Original Article
The association of LEPR rs1137101 G>A polymorphism with the risk of type 2 diabetes mellitus: from a case-control study to a meta-analysis

Haojie Wu, Weifeng Tang, Chao Liu, Ying Xue, Jianming Hou

1Shengli Clinical Medical College of Fujian Medical University, Fuzhou, Fujian Province, China; 2Department of Cardiothoracic Surgery, Affiliated People’s Hospital of Jiangsu University, Zhenjiang, Jiangsu Province, China; 3Department of Endocrinology, Fujian Provincial Hospital South Branch, Fuzhou, Fujian Province, China; 4Department of Endocrinology, Fujian Provincial Hospital, Fuzhou, Fujian Province, China

Received May 7, 2018; Accepted January 9, 2019; Epub April 15, 2019; Published April 30, 2019

Abstract: The leptin receptor (LEPR) is associated with food intake and energy expenditure. Thus, LEPR may be a key agent in the development of type 2 diabetes mellitus (T2DM). In this study, we explored the relationship of the LEPR rs1137101 G>A polymorphism with the susceptibility of T2DM. A total of 502 subjects with T2DM and 782 healthy controls were included in our study between October 2014 and May 2016. A genotyping analysis was performed using the SNPscan™ genotyping assay. This case-control study indicated there was no significant difference between the T2DM cases and the controls in the allele or genotype frequencies of the LEPR rs1137101 G>A polymorphism. To shed some light on this issue, we carried out a meta-analysis of 22 studies involving 6,103 T2DM cases and 6,618 controls. The results of the meta-analysis suggest that the LEPR rs1137101 G>A polymorphism is not associated with the susceptibility of T2DM. A stratification analysis based on ethnicity also had similar findings. In conclusion, this study suggests that the LEPR rs1137101 G>A polymorphism is not associated with the development of T2DM.

Keywords: Leptin, leptin receptor, polymorphism, type 2 diabetes mellitus, meta-analysis

Introduction
Type 2 diabetes mellitus (T2DM), a type of chronic metabolic disease, is characterized by a disorder of energy metabolism, which is caused by impaired insulin secretion and/or insulin resistance. The etiology of T2DM remains unclear. Many environmental and polygenic factors may contribute to the development of T2DM. A number of case-control studies have focused on the relationship of insulin signal transduction and glucolipid metabolism-related candidate genes with the risk of T2DM [1, 2]. The interaction of different genes and environmental risk factors may result in abnormal protein expression, and then alter signal transduction or energy metabolism, lead to a metabolism disorder, and eventually cause T2DM. Various adipocytokines expressed and secreted by adipose tissue are considered as inflammatory, immune or hormonal signalers involving aberrant insulin signal and glucolipid metabolism, and finally promote the development of T2DM [3, 4].

Leptin (LEP), a polypeptide hormone, is made by adipose cells and is antagonized by the action of ghrelin. In obese individuals, a decreased sensitivity to leptin is observed, which leads to an inability to detect satiety despite high energy stores [5]. LEP can modulate insulin activation as well as insulin secretion [6, 7]. LEP binds to the hypothalamic leptin receptor (LEPR) and helps to regulate energy balance by decreasing food intake, inhibiting hunger and increasing energy expenditure [8].

LEP interacts with LEPR and then activates a number of signal pathways. LEP resistance could be induced by defects in some parts of these processes, involving the JAK/STAT, PI3K, MAPK and mTOR signal pathways [9]. Mutations
in the LEPR gene might prevent the activation of STAT and lead to hyperphagia and obesity [10]. LEP resistance could also be caused by the PI3K signal pathway [11]. The PI3K signal pathway may also be activated by insulin receptors, so LEP and insulin together regulate the energy homeostasis of the body. The insulin-PI3K pathway can make pro-opiomelanocortin neurons insensitive to LEP through hyperpolarization [12]. Nowadays, accumulating evidence indicates that LEPR is also located in the peripheral regions [13, 14]. The variants of the LEPR gene may influence the risk of T2DM.

Recently, some case-control studies have focused on the association between LEPR variants and the risk of T2DM [15-32]. However, the results remain controversial. In this study, we selected a functional polymorphism (rs1137101 G>A) in the LEPR gene to explore its association with the susceptibility to T2DM in an Eastern Chinese Han population. Then, given the accumulating evidence and to shed some light on this issue, we carried out an extensive meta-analysis of this relationship between the LEPR rs1137101 G>A polymorphism and T2DM susceptibility.

