ASSOCIATIONS BETWEEN 45T/G POLYMORPHISM OF THE ADIPONECTIN GENE AND PLASMA ADIPONECTIN LEVELS WITH TYPE 2 DIABETES

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SUMMARY

1. The purpose of the present study was to investigate the association between the single nucleotide polymorphism (SNP) 45T/G and plasma adiponectin levels and the prevalence of Type 2 diabetes mellitus (T2DM) in Uygurs of the Xinjiang region, China.

2. We performed a cross-sectional survey in a representative sample of 151 Uygur adults aged 24–80 years. The polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method was used to determine the distribution of allele and genotype frequency of the SNP45 T/G polymorphism (exon 2) in the adiponectin gene. An ELISA was used to determine plasma adiponectin levels. Logistic regression was used to screen risk factors for T2DM.

3. Compared with the normal glucose tolerance (NGT) group, the T2DM group exhibited a higher distribution of the TG + GG genotype, G allele frequency and lower plasma adiponectin concentrations in TG + GG genotype carriers compared with those with the TT genotype. Compared with SNP45 T carriers, in the NGT group, G carriers had higher levels of systolic and diastolic blood pressure, low density lipoprotein (<0.05) and total cholesterol (<0.005). In the T2DM group, G carriers had lower levels of homeostasis model assessment (HOMA) of insulin sensitivity (<0.05) and higher levels of HOMA of insulin resistance (<0.05).

4. Adiponectin SNP 45 is positively correlated with the prevalence of T2DM in Uygurs of Xinjiang. The G allele carriers who have reduced plasma concentrations of adiponectin may have associated insulin resistance.

Key words: adiponectin, single nucleotide polymorphism 45 T/G, Type 2 diabetes, Uygurs.

INTRODUCTION

Adiponectin is an adipose tissue-derived protein with important metabolic effects.1 Reduced plasma levels of adiponectin have been demonstrated in individuals with obesity,2 insulin resistance3 and Type 2 diabetes mellitus (T2DM)2 and may be used to predict the overall risk of developing insulin resistance4 and T2DM.5 The inverse association between visceral adipose tissue and adiponectin is stronger in African Americans compared with Hispanics, a finding that suggests possible ethnic differences in the effects of visceral obesity.6

Recently, genome-wide scans in humans have mapped a susceptibility locus for T2DM and metabolic syndrome to chromosome 3q27, where the gene encoding adiponectin is located.7,8 A single nucleotide polymorphism (SNP; T/G at nucleotide 94; SNP45) was found to be associated with obesity and insulin resistance.9 However, the relationship between circulating adiponectin concentrations and the adiponectin SNP45 T/G polymorphism in exon 2 is still essentially unknown. The aim of the present study was to examine the correlation between the SNP45 T/G polymorphism in the adiponectin gene and the prevalence of T2DM in Uygurs of the Xinjiang region, China. Plasma adiponectin concentrations were also determined in different genotype carriers to elucidate the role, if any, of plasma adiponectin levels in the adiponectin gene polymorphism-linked incidence of T2DM.

METHODS

Study population

The present study was a cross-sectional survey performed in a representative sample of 151 Uygur adults aged 24–80 years who were chosen at random. Ninety-four (38 males, 56 females) non-diabetic subjects with a mean (±SD) age of 44.37 ± 9.69 years were recruited from Kashi, Xinjiang, to act as the control, normal glucose tolerance (NGT) group. Inclusion criteria were as follows: (i) no previous history of hypertension; (ii) no family history of T2DM in first- and second-degree relatives; and (iii) patients with Type 2 diabetes and individuals with impaired glucose tolerance (IGT) were removed following an oral glucose tolerance test (OGTT). The diagnosis of T2DM was made according to the 1997 guidelines of the American Diabetes Association10 and 57 unrelated Uygur patients with T2DM (28 males and 29 females; mean age 50.76 ± 9.22 years) were enrolled in the presents study. The study protocol was approved by the Ethical Committee for Clinical Trials.
of the First Affiliated Hospital of Xijinjiang Medical University and informed consent was provided by all participants.

**Distribution of allele and genotype frequencies of the SNP45 T/G polymorphism in exon 2 of the adiponectin gene**

The polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method was used to determine the distribution of allele and genotype frequencies of the SNP45 T/G polymorphism in exon 2 of the adiponectin gene. Overnight fasting blood samples were obtained from all subjects and genomic DNA was isolated from peripheral blood leukocytes. The DNA fragments containing SNP45 were amplified by PCR from genomic DNA using the forward primers 5′-GCA GCT CCT AGA AGT AGA CTC TGC TG-3′ and the reverse primers 5′-GCA GGT CTG TGA TGA AAG AGG CC-3′. The PCR was performed on 30 ng DNA in 20 μL containing 10 mmol/L Tris HCl, 50 mmol/L KCl, 1.5 mmol/L MgCl₂, pH 8.3, 0.2 mmol/L dNTP, 0.4 μmol/L forward and reverse primers and 0.035 U/μL Taq polymerase for 35 cycles (30 s at 94°C, 30 s at 60°C, 30 s at 72°C). The PCR fragments (372 bp) were digested using the restriction enzyme SmaI (recognition site: CCC → GGG). Through agarose gel electrophoresis, genotypes were inferred by comparing the length of the restriction fragment. There were three genotypes identified: (i) wild type TT (372 bp); (ii) heterozygous TG (restriction fragments 209 and 163 bp); and (iii) homozygous mutant GG (restriction fragments 209 and 163 bp).

