Original Article
Polymorphisms in the GCKR are associated with serum lipid traits, the risk of coronary artery disease and ischemic stroke

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Abstract: The present study was to determine the association of two single nucleotide polymorphisms (SNPs) in the glucokinase regulator gene (GCKR) and serum lipid levels, and the risk of coronary artery disease (CAD) and ischemic stroke (IS). Genotypes of the GCKR rs1260326 and rs8179206 in 1736 unrelated subjects (CAD, 584; IS, 555; and healthy controls; 597) were determined by the Snapshot technology platform. The genotypic and allelic frequencies of rs1260326 and rs8179206 were not different among the three groups (P > 0.05). The subjects with rs1260326TT genotype had higher serum low-density lipoprotein cholesterol (LDL-C) levels in controls, and higher triglyceride (TG) levels in CAD patients than the subjects with CC and CT genotypes after adjustment for age, sex, body mass index, blood pressure, alcohol consumption, and cigarette smoking (P < 0.05). The rs1260326TT genotype was also associated with decreased risk of IS in females (OR = 0.37, 95% CI: 0.18-0.76, P = 0.007). The present study shows that the GCKR rs1260326TT genotype is associated with high LDL-C in controls, high TG levels in CAD patients, and a decreased risk of IS in females.

Keywords: Glucokinase regulator, single nucleotide polymorphism, lipids, coronary artery disease, ischemic stroke

Introduction

Both coronary artery disease (CAD) and ischemic stroke (IS) are the leading causes of morbidity and death in the developed countries, and are also the major cause of long-term disability in survivors [1, 2]. Atherogenic dyslipidemia characterized by low levels of high-density lipoprotein cholesterol (HDL-C) and apolipoprotein (Apo) A1, high levels of total cholesterol (TC), triglyceride (TG) and low-density lipoprotein (LDL) particle number is highly associated with increased incidence of the cardiovascular disease [3] and IS [4, 5]. In addition, genetic factors are estimated to account for about 50-80% of the variation in serum lipid levels [6], and 30-60% of the incidence of CAD and IS [7]. Therefore, single nucleotide polymorphisms (SNPs) in the lipid-related genes may have some associations with serum lipid levels, and the risk of CAD and IS [8].

Glucokinase regulator gene (GCKR) located on chromosome 2p23, consists of 19 exons and encodes for the glucokinase regulator protein [9]. It regulates the glucokinase’s (GCK) activity in the hepatocytes and pancreatic beta cells [10], thus may play a pivotal role in glucose homeostasis [11]. Animal studies indicated that GCKR knockout mice have decreased GCK activity, and leading to elevated glucose levels. Adenoviral mediated over-expression of GCKR in murine liver increases GCK activity and lowered fasting blood glucose as well as increased TG levels [11-14]. Molecular mechanism showed GCKR rs1260326 in central pathways regulating both hepatic TG and glucose metabolism in humans [14].

Recently, a genome-wide association study (GWAS) identified GCKR as a potential locus for modulating TG [15]. Subsequent population-based studies and meta-analysis and fine-mapping studies, confirmed that the minor alleles of rs780094 and rs1260326 (Pro446Leu) were associated with higher levels of TG and lower fasting glucose [14, 16-21]. Consistent with this
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notion and the observed associations, the rs1260326 446Leu was recently reported in vitro to increase hepatic GCK activity. However, the associations between the SNPs in GCKR and serum lipid levels, especially the risk of CAD and IS are still needed to determine in different populations. Therefore, the aim of the present study was to detect the association of GCKR rs1260326 and rs8179206 SNPs and serum lipid levels and the risk of CAD and IS in the Guangxi Han population.

Materials and methods

Study population

A total of 584 patients with CAD and 555 patients with IS were recruited from hospitalized patients in the First Affiliated Hospital, Guangxi Medical University. All of the enrolled CAD patients were evaluated by coronary angiography due to suspected CAD or unrelated conditions requiring angiographic evaluation; the coronary angiograms were analyzed by two experienced interventional cardiologists. CAD was defined as significant coronary stenosis (≥ 50%) in at least one of the three main coronary arteries or their major branches (branch diameter ≥ 2 mm). Subjects with congenital heart disease and type I diabetes mellitus were excluded. All of the enrolled IS patients received a strict neurological examination and brain magnetic resonance imaging. The diagnosis of IS was according to the International Classification of Diseases (9th Revision). Patients with a transient ischemic attack, embolic brain infarction, stroke caused by inflammatory disease, cardioembolic stroke, autoimmune disease, or serious chronic diseases were excluded from this study. Subjects with a past history of CAD were also excluded from the study [22]. A total of 597 healthy controls matched by age, gender, and geographical area were included. The controls were judged to be free of CAD and IS by questionnaires, medical history, and clinical examination. All individuals enrolled were from the Han population in Guangxi, China. A standard questionnaire was used to ascertain general information and medical history from all participants. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University. Informed consent was obtained from all subjects after receiving a full explanation of the study.

