Association of the adiponectin gene (ADIPQ) +45 T > G polymorphism with the metabolic syndrome among Han Chinese in Sichuan province of China

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The metabolic syndrome is a cluster of abnormalities characterized by obesity, impaired glucose metabolism, hypertension or elevated blood pressure, and dyslipidemia. The aim of this study was to investigate the association of the adiponectin gene (ADIPQ) +45 T > G polymorphism with the metabolic syndrome among Han Chinese in Sichuan province of China. A case-control design was used including 116 patients with the metabolic syndrome and 108 unrelated controls, matched on age and gender. The ADIPOQ +45G allele (TG+GG) had a significant association with risk of the metabolic syndrome (odds ratio=1.88, 95% confidence interval: 1.03-3.44, \( p = 0.039 \)) adjusted for education, physical activity, family history of related diseases, smoking and drinking, compared with subjects with TT genotype. The association between the ADIPOQ +45 T>G polymorphism and the metabolic syndrome was independent of multiple confounders.

Key Words: the metabolic syndrome, ADIPOQ, polymorphism, case-control study, China

INTRODUCTION

The metabolic syndrome (MS) is a cluster of physiological and biochemical abnormalities, including four major components: obesity, especially central obesity; impaired glucose metabolism such as type 2 diabetes mellitus (T2DM), impaired fasting glucose, or impaired glucose tolerance (IGT); hypertension or elevated blood pressure; and dyslipidemia with hypertriglyceridemia and/or hypoalphalipoproteinemia.1 MS is associated with the development of T2DM and of cardiovascular disease (CVD),2 as well as with an increased all-cause mortality.3

Adiponectin is a relatively abundant serum protein secreted by adipocytes.4 Plasma levels of adiponectin is lower in people with obesity;5 hypertension,6 T2DM and coronary heart disease (CHD),7 compared to those in healthy subjects. Some studies showed that the level of adiponectin is correlated with insulin resistance,8 and with glucose and lipid metabolism.9 Furthermore, adiponectin has been reported to be independently associated with MS.10,11

The adiponectin gene (ADIPOQ) is located on chromosome 3q27, it consists of three exons and two introns,12 and has been linked to CHD,13 T2DM14 and MS.15 The +45 T > G polymorphism is one of the most common variants of the exon 2 of the ADIPOQ gene and it is reported to be associated with serum levels of adiponectin, insulin sensitivity, obesity and T2DM.16-18 However, whether the +45 T > G polymorphism is associated with MS still remains unclear.16, 19

Previous studies showed a low prevalence of hypertension among Yi farmers20,21 in remote mountainous areas of southwestern China where the largest Yi community is located. In addition, Wang et al 22 reported that the age- and sex-adjusted prevalence of MS was 9.9 times higher in Yi migrants (23.8%) and 6.3 times higher in Han people (22.5%) compared with the Han population.
ple (15.2%) than in Yi farmers (2.4%) and assumed, therefore, lifestyle strongly influenced development of MS in Han and Yi Chinese. In addition, it is well known that MS is also influenced by genetic factors. This study was therefore designed to examine the possible association of the ADIPOQ +45 T > G polymorphism with MS among Han Chinese in Sichuan province of China to further explore the mechanisms that may affect MS in Han Chinese.

MATERIALS AND METHODS
Participants classification and definition
A case-control study comprising of 224 Han Chinese aged over 20 years living in Xichang City, southwestern China was carried out based on a cross-sectional survey from 2007 to 2008.22 Both parents of the study participant should be of Han ethnicity, and inhabits of the city for more than five years. The participants included 116 patients of MS and 108 unrelated controls matched with regard to age (± 3 years) and gender.

According to the Chinese Diabetes Society in 2004, individuals satisfying three or more of the following components were diagnosed as having the MS: (1) body mass index (BMI) ≥25.0 kg/m²; (2) serum levels of triglyceride ≥150 mg/dL (1.7 mmol/L) and/or serum levels of high-density lipoprotein-cholesterol (HDLC) < 35 mg/dL (0.9 mmol/L) in males and <39 mg/dL (1.0 mmol/L) in females; (3) blood pressure ≥140/90 mmHg and/or history of treatment for previously diagnosed hypertension; (4) fasting plasma glucose (FPG) ≥110 mg/dL (6.1 mmol/L) and/or two-hour plasma glucose (2hPG) ≥140 mg/dL (7.8 mmol/L) after meals and/or history of previously diagnosed T2DM.