Materials and methods

Subjects

A total of 502 subjects with T2DM and 782 healthy controls were included in our study between October 2014 and May 2016. All participants were of Chinese Han ethnicity and from Fujian and Jiangsu provinces in China. Additionally, all T2DM cases recruited in the study were patients who had been diagnosed at the People’s Hospital of Jiangsu University (Zhenjiang City, China) and the Affiliated Union Hospital of Fujian Medical University (Fuzhou City, China) and were confirmed as having T2DM through the World Health Organization 1999 guidelines of T2DM [33]. The control group consisted of 782 healthy individuals who had attended a routine physical examination in these hospitals mentioned above. Controls had no history of T2DM and were gender and age matched to the T2DM cases. The T2DM cases and controls were interviewed by two members of our group, and they answered a series of questions in order to document demographic characteristics and risk factors. Each subject signed an informed consent. The study was approved by the ethics committees of Fujian Medical University and Jiangsu University. Clinical information and biochemistry characteristics involving fasting plasma glucose (FPG), total cholesterol, high-density lipoprotein cholesterol (HDL-C), serum triglycerides, high-density lipoprotein cholesterol (LDL-C), systolic blood pressure, diastolic blood pressure, weight and height were obtained from their questionnaires and medical records.

DNA extraction and genotyping

Blood samples were donated by each participant. Genomic DNA was carefully extracted from leukocytes using the Promega DNA Isolation Kit (Promega, Madison, USA). Using a NanoDrop spectrophotometer, DNA quantity was assessed by absorbance at 260 nm and the purity of each DNA sample was measured using the 260/280 absorbance ratio. The genotyping analysis was performed using a polymerase chain reaction (30 ng/ul DNA), using the SNPscan™ genotyping assay (Genesky Biotechnologies Inc., Shanghai, China) according to the manufacturer’s instructions. For quality control, a repeated genotyping was done for fifty-two (4%) randomly selected DNA samples.

Statistical analysis

Continuous variables [e.g. total cholesterol, triglyceride, HDL-C and LDL-C levels, FPG, age, height, weight and body mass index (BMI)] are expressed as the means ± standard deviation (SD). Student’s t-test was used to compare the difference between the T2DM patients and the healthy controls. Comparisons of demographic characteristics (sex and age), selected variables (smoking status, drinking and BMI), and genotypes of the LEPR rs1137101 G>A between the T2DM cases and the healthy controls were conducted using a Chi-square test ($x^2$). Using logistic regression analyses, the relationship between LEPR rs1137101 G>A genotypes and the susceptibility of T2DM was estimated by calculating the crude/adjusted odds ratios (ORs) and their 95% confidence intervals (CIs). The Hardy-Weinberg equilibrium (HWE) was measured by an online goodness-of-fit $x^2$ test (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). All statistical analyses were conducted using the SAS 9.4 software (SAS Institute, Cary, NC, USA).

Meta-analysis

We searched PubMed, Embase, the Chinese National Knowledge Infrastructure (CNKI), and
LEPR rs1137101 G>A polymorphism and T2DM

Table 1. Distribution of selected demographic variables and risk factors in type 2 diabetes cases and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n=502)</th>
<th>Controls (n=782)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>227</td>
<td>45.22</td>
<td>0.113</td>
</tr>
<tr>
<td>≥65</td>
<td>275</td>
<td>54.78</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.819</td>
</tr>
<tr>
<td>Male</td>
<td>332</td>
<td>66.14</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>170</td>
<td>33.86</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td>0.264</td>
</tr>
<tr>
<td>Ever</td>
<td>169</td>
<td>33.67</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>333</td>
<td>66.33</td>
<td></td>
</tr>
<tr>
<td>Alcohol use</td>
<td></td>
<td></td>
<td>0.263</td>
</tr>
<tr>
<td>Ever</td>
<td>49</td>
<td>9.76</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>453</td>
<td>90.24</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥24</td>
<td>292</td>
<td>58.17</td>
<td></td>
</tr>
<tr>
<td>&lt;24</td>
<td>210</td>
<td>41.83</td>
<td></td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>135.08 (+17.83)</td>
<td>134.02 (+17.71)</td>
<td>0.297</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>79.79 (+10.35)</td>
<td>80.06 (+10.02)</td>
<td>0.649</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>8.08 (+2.76)</td>
<td>5.13 (+0.49)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.74 (+1.14)</td>
<td>1.55 (+0.96)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.61 (+1.24)</td>
<td>4.88 (+1.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.13 (+0.37)</td>
<td>1.30 (+0.37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.00 (+1.07)</td>
<td>3.14 (+0.82)</td>
<td>0.010</td>
</tr>
</tbody>
</table>

*Two-sided x² test and student t test; Bold values are statistically significant (P<0.05); BMI, body mass index; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

The China Biology Medicine (CBM) databases using the words: (leptin receptor or LEPR) and (polymorphism or variant or SNP) and (Type 2 diabetes mellitus or T2DM or diabetic). The studies were eligible when they met the major criteria: (a) case-control studies for humans, (b) studies published by April 30, 2017, (c) studies providing genotype frequencies. (d) the genotype frequencies were in accord with HWE in the controls (P>0.05). In order to provide an extensive review, the references in the included publications were also searched to retrieve any potential data which might have been ignored in our initial search.

