 (0.61-0.98)</td>
</tr>
<tr>
<td>Homozygote model</td>
<td>0.93 (0.56-1.54)</td>
<td>0.777</td>
<td>0.89 (0.52-1.50)</td>
</tr>
<tr>
<td>Dominant model</td>
<td>0.81 (0.65-1.01)</td>
<td>0.061</td>
<td>0.80 (0.64-1.00)</td>
</tr>
<tr>
<td>Recessive model</td>
<td>1.02 (0.62-1.68)</td>
<td>0.925</td>
<td>0.98 (0.58-1.65)</td>
</tr>
<tr>
<td>LEPR rs1137100 G&gt;A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additive model</td>
<td>1.04 (0.82-1.32)</td>
<td>0.747</td>
<td>0.98 (0.76-1.25)</td>
</tr>
<tr>
<td>Homozygote model</td>
<td>2.05 (1.09-3.86)</td>
<td>0.027</td>
<td>1.98 (1.01-3.92)</td>
</tr>
<tr>
<td>Dominant model</td>
<td>1.12 (0.89-1.40)</td>
<td>0.345</td>
<td>1.05 (0.83-1.34)</td>
</tr>
<tr>
<td>Recessive model</td>
<td>2.04 (1.09-3.83)</td>
<td>0.026</td>
<td>2.02 (1.03-3.97)</td>
</tr>
<tr>
<td>LEPR rs1137101 G&gt;A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additive model</td>
<td>0.87 (0.68-1.13)</td>
<td>0.305</td>
<td>0.82 (0.62-1.07)</td>
</tr>
<tr>
<td>Homozygote model</td>
<td>1.79 (0.77-4.19)</td>
<td>0.177</td>
<td>1.59 (0.64-3.94)</td>
</tr>
<tr>
<td>Dominant model</td>
<td>0.93 (0.72-1.19)</td>
<td>0.540</td>
<td>0.86 (0.66-1.12)</td>
</tr>
<tr>
<td>Recessive model</td>
<td>1.86 (0.80-4.34)</td>
<td>0.150</td>
<td>1.68 (0.68-4.18)</td>
</tr>
</tbody>
</table>

*Adjusted for smoking, drinking, BMI, age, sex, hypertension, T2DM and hyperlipidemia; Bold values are statistically significant (P < 0.05). CAD: coronary artery disease; MI: myocardial infarction.
has been suggested that the LEP rs2167270 G>A SNP is in linkage disequilibrium with promoter region variation, which might influence gene transcription [26]. Hager et al. reported that the presence of the LEP rs2167270 A allele decreased BMI among obese females, suggesting that LEP rs2167270 A allele carriers are more sensitive to satiety signals via the LEP protein [27]. Additionally, a recent study reported that the LEP rs2167270 A allele may decrease the circulating LEP level, body weight and BMI, and consequently, may be a protective factor against obesity in females [26]. Obesity and being overweight are considered risk factors in the pathogenesis of CAD [2]. Here, we found that the LEP rs2167270 G>A polymorphism was associated with a reduced CAD risk. This finding might be interpreted to mean that the LEP rs2167270 G→A variant reduces the risk of CAD by decreasing the BMI. In the future, more epidemiologic studies considering detailed lifestyle and environmental factors are required to confirm these findings.

The LEPR rs1137100 G>A polymorphism, a missense SNP, is located on the translation region of the gene, and this common functional polymorphism (Arg109Lys; rs1137100) in the LEPR gene is a G→A missense substitution that results in an Arg to Lys substitution in the exon. The A allele of LEPR rs1137100 may be associated with the increased risk of T2DM [28] and higher BMI [29]. In the present study, the association between the LEPR rs1137100 G>A polymorphism and an increased risk of CAD was found. Thus, the LEPR rs1137100 G→A variant may increase the BMI and consequently raise the risk of CAD. More epidemiologic studies in a larger cohort with functional analysis are needed to reinforce our findings and elucidate the biological role of the LEPR rs1137100 G>A polymorphism in CAD.

Notably, our case-control study has some limitations. First, patients and controls were enrolled from a local hospital; thus, bias might have occurred. Secondly, the LEP/LEPR SNPs studied were selected from related publications. In the future, a fine-mapping study should be carried out to obtain an extensive view of these genes. Thirdly, it would be interesting to define the relationships of LEP/LEPR SNPs with the serum protein levels. However, because these proteins were not assessed in this study, we could not conduct such a correlation analysis as part of this investigation. Finally, due to a lack of detailed information on cardiovascular events, we could not determine the roles of the LEP rs2167270 G>A, rs7799039 A>G and LEPR rs1137100 G>A variants in CAD progression and prognosis.

In summary, the results of the present study indicate that the LEP rs2167270 G>A polymorphism may be considered a protective factor against CAD among the eastern Chinese Han population, whereas LEPR rs1137100 G>A may be associated with an increased risk of CAD in this population. Future studies that take into account detailed lifestyle and environmental factors are needed to confirm our findings.

Acknowledgements

We appreciate all the study participants. We wish to thank Dr. Yan Liu (Genesky Biotechnologies Inc., Shanghai, China) for technical support. This study was supported in part by the National Natural Science Foundation of China (81670438).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Liangwan Chen, Department of Cardiac Surgery, Fujian Medical University Union Hospital, Fuzhou 350001, Fujian Province, China. Tel: (86) 591-83357896; Fax: (86) 591-87113828; E-mail: chenliangwan@tom.com

References

in the activation of immune cells. Mediators Inflamm 2010; 2010: 568343.


[26] Hart Sailsor ML, Folsom AR, Ballantyne CM, Hoelscher DM, Jackson AS, Linda Kao WH, Pankow JS and Bray MS. Genetic variation and
LEP/LEPR polymorphisms and CAD