Genotyping and biochemical analysis

All of the biochemical assays and genotyping in CAD and IS patients were performed after hospitalization, and all of the venous blood samples were obtained from the patients and con-

Table 1. Baseline characteristics of the participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n = 597)</th>
<th>Case</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>控制 (n = 597)</td>
<td>CAD (n = 584)</td>
<td>IS (n = 555)</td>
<td></td>
</tr>
<tr>
<td>Male/female</td>
<td>434/163</td>
<td>428/156</td>
<td>405/150</td>
<td>0.861</td>
</tr>
<tr>
<td>Age, years</td>
<td>61.32±9.76</td>
<td>62.23±10.63</td>
<td>62.86±12.11</td>
<td>0.121</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.41±2.86</td>
<td>23.84±3.38</td>
<td>24.71±22.04</td>
<td>0.000</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>130.60±20.01</td>
<td>133.03±23.33</td>
<td>147.72±22.15</td>
<td>0.059</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>82.94±13.37</td>
<td>79.18±14.21</td>
<td>83.78±12.97</td>
<td>0.000</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>49.81±14.70</td>
<td>53.49±18.08</td>
<td>64.02±17.92</td>
<td>0.000</td>
</tr>
<tr>
<td>Cigarette smoking, n (%)</td>
<td>236 (39.5)</td>
<td>250 (42.8)</td>
<td>233 (42.0)</td>
<td>0.253</td>
</tr>
<tr>
<td>Alcohol consumption, n (%)</td>
<td>235 (39.4)</td>
<td>131 (22.4)</td>
<td>157 (28.3)</td>
<td>0.000</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.93±1.11</td>
<td>4.53±1.19</td>
<td>4.52±1.15</td>
<td>0.000</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.42±1.87</td>
<td>1.66±1.11</td>
<td>1.67±1.36</td>
<td>0.007</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.90±0.50</td>
<td>1.14±0.34</td>
<td>1.23±0.40</td>
<td>0.000</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.74±0.80</td>
<td>2.71±1.00</td>
<td>2.68±0.90</td>
<td>0.550</td>
</tr>
<tr>
<td>Apolipoprotein (Apo) A1, g/L</td>
<td>1.41±0.28</td>
<td>1.04±0.52</td>
<td>1.02±0.22</td>
<td>0.000</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>0.91±0.22</td>
<td>0.91±0.27</td>
<td>0.89±0.25</td>
<td>0.995</td>
</tr>
<tr>
<td>ApoA1/ApoB</td>
<td>1.63±0.49</td>
<td>1.36±2.45</td>
<td>1.26±0.60</td>
<td>0.013</td>
</tr>
</tbody>
</table>

CAD, coronary artery disease; IS, ischemic stroke; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
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trols after at least 12 h of fasting. Genomic DNA was isolated from peripheral blood leukocytes using the phenol-chloroform method. We selected the SNPs across the region of the GCKR from NCBI dbSNP Build 132 (http://www. Ncbi.nlm.nih.gov/SNP/). Genotyping of the rs1260326 and rs8179206 SNPs was performed by the Snapshot technology platform. The levels of serum TC, TG, HDL-C, and LDL-C in the samples were determined by enzymatic methods with commercially available kits. Serum ApoA1 and ApoB levels were detected by an immunoturbidimetric immunoassay using a commercial kit.

Diagnostic criteria

The normal values of serum TC, TG, HDL-C, LDL-C, ApoA1, ApoB and ApoA1/ApoB ratio in our Clinical Science Experiment Center were 3.10-5.17, 0.56-1.70, 0.91-1.81, 2.70-3.20 mmol/L, 1.00-1.78, 0.63-1.14 g/L, and 1.00-2.50; respectively. The individuals with TC > 5.17 mmol/L and/or TG > 1.70 mmol/L were defined as hyperlipidemic. Hypertension was diagnosed according to the criteria from the 1999 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension [23]. The diagnostic criteria of overweight and obesity were according to the Cooperative Meta-analysis Group of China Obesity Task Force. Normal weight, overweight and obesity were defined as a BMI < 24, 24-28 and > 28 kg/m², respectively [24]. Dyslipidemia was defined according to World Health Organization criteria: TG ≥ 1.7 mmol/L and HDL-C < 0.9 mmol/L for men or < 1.0 mmol/L for women.