Two authors (H. Wu and W. Tang) carefully extracted the corresponding information from the included case-control studies. In case of disagreement among the two reviewers, a consensus was reached after a thorough discussion. In the event that a publication presented several subgroups, they were treated as different case-control studies. The major information extracted included: (a) the first author’s name, (b) the publication year, (c) the ethnicity of the study population, (d) the sample size of the T2DM cases and controls, (d) the diagnostic criteria, (e) the genotyping method and (f) LEPR rs1137101 G>A genotypes number.

The association of the LEPR rs1137101 G>A polymorphisms with T2DM susceptibility was assessed using the crude ORs with their 95% CIs. The potential heterogeneity among the eligible studies was determined by a Chi-square based I² test. A P<0.1 or I²>50% indicated significant heterogeneity across studies, and a random-effects model (the DerSimonian and Laird method) [34] was applied to calculate the results; otherwise, the fixed-effects model (the Mantel-Haenszel method) [35] was used. Publication bias among the included studies
was examined with the funnel plot and Egger’s linear regression test [36]. A \( P < 0.1 \) was defined as evidence of bias. We carried out the sensitivity analysis by omitting an individual study in turn and recalculating the remainders. The statistical analysis was performed with Stata software (Stata Corporation, College Station, TX), version 12.0.

Results

Baseline characteristics

The anthropometric data, biochemistry characteristics, demographics and risk factor are summarized in Table 1. The information on the LEPR rs1137101 G>A locus is listed in Table 2. In the present case-control study, the success ratio of genotyping was 99.53% for LEPR rs1137101 G>A in the 1284 samples. In our controls, the minor allele frequency (MAF) was similar to what is found in the basic database of the Chinese population. In addition, the genotype frequencies of the LEPR rs1137101 G>A polymorphism were in accord with HWE in the controls (\( P > 0.05 \)).

Association of LEPR rs1137101 G>A polymorphisms with T2DM

The LEPR rs1137101 G>A genotype distributions are summarized in Table 3. In the single locus analysis, the genotype frequencies of LEPR rs1137101 G>A were 74.60% (GG), 23.19% (GA), and 2.22% (AA) in T2DM patients and 75.83% (GG), 23.02% (GA), and 1.15% (AA) in controls. The LEPR rs1137101 G>A polymorphism was not associated with T2DM susceptibility (GA vs. GG: adjusted OR 1.00, 95% CI 0.77-1.32, \( P = 0.975 \); GA vs. GG: adjusted OR 2.14, 95% CI 0.87-5.27, \( P = 0.099 \); GA/AA vs. GG: adjusted OR 1.07, 95% CI 0.83-1.40, \( P = 0.597 \); AA vs. GA/GG: adjusted OR 2.17, 95% CI 0.88-5.33, \( P = 0.093 \); Table 3).

Meta-analysis of LEPR rs1137101 G>A polymorphisms with T2DM

The initial search yielded 1,416 potentially relevant studies. According to the major inclusion criteria, our case-control study and twenty-one publications [15-31] were included in this meta-analysis. As shown in Table 4, some publications included several independent case-control studies [17, 20, 27], and we treated them separately. Figure 1 summarizes the flow chart of study selection. Finally, there were eighteen publications (including twenty-one case-control studies) [15-22, 25-30, 32] and our case-control study on the relationship of LEPR rs1137101 G>A polymorphism with risk of T2DM. In terms of the subjects in these studies, eighteen were Asians and four were Caucasians. Among the 22 case-control stud-

\[\text{Table 2. Primary information for LEPR polymorphisms} \]

<table>
<thead>
<tr>
<th>Genotyped SNPs</th>
<th>Chromosome</th>
<th>Chr Pos (NCBI Build 37)</th>
<th>Region</th>
<th>MAF (^a) for Chinese in database</th>
<th>MAF in our controls (n=782)</th>
<th>( P ) value for HWE (^b)</th>
<th>Genotyping method</th>
<th>Genotyping value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEPR rs1137101 G&gt;A</td>
<td>1</td>
<td>66058513</td>
<td>Exon 6</td>
<td>0.111</td>
<td>0.127</td>
<td>0.253</td>
<td>SNPsan</td>
<td>99.53</td>
</tr>
</tbody>
</table>

\(^a\)MAF: minor allele frequency; \(^b\)HWE: Hardy-Weinberg equilibrium.

\[\text{Table 3. Logistic regression analyses of the association between the LEPR rs1137101 G>A polymorphisms and the risk of type 2 diabetes} \]