This study was approved by the institutional review board of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing. Informed consent was obtained from all participants.

Measurements
To evaluate demographic, socioeconomic and lifestyle information, the participants were interviewed by local trained physicians with a questionnaire that collected data including age, gender, ethnicity, education, occupation, family history of related diseases, smoking, drinking, as well as information related to diagnosis and treatment for hypertension and diabetes. Education for this study was categorized into three levels, ie, illiteracy or primary school, high school and university. Physical activity was categorized based on the participant’s occupation, as light, moderate or heavy, respectively. Family history of related diseases was categorized as any of their biological parents or siblings with hypertension, diabetes or dyslipidemia. Smoking was defined as never smoker, or ever smoker if the subject was a current smoker or a former smoker. Drinking was defined as never drinking alcoholic beverages, or ever drinker if the subject was a current drinker or a former drinker.

Blood pressure was measured three consecutive readings after a 10-min resting period in a sitting position to the nearest 2 mmHg on the right arm using a standard mercury sphygmomanometer. Both body height and weight were measured with the participants in light clothing and without shoes. Obesity was assessed by body mass index (BMI, as weight in kilograms divided by the square of height in meters).

Biochemical analysis
Venous blood was obtained after overnight fasting, and processed at the examination center and then shipped to a central clinical laboratory in Beijing where all the samples were kept at -40°C before analysis. FPG level was measured by the glucose oxidase method. Both of HDL-C and triglyceride were measured by enzymatic methods. All biochemical analyses were performed using Hitachi 7600 automatic analyzer (Boehringer Mannheim, Mannheim, Germany).

Genotyping of the ADIPOQ +45 T > G polymorphism
The ADIPOQ +45 T > G polymorphism (rs2241766) was genotyped by polymerase chain reaction (PCR) and restriction fragment length polymorphism (PCR-RFLP). The polymorphism was amplified using the following primers: forward, 5’ -GCAGCTTCTAGAAGTAGAC TCTGCTG -3’ and reverse, 5’ -GCAGGTCTGTGAT GAAAGAGGCC -3’ (Yingjun Biotech Co Ltd, Shanghai, China). Polymerase chain reaction was performed in a total volume of 25 μl mixture containing 2.0 μl DNA, 0.5 μl forward primer, 0.5 μl reverse primer, 0.7 μl deoxynucleotide triphosphate (dNTP), 1.8 μl MgCl₂, 2.5 μl 10×buffer, 0.5μl of Taq DNA polymerase and 16.5 μl of ddH₂O. The amplification conditions were as follows: 94°C for 5 min, followed by 35 cycles of 1 min at 94°C, 1 min at 59°C and 1 min at 72°C, and ending with a single 5 min extension step at 72°C. The PCR products were run on 2% agarose gels electrophoresis to evaluate overall amplification efficiency. Then they were digested with the enzyme Smal (Biolabs Inc., New England, U.S.) at 25°C for 4 hours. Reaction mixture contained the PCR products 10 μl, 0.3μl of Smal, 2μl 10×buffer and 8μl ddH₂O. The digestion products were resolved by 3% agarose gel electrophoresis and viewed under an UV transilluminator. The TT homozygote yielded the 372 bp uncut fragment only, the TG heterozygote yielded the 372, 209 and 163bp fragments, while the GG homozygote yielded the 209 and 163bp fragments.

Statistical analysis
Continuous variables are presented as means ± standard deviation (SD) and difference between the cases and controls was compared using Student’s t-test for normally-distributed variables or non-parametric Mann-Whitney U-test for non-normally-distributed ones. Test for Hardy-Weinberg equilibrium and comparison of allele and genotype distribution between MS cases and controls were assessed by chi-square test. Odds ratios (OR) and their 95% confidence intervals (CI) were calculated using the Mantel-Haenszel method. Multiple logistic regression analysis was performed to estimate an association of the +45 T > G polymorphism with the risk of MS. A p value less than 0.05 was considered statistically significant. All data analyses were performed using SAS version 9.2 software (SAS Institute Inc., Cary, NC, USA).

RESULTS
Demographic characteristics of 116 MS cases and 108 controls are shown in Table 1. Mean age of the cases and controls were 51.6±11.6 years and 51.8±11.5 years, respectively (p=0.890). The cases had significantly higher BMI, systolic blood pressure, diastolic blood pressure, triglyceride and total cholesterol, but lower HDL-C than those in the controls. No significant differences in FPG and low-density lipoprotein-cholesterol (LDL-C) between the two groups were found (p > 0.05).