Statistical analyses

All statistical analyses were performed using the statistical software package SPSS 16.0 (SPSS Inc. Chicago, IL, USA). A standard goodness-of-fit test was used to test the Hardy-Weinberg equilibrium. A chi-square analysis was used to evaluate the difference in genotype distribution and sex ratio between the groups. The general characteristics between the cases and controls were tested using Student’s unpaired t-test. The association between genotypes and serum lipid parameters was tested by analysis of covariance (ANCOVA). Sex, age, body mass index (BMI), blood pressure, alcohol consumption, and cigarette smoking were adjusted for the statistical analysis. ORs and 95% CIs were calculated using unconditional logistic regression. A two-
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Results

Baseline characteristics

The baseline characteristics of participants in this study are presented in Table 1. The mean age, ratio of males to females, serum LDL-C and ApoB levels, and the percentages of subjects who smoked cigarette were not different between controls and CAD or IS patients (P > 0.05 for all). The CAD patients had higher BMI, pulse pressure and serum TG levels, but lower levels of diastolic blood pressure, serum TC, HDL-C, ApoA1, the ratio of ApoA1 to ApoB, and the percentages of subjects who consumed alcohol (P < 0.05-0.001) than the controls. The IS patients had higher BMI, systolic blood pressure, pulse pressure and serum TG levels, and lower levels of serum TC, HDL-C, ApoA1, the ratio of ApoA1 to ApoB, and the percentages of subjects who consumed alcohol (P < 0.05-0.001) than the controls.
Table 5. Association of the genotypes and serum lipid levels in the controls and cases

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control</th>
<th>CAD</th>
<th>IS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TC (mmol/L)</td>
<td>TG (mmol/L)</td>
<td>HDL-C (mmol/L)</td>
</tr>
<tr>
<td>rs1260326</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>4.85±1.20</td>
<td>1.49±2.18</td>
<td>1.90±0.49</td>
</tr>
<tr>
<td>CT</td>
<td>4.96±1.01</td>
<td>1.34±1.84</td>
<td>1.93±0.52</td>
</tr>
<tr>
<td>TT</td>
<td>5.02±1.18</td>
<td>1.42±1.19</td>
<td>1.83±0.47</td>
</tr>
<tr>
<td>P</td>
<td>0.353</td>
<td>0.665</td>
<td>0.213</td>
</tr>
<tr>
<td>rs8179206</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>4.93±1.12</td>
<td>1.43±1.90</td>
<td>1.89±0.50</td>
</tr>
<tr>
<td>GA</td>
<td>4.93±1.00</td>
<td>0.91±0.26</td>
<td>2.01±0.47</td>
</tr>
<tr>
<td>GG</td>
<td>6.43</td>
<td>1.42</td>
<td>1.31</td>
</tr>
<tr>
<td>P</td>
<td>0.998</td>
<td>0.362</td>
<td>0.292</td>
</tr>
</tbody>
</table>

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
Genotypic and allelic frequencies

The genotypes of rs1260326 and rs8179206 SNPs were in Hardy-Weinberg equilibrium in the controls and patients (Table 2). There were no significant differences in the genotypic and allelic frequencies between controls and CAD or IS patients (Table 2; \( P > 0.05 \)). When the analysis was stratified by gender, the genotypic frequency of the rs1260326 SNP was significantly different between control and IS patients, but not in males (Table 3; \( P < 0.05 \)).

GCKR polymorphisms and the risk of CAD and IS

There was no significant association of rs1260326 and rs8179206 SNPs and the risk of CAD or IS in different genetic models (Table 2). But the TT genotype of the rs1260326 SNP was associated with decreased risk of IS in females (OR = 0.37, 95% CI: 0.18-0.76, \( P = 0.007 \); Table 3).