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (n=502)</th>
<th>Controls (n=782)</th>
<th>Crude OR (95% CI)</th>
<th>( P )</th>
<th>Adjusted OR (^a) (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEPR rs1137101 G&gt;A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>370</td>
<td>74.60</td>
<td>593</td>
<td>75.83</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>GA</td>
<td>115</td>
<td>23.19</td>
<td>180</td>
<td>23.02</td>
<td>1.01 (0.77-1.32)</td>
<td>0.956</td>
</tr>
<tr>
<td>AA</td>
<td>11</td>
<td>2.22</td>
<td>9</td>
<td>1.15</td>
<td>1.93 (0.79-4.70)</td>
<td>0.149</td>
</tr>
<tr>
<td>GA+AA</td>
<td>126</td>
<td>25.40</td>
<td>189</td>
<td>24.17</td>
<td>1.07 (0.82-1.39)</td>
<td>0.617</td>
</tr>
<tr>
<td>GG+GA</td>
<td>485</td>
<td>97.78</td>
<td>773</td>
<td>98.85</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>AA</td>
<td>11</td>
<td>2.22</td>
<td>9</td>
<td>1.15</td>
<td>1.95 (0.80-4.74)</td>
<td>0.141</td>
</tr>
<tr>
<td>A allele</td>
<td>137</td>
<td>13.81</td>
<td>198</td>
<td>12.66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Adjusted for age, sex, smoking status, alcohol use and BMI status. Bold values are statistically significant (\( P < 0.05 \)).
Table 4. Characteristics of the included studies in this meta-analysis