As shown in Table 2, frequencies of the TT, TG and GG genotypes were 61.2%, 34.5% and 4.3% in the cases, and 70.4%, 25.9% and 3.7% in the controls, respectively. Because only five cases and four controls were homozygous for the rare G-allele, we combined them with those having the TG genotype in all statistical analysis. Compared with TT, there was no difference in distribution of the TG, GG and TG+GG genotypic frequencies between cases and controls. In addition, no difference was observed in frequency of allele between the two groups. Frequencies of the genotype were in Hardy-Weinberg equilibrium in both groups.

Results of multiple logistic regression analysis showed that the ADIPOQ +45 T > G polymorphism was an independent risk factor for MS (Figure 1). After adjustment for education, physical activity, family history of related diseases, smoking and drinking, carriers of the G allele (TG+GG) had a 1.88-fold higher risk for developing MS (OR=1.88, 95% CI: 1.03-3.44, p=0.039) than subjects with TT genotype.

Table1. Demographic characteristics of the study participants †

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n=116)</th>
<th>Controls (n=108)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51.6±11.6</td>
<td>51.8±11.5</td>
<td>0.890</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.2±2.21</td>
<td>21.5±2.69</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>144±19.1</td>
<td>112±12.0</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>93.0±12.2</td>
<td>74.4±8.60</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>6.3±4.1</td>
<td>5.6±1.77</td>
<td>0.198</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>3.4±4.06</td>
<td>1.15±0.67</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.0±0.99</td>
<td>4.45±0.75</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>0.95±0.24</td>
<td>1.31±0.29</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.54±1.55</td>
<td>2.62±0.64</td>
<td>0.336</td>
</tr>
</tbody>
</table>

† Data indicated as mean ± standard deviation. SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol.

Table2. Distribution of the frequencies of the genotype, combined genotype and allele between cases and controls

<table>
<thead>
<tr>
<th>Genotype/allele</th>
<th>Cases (n=116) n (%)</th>
<th>Controls (n=108) n (%)</th>
<th>p-value</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>71 (61.2)</td>
<td>76 (70.4)</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>40 (34.5)</td>
<td>28 (25.9)</td>
<td></td>
<td>1.53†</td>
<td>0.86-2.74</td>
</tr>
<tr>
<td>GG</td>
<td>5 (4.3)</td>
<td>4 (3.7)</td>
<td>0.349 *</td>
<td>1.34 †</td>
<td>0.35-5.18</td>
</tr>
<tr>
<td>TG+GG</td>
<td>45 (38.8)</td>
<td>32 (29.6)</td>
<td>0.149 **</td>
<td>1.24 †</td>
<td>0.86-2.63</td>
</tr>
<tr>
<td>T</td>
<td>182 (78.4)</td>
<td>180 (83.3)</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>50 (21.6)</td>
<td>36 (16.7)</td>
<td>0.190 ***</td>
<td>1.37 ‡</td>
<td>0.85-2.21</td>
</tr>
</tbody>
</table>

p-value * for the difference in the frequencies of TT, TG and GG genotype between cases and controls
p-value ** for the difference in the frequencies of TT and TG+GG genotype between cases and controls
p-value *** for the difference in the frequencies of the T and G allele between cases and controls
OR†: TG and GG vs. TT
OR‡: TG+GG vs. TT
OR§: G vs. T

DISCUSSION

This study showed that the ADIPOQ +45 T > G polymorphism was independently associated with MS, and that the G allele had a corresponding significant association with the MS.

MS is a complex disorder and its main pathophysiological mechanism is mediated by insulin resistance and central obesity.24–25 Insulin resistance is considered to be at the core of the syndrome, while central obesity is its most prevalent clinical manifestation.26 Both genetic and environmental factors contribute to the development of MS.27,28 Some studies have provided evidence that the ADIPOQ +45 T> G polymorphism is associated with MS components.16–18 However, a number of studies have mainly focused on the association of the polymorphism with one or two components of MS, such as obesity or and T2DM. To our knowledge, a limited number of published work has been found on association of the +45 T > G polymorphism with MS. In addition, environmental factors such as smoking, drinking, physical activity and family income might have predominantly contributed to the progression of MS.22,27 Therefore, a case-control study was designed to adjust confounding effect of environmental factors on MS.