Genotypes and serum lipid levels

As shown in Table 4, the subjects with TT genotype of rs1260326 in the total population had lower serum HDL-C levels than those with CT/CC genotypes (\( P = 0.018 \)). For controls, the subjects with TT genotype of rs1260326 were associated with higher serum LDL-C than the subjects with CC genotype (\( P = 0.022 \)). For the CAD patients, the subjects with TT genotype of rs1260326 were associated with higher serum TG levels than the subjects with CC genotype (\( P = 0.038 \); Table 5). There was no significant association of rs8179206 SNP and serum lipid levels (Tables 4 and 5).

Discussion

The present study showed that the TT genotype of rs1260326 SNP was associated with increased risk of dyslipidemia and decreased serum HDL-C levels in the total population [17, 19, 25]. We also found that the subjects with TT genotype of rs1260326 SNP had higher serum LDL-C levels in controls and higher serum TG levels in CAD patients than the subjects with CC/CT genotypes. The results of the present study in the CAD patients were consistent with those of several previous studies in the Finnish, Swedish, Danish, Amish, and French populations [15, 16, 24, 26]. The association between variants in GCKR and TG was initially identified through an agnostic GWAS approach [15]. Further, some studies showed that the rs1260326 SNP was inversely associated with TG levels and glucose levels, and affected GCK activity in liver through diminished regulation by Fructose 6-phosphate [10]. This is predicted to increase glycolytic flux and hence glucose uptake by the liver. This enhanced rate of glycolysis may raise levels of malonyl-CoA, leading to increase TG levels of de novo lipogenesis and cholesterol synthesis and export. This perturbation of hepatic metabolism could account for the reduced glucose and raised TG seen in TT genotype of rs1260326 SNP [13, 27]. Elevated fasting TG levels are also associated with reduced levels of HDL-C, which is suggested to mark reduced removal of cholesterol from the arterial wall [28]. In this study, no such association was found in the controls and IS patients. It is possible that the impact of uncertain variant and a genetic background and lifestyle and diet caused the associations in the CAD group [21, 28, 29]. Different populations have different relationships between polymorphisms and serum lipid levels, which suggested our sample size may be not enough, further investigations are expended to confirm these interactions.

The SNPs of GCKR rs780093 and rs780094 were strong LD with rs1260326 SNP [16, 26, 30]. The SNP of rs8179206 was LD with rs1260326 (\( D' = 0.252; r^2 = 0.001 \)). The results of several previous association studies between GCKR polymorphisms and atherosclerosis-related diseases are not entirely consistent. Shen et al. [26] showed that the GCKR rs1260326 T allele was associated with higher atherogenic risk. Lian et al. [30] demonstrated GCKR rs780093 as a risk factor for CAD in individuals aged 65 and older. On the contrary, Varbo et al. [19] found that the GCKR rs1260326 SNP did not influence the risk of ischemic heart disease or myocardial infarction. In the Ludwigshafen Risk and Cardiovascular Health Study, no association was found with respect to coronary stenosis [31]. Járomi et al. [32] could not detect any association of rs1260326 with the susceptibility of stroke. Bi et al. [33] also found that the rs780094 SNP had no association with the incidence of CAD or stroke.

In the present study, we showed that the rs1260326T allele frequency (42.3%) was simi-
lar with that in HapMap CEU (European) population (42.0%). However, the T allele frequency of rs1260326 SNP is different among Chinese population (63.4%), Sub-Saharan African (10.3%), and Japanese population (59.3%) in the HapMap project database. Studies have found that GCKR is associated with increased levels of TG, unfavorable factor for CAD concurrent with lower levels of fasting plasma glucose, favorable factor for CAD [14, 17]. In the present study, although the rs1260326 SNP associated with unfavorable serum lipid profiles, we found that the TT genotype associated with decreased IS risk, thus, the lower glucose levels may also help to explain the inconsistent association of GCKR rs1260326 SNP and the risk of IS. The ambiguous results propose that the risk of CAD and IS associated with GCKR may vary based on the proportion of variance of the subjects’ glycemic and lipid traits. In addition, the number of subjects for minor allele of SNPs was too small to interpret the associations of SNPs and the risk of diseases. Therefore, larger sample size and multi-ethnic population studies are needed to confirm our results.

Conclusion

Our data indicate that the TT genotype of rs1260326 SNP is associated with increased serum LDL-C levels in controls, increased serum TG levels in CAD patients, and decreased serum HDL-C levels in the total population, but with decreased risk of IS in females.

Acknowledgements

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Disclosure of conflict of interest

None.

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