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Ethnicity</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>Diagnostic criteria</th>
<th>Genotype Method</th>
<th>Age</th>
<th>BMI</th>
<th>case</th>
<th>control</th>
<th>Source of control</th>
<th>Quality score of Newcastle-Ottawa scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roszkowski-</td>
<td>2014</td>
<td>Poland</td>
<td>Caucasians</td>
<td>190</td>
<td>768</td>
<td>NA</td>
<td>PCR-RFLP</td>
<td>47.2±5.3</td>
<td>45.2±5.3</td>
<td>average: 45.2</td>
<td>NA</td>
<td>NA</td>
<td>6</td>
</tr>
<tr>
<td>et al.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2017</td>
<td>28.02±4.0</td>
<td>23.9±3.1</td>
<td>2009</td>
<td>48.94±11.56</td>
<td>WHO1999</td>
</tr>
<tr>
<td>Jiang et al.</td>
<td>2014</td>
<td>China</td>
<td>Asians</td>
<td>188</td>
<td>399</td>
<td>WHO1999</td>
<td>MALDI-TOF MS</td>
<td>67.9±6.7</td>
<td>68.1±6.1</td>
<td>24.1±4.0</td>
<td>23.9±3.9</td>
<td>81</td>
<td>6</td>
</tr>
<tr>
<td>Jiang et al.</td>
<td>2014</td>
<td>China</td>
<td>Asians</td>
<td>181</td>
<td>399</td>
<td>WHO1999</td>
<td>MALDI-TOF MS</td>
<td>68.2±6.1</td>
<td>68.1±6.1</td>
<td>24.1±4.0</td>
<td>23.9±3.9</td>
<td>120</td>
<td>66</td>
</tr>
<tr>
<td>Mohammadzadeh et al.</td>
<td>2013</td>
<td>Iran</td>
<td>Caucasians</td>
<td>144</td>
<td>147</td>
<td>ADA1997</td>
<td>PCR-RFLP</td>
<td>54.3±8.85</td>
<td>52.5±7.31</td>
<td>27.6±4.50</td>
<td>28.0±4.01</td>
<td>2002</td>
<td>6</td>
</tr>
<tr>
<td>Gan et al.</td>
<td>2012</td>
<td>China</td>
<td>Asians</td>
<td>148</td>
<td>172</td>
<td>NA</td>
<td>PCR-RFLP</td>
<td>53.1±10.73</td>
<td>52.8±7.90</td>
<td>24.3±1.76</td>
<td>24.8±1.73</td>
<td>2004</td>
<td>6</td>
</tr>
<tr>
<td>Gan et al.</td>
<td>2012</td>
<td>China</td>
<td>Asians</td>
<td>153</td>
<td>172</td>
<td>NA</td>
<td>PCR-RFLP</td>
<td>52.2±10.74</td>
<td>52.8±7.90</td>
<td>25.1±2.67</td>
<td>24.8±1.73</td>
<td>2004</td>
<td>6</td>
</tr>
<tr>
<td>Han et al.</td>
<td>2008</td>
<td>Korea</td>
<td>Asians</td>
<td>407</td>
<td>345</td>
<td>ADA</td>
<td>TaqMan</td>
<td>59.7±6.2</td>
<td>64.4±3.36</td>
<td>24.7±3.06</td>
<td>23.9±3.23</td>
<td>2001</td>
<td>5</td>
</tr>
<tr>
<td>Park et al.</td>
<td>2006</td>
<td>Korea</td>
<td>Asians</td>
<td>775</td>
<td>688</td>
<td>ADA</td>
<td>TaqMan</td>
<td>58.9±10.5</td>
<td>64.2±4.2</td>
<td>24.4±2.9</td>
<td>23.6±3.1</td>
<td>2001</td>
<td>5</td>
</tr>
<tr>
<td>Zheng et al.</td>
<td>1999</td>
<td>China</td>
<td>Asians</td>
<td>166</td>
<td>193</td>
<td>ADA</td>
<td>PCR-RFLP</td>
<td>56.4±9.7</td>
<td>53.0±11.7</td>
<td>26.7±3.67</td>
<td>25.6±4.33</td>
<td>2001</td>
<td>5</td>
</tr>
<tr>
<td>Ying et al.</td>
<td>2009</td>
<td>China</td>
<td>Asians</td>
<td>225</td>
<td>111</td>
<td>WHO1999</td>
<td>MALDI-TOF MS</td>
<td>55.7±6.3</td>
<td>50.4±7.2</td>
<td>Average: 25.4</td>
<td>20.5±2.3</td>
<td>2001</td>
<td>5</td>
</tr>
<tr>
<td>Shi et al.</td>
<td>2012</td>
<td>China</td>
<td>Asians</td>
<td>333</td>
<td>395</td>
<td>WHO1999</td>
<td>MALDI-TOF MS</td>
<td>60.6±11.06</td>
<td>47.5±6.25</td>
<td>NA</td>
<td>NA</td>
<td>2001</td>
<td>5</td>
</tr>
<tr>
<td>Al-Harthth et al.</td>
<td>2013</td>
<td>Saudi</td>
<td>Overview</td>
<td>150</td>
<td>130</td>
<td>NA</td>
<td>DNA sequencing</td>
<td>45-65 years</td>
<td>45-65 years</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>2001</td>
<td>5</td>
</tr>
<tr>
<td>Takahashi-Yasuno et al.</td>
<td>2004</td>
<td>Japan</td>
<td>Asians</td>
<td>220</td>
<td>377</td>
<td>NA</td>
<td>PCR-RFLP</td>
<td>51.5±10.5</td>
<td>47.5±6.25</td>
<td>NA</td>
<td>NA</td>
<td>2001</td>
<td>5</td>
</tr>
<tr>
<td>Liao et al.</td>
<td>2004</td>
<td>China</td>
<td>Asians</td>
<td>999</td>
<td>45</td>
<td>ADA</td>
<td>Allele-specific</td>
<td>Average: 60.2</td>
<td>NA</td>
<td>Average: 25.2</td>
<td>NA</td>
<td>2001</td>
<td>5</td>
</tr>
<tr>
<td>Fang et al.</td>
<td>2011</td>
<td>China</td>
<td>Asians</td>
<td>239</td>
<td>287</td>
<td>WHO1999</td>
<td>PCR-RFLP</td>
<td>58.2±11.51</td>
<td>48.9±11.56</td>
<td>24.5±3.40</td>
<td>23.3±2.95</td>
<td>2001</td>
<td>5</td>
</tr>
<tr>
<td>Li et al.</td>
<td>2015</td>
<td>China</td>
<td>Asians</td>
<td>126</td>
<td>185</td>
<td>NA</td>
<td>PCR-RFLP</td>
<td>50.7±6.2</td>
<td>42.5±2.9</td>
<td>NA</td>
<td>NA</td>
<td>2001</td>
<td>5</td>
</tr>
<tr>
<td>Li et al.</td>
<td>2015</td>
<td>China</td>
<td>Asians</td>
<td>108</td>
<td>185</td>
<td>NA</td>
<td>PCR-RFLP</td>
<td>55.7±3.4</td>
<td>42.5±2.9</td>
<td>NA</td>
<td>NA</td>
<td>2001</td>
<td>5</td>
</tr>
<tr>
<td>Xie et al.</td>
<td>2002</td>
<td>China</td>
<td>Asians</td>
<td>184</td>
<td>86</td>
<td>ADA</td>
<td>PCR-RFLP</td>
<td>60.5±11.6</td>
<td>38.4±10.6</td>
<td>24.25±2.85</td>
<td>23.3±2.54</td>
<td>2001</td>
<td>5</td>
</tr>
<tr>
<td>Wu et al.</td>
<td>2017</td>
<td>China</td>
<td>Asians</td>
<td>241</td>
<td>270</td>
<td>WHO1999</td>
<td>TaqMan</td>
<td>68.2±6.0</td>
<td>67.9±6.4</td>
<td>25.8±3.4</td>
<td>20.6±2.0</td>
<td>2001</td>
<td>5</td>
</tr>
<tr>
<td>Our study</td>
<td>2017</td>
<td>China</td>
<td>Asians</td>
<td>502</td>
<td>782</td>
<td>WHO1999</td>
<td>SNPscan</td>
<td>65.20±9.51</td>
<td>64.67±9.80</td>
<td>24.95±3.64</td>
<td>23.51±2.94</td>
<td>2001</td>
<td>5</td>
</tr>
<tr>
<td>Li et al.</td>
<td>2001</td>
<td>China</td>
<td>Asians</td>
<td>36</td>
<td>104</td>
<td>ADA</td>
<td>PCR-RFLP</td>
<td>58.8±8.14</td>
<td>58.8±8.07</td>
<td>26.17±2.47</td>
<td>24.93±3.56</td>
<td>2001</td>
<td>5</td>
</tr>
</tbody>
</table>