Age and gender which are independently associated with MS may confound an association of the ADIPOQ +45 T > G polymorphism with MS.22 Prevalence of MS varies with gender and increases with increasing age.22,29 Besides, diagnostic criteria for MS for men and women are different. Therefore, a case-control design is needed.
to match age and gender between MS cases and the controls.

Heid et al. reported that frequencies of the TG and GG genotypes were 21% and 1%, respectively in healthy Caucasian population. We found that frequencies of TG and GG genotypes were 30% and 4%, respectively in Han Chinese (data not shown). Furthermore, the +45 T > G mutation showed higher frequencies of TG and GG genotypes (41 and 6%, respectively) among Han Chinese in eastern China, compared with our study. This implies that the +45 T > G mutation rate had a different distribution among different ethnic and regional populations. Therefore, case-control studies on an association of the genotypes with MS among different populations may show inconsistent results.

In the present study, after adjusting for environmental factors that may confound expression of the phenotype, such as education, physical activity, family history of related diseases, smoking and drinking, our results showed that the G-allele carriers had an approximately two-fold higher risk for an association with MS in cases than that in controls. This finding was consistent with that found in some of the previous studies. The 45G allele had a significantly increased risk for obesity and reduced insulin sensitivity in Germans, and an increased risk for T2DM in the Japanese and other populations in the STOP-NIDDM trial. However, there are some inconsistent findings showing that the 45G allele appeared to have a protective role on MS, whereas, +45T allele is likely to be a risk factor. The +45T allele is a risk factor for developing obesity and insulin resistance syndrome in Italians. Similar results have been reported in populations from Taiwan and Sweden, respectively.

These inconsistent results may depend on several causes. First of all, the ADIPOQ gene is only one gene regulating the traits of MS. Moreover, MS is not only associated with genetic factors, but also with environmental factors. Dietary intake is recognized as an important environmental factor in affecting predisposition to MS, and its effect on adiponectin concentrations has been reported in a previous study. In addition, some studies reported that an interaction between genetic predisposition and dietary fat intake may contribute to the development of MS. We, therefore, assume that the effect of a single gene variant on MS will be weak.

Some limitations of this study should be interpreted. Firstly, the study has a case-control design, therefore we can’t affirm that after the investigation some control subjects did not develop MS. Secondly, plasma level of adiponectin was not measured and an association of adiponectin level with the ADIPOQ +45 T>G polymorphism and MS couldn’t be analyzed. Thirdly, only one variant within the ADIPOQ gene was explored for its association with MS, and not all of the single nucleotide polymorphisms (SNPs) reported in the literature. Therefore, we can’t explore the effect of gene-gene interactions on MS. In addition, no detailed information about dietary components was available in this study population, so the effect of gene-diet interactions on the MS can’t be identified.

In conclusion, the ADIPOQ +45 T>G polymorphism is independently associated with MS, and the G allele has a significant association with the risk of MS among Han Chinese. It is implied that the ADIPOQ +45 T>G polymorphism may be a genetic contributor to MS.

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AUTHOR DISCLOSURES
All authors have no conflicts of interest to declare.

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中國四川省漢族居民脂聯素基因+45 T > G 多態性與代謝綜合征的關聯

代謝綜合征是多種異常組分集聚在同一個體的臨床綜合征，異常組分主要包括肥胖、糖代謝受損、高血壓或血壓升高以及血脂紊亂。本研究的目的是探討中國四川省漢族居民脂聯素基因+45 T > G 多態性與代謝綜合征的關聯。應用病例-對照研究的方法，以確診的 116 例代謝綜合征患者為病例組，按照年齡和性別進行頻數匹配，選取 108 例非代謝綜合征且無血緣關係者作為對照組。研究結果顯示，在調整了教育程度、體力勞動強度、相關疾病家族史、吸煙和飲酒情況後，與攜帶 TT 基因型的人相比，脂聯素基因第 45 位的 G 等位基因 (TG+GG) 是代謝綜合征的危險因子 (OR=1.88，95%CI=1.03-3.44，p=0.039)。此結果表明，控制干擾因素後，脂聯素基因+45 T > G 多態性仍是代謝綜合征的獨立危險因素。

關鍵字：代謝綜合征、脂聯素基因、基因多態性、病例-對照研究、中國