**Biological measurements**

We chose 78 Uygurs (42 NGT subjects, 36T2DM patients) at random from the 151 Uygur adults from the Xinjiang Kashi region who were recruited to the study and evaluated their plasma concentrations of adiponectin. Plasma concentrations of adiponectin were determined using an ELISA-based adiponectin kit (Bionewtrns Pharmaceutical Biotechnology, Franklin, MA, USA).

We calculated pancreatic β-cell function and insulin resistance (IR) from fasting glucose and insulin concentrations using homeostatic model assessment (HOMA-β-cell and HOMA-IR, respectively) as follows:

\[
\text{HOMA-IR} = \frac{\text{fasting glucose (mmol/L)} \times \text{fasting insulin (μU/mL))}}{22.5}
\]

\[
\text{HOMA-β-cell} = \frac{20 \times \text{fasting insulin (μU/mL)}}{\left(\text{fasting glucose (mmol/L)} - 3.5\right)}
\]

**Statistical analysis**

The distribution of the alleles of SNP45 was tested for Hardy–Weinberg equilibrium (P > 0.05). Proportions of genotypes of alleles were compared by χ² analysis. Odds ratios (ORs) and 95% confidence intervals (CI), with adjustment for age and sex, were calculated by logistic regression analysis. Differences in plasma adiponectin concentrations between individuals with different genotypes were tested by Student’s t-test. The significance level was set at 0.05 or 0.01. Logistic regression was used to analyse sex, age, heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), waist circumference (WC), waist : hip ratio (WHR), HOMA, triglycerides (TG), total cholesterol (CH), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and adiponectin (APN) SNP45 and then morbidity risk factors of diabetes were screened. All data analysis was performed using spss 11.0 software (SPSS, Chicago, IL, USA).

**RESULTS**

**Association between SNP45 in the adiponectin gene and T2DM**

The G allele and TG + GG genotype were significantly more frequent than the T allele in T2DM patients than in the NGT group (P < 0.05).

Subjects with the G/G genotype were at increased risk for T2DM (OR 9.42, 95% CI 1.63–54.2) compared with those having the T/T genotype. This finding is consistent with data showing that the G allele of SNP45 is significantly associated with T2DM (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Genotype frequency</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TT + GG</td>
<td>G</td>
</tr>
<tr>
<td>NGT</td>
<td>94</td>
<td>19 (20.2%)</td>
<td>166 (88.3%)</td>
</tr>
<tr>
<td>T2DM</td>
<td>57</td>
<td>21 (36.8%)</td>
<td>91 (79.8%)</td>
</tr>
</tbody>
</table>

Data show the number of subjects in each group, with allele distribution and genotype frequency given in parentheses.

**Table 2** Differences in plasma adiponectin concentrations between subjects with normal glucose tolerance and those with type 2 diabetes mellitus and among different genotype carriers (TT or TG + GG)

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma adiponectin (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGT</td>
<td>15.6 ± 1.8</td>
</tr>
<tr>
<td>T2DM</td>
<td>10.0 ± 1.1*</td>
</tr>
<tr>
<td>TT</td>
<td>16.1 ± 1.6</td>
</tr>
<tr>
<td>TG + GG</td>
<td>15.4 ± 1.7</td>
</tr>
<tr>
<td>T2DM</td>
<td>10.6 ± 0.6</td>
</tr>
<tr>
<td>TG + GG</td>
<td>8.96 ± 1.05†</td>
</tr>
</tbody>
</table>

Values are the mean±SEM. *P < 0.001 compared with subjects with normal glucose tolerance (NGT); †P < 0.01 compared with the TT group. T2DM, Type 2 diabetes mellitus.

**Relationship between SNP45 and plasma adiponectin levels**

Results given in Table 2 indicate that the T2DM group had significantly lower plasma adiponectin concentrations than the NGT group (P < 0.05). Further analysis revealed that the plasma adiponectin concentration of TG + GG genotype carriers was not significantly different from that of the TT genotype in the NGT group. However, in the T2DM group, plasma levels of adiponectin of the TG + GG genotype were significantly lower than those of the TT genotype (Table 2).