LEPR rs1137101 G>A polymorphism and T2DM

For the LEPR rs1137101 G>A polymorphism, there were twenty-two case-control studies with 5,869 T2DM cases, and 6,618 controls that met the inclusion criteria [15-22, 25-30, 32]. Overall, we didn’t find any association between the LEPR rs1137101 G>A polymorphism and the development of T2DM (OR, 1.06; 95% CI, 0.90-1.27; P=0.479 for A vs. G; OR, 0.92; 95% CI, 0.64-1.31; P=0.643 for AA vs. GG; OR, 1.12; 95% CI, 0.90-1.38; P=0.300 for AA+GA vs. GG and OR, 0.98; 95% CI, 0.81-1.18; P=0.814 for AA vs. GG+GA; Table 6 and Figure 2). In a subgroup analysis by ethnicity, a null association was also found among Asians (OR, 1.07; 95% CI, 0.86-1.34; P=0.534 for A vs. G; OR, 0.84; 95% CI, 0.49-1.42; P=0.506 for AA vs. GG; OR, 1.14; 95% CI, 0.90-1.45; P=0.290 for AA+GA vs. GG and OR, 0.82; 95% CI, 0.51-1.34; P=0.435 for AA vs. GG+GA) and Caucasians (OR, 1.03; 95% CI, 0.88-1.20; P=0.747 for A vs. G; OR, 1.03; 95% CI, 0.73-1.45; P=0.867 for AA vs. GG; OR, 1.02; 95% CI, 0.76-1.38; P=0.883 for AA+GA vs. GG and OR, 1.04; 95% CI, 0.83-1.30; P=0.730 for AA vs. GG+GA). For the LEPR rs1137101 G>A polymorphism, we found there was no significant publication bias (A vs. G: Begg’s test P=0.581, Egger’s linear regression test P=0.516; AA vs. GG: Begg’s test P=0.889, Egger’s linear regression test P=0.700; AA+GA vs. GG: Begg’s test P=0.415, Egger’s linear regression test P=0.392; AA vs. GG+GA: Begg’s test P=0.576, Egger’s linear regression test P=0.407, Figure 3). Sensitivity analyses were conducted by sequential omission of the included studies to determine the effect of each study on the overall evaluation. The results suggested the significance of ORs was not substantially altered by omitting any single study (Figure 4). A significant heterogeneity was evident in the allele genetic model. Therefore, subgroup analyses were performed, and the results indicated that Asians, small sample sizes (<1000), hospital-based studies and low-quality study (<7) subgroups might contribute the major heterogeneity.

Discussion

Nowadays, T2DM is a public health problem worldwide. The major characteristics of T2DM are hyperglycemia, absolute/relative insulin resistance (IR), and metabolism disturbance. The etiology of T2DM may be very complicated. It results from an interaction between an individual’s genetic components and multiple environmental factors [37]. The individual genetic mutations may play important roles in the
LEPR rs1137101 G>A polymorphism and T2DM

Table 5. Distribution of LEPR rs1137101 G>A polymorphism genotype and allele

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Case</th>
<th>Control</th>
<th>Case</th>
<th>Control</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zheng et al.</td>
<td>1999</td>
<td>148 45</td>
<td>- 132</td>
<td>34</td>
<td>- 8</td>
<td>28</td>
</tr>
<tr>
<td>Li et al.</td>
<td>2001</td>
<td>- - -</td>
<td>69 17</td>
<td>0 51</td>
<td>317</td>
<td>17  155</td>
</tr>
<tr>
<td>Xie et al.</td>
<td>2002</td>
<td>138 41</td>
<td>5 69</td>
<td>17 0</td>
<td>51 317</td>
<td>17  155</td>
</tr>
<tr>
<td>Takahashi-Yasuno et al.</td>
<td>2004</td>
<td>154 66</td>
<td>- 279</td>
<td>97</td>
<td>- -</td>
<td>-</td>
</tr>
<tr>
<td>Liao et al.</td>
<td>2012</td>
<td>796 194 8 36</td>
<td>8 1 210</td>
<td>1786</td>
<td>10 80</td>
<td>Yes</td>
</tr>
<tr>
<td>Park et al.</td>
<td>2006</td>
<td>578 177 11 523</td>
<td>148 13 199</td>
<td>1333</td>
<td>174 194</td>
<td>Yes</td>
</tr>
<tr>
<td>Han et al.</td>
<td>2008</td>
<td>311 92 4 266</td>
<td>69 8 100</td>
<td>714</td>
<td>85 601</td>
<td>Yes</td>
</tr>
<tr>
<td>Ying et al.</td>
<td>2009</td>
<td>158 66 1 89</td>
<td>21 1 68</td>
<td>382</td>
<td>23 199</td>
<td>Yes</td>
</tr>
<tr>
<td>Fang et al.</td>
<td>2011</td>
<td>169 64 5 150</td>
<td>116 20 74</td>
<td>402</td>
<td>156 416</td>
<td>Yes</td>
</tr>
<tr>
<td>Gan et al.</td>
<td>2012</td>
<td>111 33 4 121</td>
<td>47 4 41</td>
<td>255</td>
<td>55 289</td>
<td>Yes</td>
</tr>
<tr>
<td>Gan et al.</td>
<td>2012</td>
<td>89 50 14 121</td>
<td>47 4 78</td>
<td>228</td>
<td>55 289</td>
<td>Yes</td>
</tr>
<tr>
<td>Shi et al.</td>
<td>2012</td>
<td>283 49 1 310</td>
<td>80 5 51</td>
<td>615</td>
<td>90 700</td>
<td>Yes</td>
</tr>
<tr>
<td>Mohammadzadeh et al.</td>
<td>2013</td>
<td>5 59 80 5 62</td>
<td>80 219</td>
<td>69 222</td>
<td>72 72</td>
<td>Yes</td>
</tr>
<tr>
<td>Al-harithy et al.</td>
<td>2013</td>
<td>8 50 92 12 40 78</td>
<td>234</td>
<td>66 196</td>
<td>64 75</td>
<td>Yes</td>
</tr>
<tr>
<td>Roszkowska-Ganczarz et al.</td>
<td>2014</td>
<td>44 98 48 188</td>
<td>374 206 194</td>
<td>186</td>
<td>786 750</td>
<td>Yes</td>
</tr>
<tr>
<td>Jiang et al.</td>
<td>2014</td>
<td>140 34 3 263</td>
<td>75 8 40</td>
<td>314</td>
<td>91 601</td>
<td>Yes</td>
</tr>
<tr>
<td>Jiang et al.</td>
<td>2014</td>
<td>133 31 1 263</td>
<td>75 8 33</td>
<td>297</td>
<td>91 601</td>
<td>Yes</td>
</tr>
<tr>
<td>Li et al.</td>
<td>2015</td>
<td>77 48 1 136</td>
<td>47 2 50</td>
<td>202</td>
<td>51 319</td>
<td>Yes</td>
</tr>
<tr>
<td>Li et al.</td>
<td>2015</td>
<td>32 75 1 136</td>
<td>47 2 77</td>
<td>139</td>
<td>51 319</td>
<td>Yes</td>
</tr>
<tr>
<td>Meshkani et al.</td>
<td>2016</td>
<td>16 23 32 33</td>
<td>98 81 87</td>
<td>55</td>
<td>260 164</td>
<td>Yes</td>
</tr>
<tr>
<td>Wu et al.</td>
<td>2017</td>
<td>187 51 3 210</td>
<td>57 3 57</td>
<td>425</td>
<td>63 477</td>
<td>Yes</td>
</tr>
<tr>
<td>Our study</td>
<td>2017</td>
<td>370 115 11 593</td>
<td>180 9 137</td>
<td>855</td>
<td>198 1366</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*indicating GA+AA. HWE: Hardy-Weinberg equilibrium.

LEPR rs1137101 G>A polymorphism and T2DM

LEPR rs1137101 G>A polymorphism is a G→A variant which is located in exon 6 at nucleotide 668 from the start codon. A previous study supports the hypothesis that the LEPR rs1137101 G>A polymorphism is associated with obesity and could predict body composition variability [38]. Obesity plays a vital role in the development of T2DM; therefore, it is believed that LEPR signaling may be involved in the etiology of T2DM. Of late, the association between the LEPR rs1137101 G>A polymorphism and the risk of T2DM has been described in several studies; however, the findings still remain controversial. Several meta-analyses have suggested that the LEPR rs1137101 G>A polymorphism is not associated with the risk of T2DM [25, 39]. However, another meta-analysis reported that the LEPR rs1137101 G>A polymorphism is a risk factor for T2DM [26]. In the present case-control study, we found that the LEPR rs1137101 G>A polymorphism was not associated with the risk of T2DM. In addition, this potential association was also found in the subsequent meta-analysis. In the future, more studies should be performed to explore the association between the LEPR rs1137101 G>A polymorphism and T2DM risk.
**Table 6. Meta-analysis of the LEPR rs1137101 G>A polymorphism and T2DM**