**Relationship between SNP45 and anthropometric and biological indices**

In the NGT group, compared with SNP45 T carriers, G carriers had higher levels of SBP, DBP, LDL (all P < 0.05) and CH (P < 0.005). There were no significant differences in HR, BMI, WC, WHR,
HOMA, TG and HDL between these two genotype subgroups (Figs 1,2).

In the T2DM group, compared with SNP45 T carriers, G carriers had lower HOMA of insulin sensitivity (HOMA-ISI) \((P < 0.05)\) but higher HOMA-IR \((P < 0.05)\). There were no significant differences in the other indices between these two genotype subgroups (Fig. 3).

The results suggested that adiponectin gene SNP45 may affect blood lipid metabolism and that the G allele may be associated with insulin resistance in Uygurs.

**DISCUSSION**

Adipose tissues express a variety of secretory proteins of potential importance for metabolic and vascular diseases. The recently described adiponectin is a 244 amino acid adipocyte-derived protein\(^{12}\) derived solely from adipose tissue in humans. However, the levels of adiponectin are paradoxically reduced in obesity, despite the abundance of adipose tissue in obesity.\(^{12}\) Both T2DM and insulin resistance are associated with decreased adiponectin levels.\(^{3,13}\)

Furthermore, administration of adiponectin has been shown to enhance insulin sensitivity in animal models.\(^{14,15}\) These observations suggest that adiponectin plays an important role in the regulation of insulin signalling and insulin sensitivity.

The adiponectin gene is localized to human chromosome 3q27,\(^{7}\) a region identified as a susceptible locus for metabolic syndrome and T2DM in Caucasians.\(^{8}\) Screening for this gene in Japanese\(^{16}\) and Caucasian populations\(^{17}\) has uncovered more than 10 SNP. The SNP45 was significantly associated with risk of T2DM in Japanese,\(^{16}\) although not French, populations.\(^{17}\) In a study based on German populations,\(^{18}\) the +45 SNP was significantly associated with obesity and insulin resistance among subjects without a family history of diabetes. These observations give us a clue that adiponectin SNP45 may play a different role in the pathogenesis of T2DM in various ethnic groups. The present study found that, among Uygurs of the Xinjiang region, the T2DM group had a higher distribution of the TG+GG genotype and G allele frequency than the NGT group. Thus, it is reasonable to speculate that the adiponectin SNP45 polymorphism may be correlated with the prevalence of T2DM in the Uygurs of the Xinjiang region. The G allele may be the ultimate risk factor for T2DM in Uygurs. Further studies are required to determine the mechanism by which SNP45 influences insulin sensitivity and, consequently, the risk of T2DM. It was demonstrated recently that replenishment of adiponectin may ameliorate insulin resistance in animal models of T2DM resulting from a high-fat diet.\(^{18}\) Administration of physiological doses of...
adiponectin lessened triglyceride accumulation in skeletal muscle and liver by facilitating fatty acid combustion and energy dissipation.\textsuperscript{18,19} Therefore, deficiencies in adiponectin would presumably trigger insulin resistance, en route to the development of T2DM. In the present study, patients with T2DM carrying the G allele of SNP45, a putative at risk allele, exhibited much lower plasma adiponectin concentrations than T2DM patients carrying the T allele. This suggests that the SNP45 polymorphism may affect insulin resistance, possibly through changes in mRNA stability, levels of adiponectin and eventually reduced plasma adiponectin concentrations. Our findings are similar those reported by Yang et al.,\textsuperscript{20} who found that subjects the TT genotype at SNP45 was associated with insulin resistance. Menzaghi et al.,\textsuperscript{21} reported that subjects with the TT genotype at SNP276 had a higher serum adiponectin level than subjects with the other genotypes. However, no difference was observed in serum adiponectin levels at baseline among the SNP45 genotypes in that study. Gonzalez-Sanchez et al.,\textsuperscript{22} reported that the G allele of SNP45 was associated with a higher prevalence of IGT. However, this substitution did not result in any change in serum adiponectin concentrations. The G/G genotype of SNP276 of the adiponectin gene resulted in reduced serum adiponectin levels and was associated with increased risk for IGT in Spanish subjects.\textsuperscript{22} These discrepancies among association studies may be explained, at least in part, by differences in sample size or even in the linkage disequilibrium structure at this locus in different populations.

Nevertheless, direct evidence to support SNP45-induced regulation of adiponectin expression is still lacking and warrants further investigation, including direct measurement of adiponectin expression in white adipose tissues obtained from individuals with the SNP45 genotypes mentioned in the present study.

In summary, the adiponectin SNP45 polymorphism may be closely correlated with the prevalence of T2DM in Uygurs of the Xinjiang region in China. The incidence and morbidity of T2DM are expected to be much higher in G allele carriers, which may be underscored by alterations in adiponectin expression and plasma concentrations in these G allele carriers.

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REFERENCES