<table>
<thead>
<tr>
<th></th>
<th>No. of cases/ controls</th>
<th>A vs. G</th>
<th></th>
<th>AA vs. GG</th>
<th></th>
<th>AA+GA vs. GG</th>
<th></th>
<th>AA vs. GA+GG</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>$\hat{\tau}$</td>
<td>P (Q-test)</td>
<td>OR (95% CI)</td>
<td>P</td>
<td>$\hat{\tau}$</td>
<td>P (Q-test)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>5,869/6,618</td>
<td>1.06</td>
<td>0.90-1.27</td>
<td>0.479</td>
<td>77.3%</td>
<td>&lt;0.001</td>
<td>0.92</td>
<td>0.64-1.31</td>
<td>0.643</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asians</td>
<td>5,231/5,195</td>
<td>1.07</td>
<td>0.86-1.34</td>
<td>0.534</td>
<td>82.0%</td>
<td>&lt;0.001</td>
<td>0.84</td>
<td>0.49-1.42</td>
<td>0.506</td>
</tr>
<tr>
<td>Caucasians</td>
<td>6,38/1,423</td>
<td>1.03</td>
<td>0.88-1.20</td>
<td>0.747</td>
<td>0.0%</td>
<td>0.929</td>
<td>1.03</td>
<td>0.73-1.45</td>
<td>0.867</td>
</tr>
<tr>
<td><strong>Sample size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1000</td>
<td>3,593/5,103</td>
<td>1.07</td>
<td>0.86-1.33</td>
<td>0.522</td>
<td>80.8%</td>
<td>&lt;0.001</td>
<td>0.89</td>
<td>0.59-1.35</td>
<td>0.587</td>
</tr>
<tr>
<td>≥1000</td>
<td>2,276/1,515</td>
<td>1.05</td>
<td>0.90-1.23</td>
<td>0.504</td>
<td>0.0%</td>
<td>0.846</td>
<td>1.10</td>
<td>0.62-1.96</td>
<td>0.751</td>
</tr>
<tr>
<td><strong>Source of control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population-based</td>
<td>1,727/1,088</td>
<td>0.86</td>
<td>0.70-1.06</td>
<td>0.152</td>
<td>21.2%</td>
<td>0.283</td>
<td>0.70</td>
<td>0.39-1.28</td>
<td>0.248</td>
</tr>
<tr>
<td>Hospital-based</td>
<td>4,142/5,530</td>
<td>1.12</td>
<td>0.91-1.37</td>
<td>0.291</td>
<td>80.4%</td>
<td>&lt;0.001</td>
<td>0.99</td>
<td>0.65-1.51</td>
<td>0.962</td>
</tr>
<tr>
<td><strong>Quality score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥7</td>
<td>1,838/2,492</td>
<td>1.10</td>
<td>0.95-1.26</td>
<td>0.195</td>
<td>0.0%</td>
<td>0.856</td>
<td>1.22</td>
<td>0.79-1.87</td>
<td>0.363</td>
</tr>
<tr>
<td>&lt;7</td>
<td>4,031/4,126</td>
<td>1.05</td>
<td>0.82-1.36</td>
<td>0.691</td>
<td>85.0%</td>
<td>&lt;0.001</td>
<td>0.77</td>
<td>0.46-1.29</td>
<td>0.319</td>
</tr>
</tbody>
</table>
In addition, in our study, some limitations should be considered. First, the hospital-based design of our case-control study might lead to bias. Secondly, significant heterogeneity was found in some genetic models. Although the results of the sensitivity analyses were not substantially changed compared with the crude ORs and CIs, our findings should be interpreted with much caution. Thirdly, we only focused on the LEPR rs1137101 G>A polymorphism, which could not provide an extensive view of the genetic risk in T2DM.
the \textit{LEPR} gene. A fine-mapping study or genome wide association study should be carried out to explore the potential association between the \textit{LEPR} SNPs and the risk of T2DM. Finally, data on plasma-soluble \textit{LEPR} levels were unavailable, which left us unable to compare the plasma-soluble \textit{LEPR} levels across different genotypes.

In conclusion, this case-control study along with a comprehensive meta-analysis suggests that the \textit{LEPR} rs1137101 G>A polymorphism is not associated with the susceptibility of T2DM. Nevertheless, for practical reasons, further investigations with detailed environmental data and large sample sizes are needed to confirm or refute these results.

\textbf{Acknowledgements}

We appreciate all subjects who participated in this study. We wish to thank Dr. Yan Liu (Genesky Biotechnologies Inc., Shanghai, China) for technical support. This study was supported in part by the Young and Middle-aged Talent Training Project of the Health Development Planning Commission in Fujian Province (2016-ZQN-25 and 2014-ZQN-JC-11), the Medical Innovation Project of Fujian Province (2014-CX-15 and 2014-CX-18), the Nursery Garden Project of Fujian Medical University (2015MP020), and the Science and Technology Project of Fujian Province (2060203).

\textbf{Disclosure of conflict of interest}

None.

\textbf{Address correspondence to:} Jianming Hou, Department of Endocrinology, Shengli Clinical Medical College of Fujian Medical University, Fuzhou 350000, Fujian Province, China. E-mail: hjm996@126.com

\textbf{References}

LEPR rs1137101 G>A polymorphism and T2DM


[24] Murugesan D, Arunachalam T, Ramamurthy V and Subramanian S. Association of polymor-