Adiponectin SNP276 is associated with obesity, the metabolic syndrome, and diabetes in the elderly1–3

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ABSTRACT

Background: Genetic variations of the human adiponectin gene are associated with metabolic phenotypes, including obesity, insulin sensitivity, and diabetes. However, these associations have not been examined in an elderly population.

Objective: The objective of the study was to investigate whether the genetic variants of adiponectin are associated with any metabolic phenotype in the elderly.

Design: In a population-based, case-control genetic association study, a total of 1438 subjects >65 yr old were recruited from the community. The phenotypes of the metabolic syndrome (MetS) were measured. Four single-nucleotide polymorphisms (SNP) were genotyped by mass spectrometry.

Results: The G allele of SNP276 in intron 2 was associated with a reduced risk of obesity, MetS, and diabetes mellitus. The GT genotype relative to the GG genotype had an age- and sex-adjusted odds ratio of 1.32 for obesity [body mass index (BMI; in kg/m²) ≥ 25; P = 0.014] and of 1.33 (P = 0.011) and 1.47 (P = 0.001) for MetS according to modified National Cholesterol Education Program and International Diabetes Federation criteria, respectively. The age-, sex-, and BMI-adjusted odds ratio of diabetes mellitus for the GT and TT genotypes relative to the GG genotype were 1.28 (P = 0.042) and 1.72 (P = 0.013), respectively, and there was an obvious dosage effect (P for trend = 0.004). In linear regression after adjustment for age, sex, and BMI, the GT and TT genotypes were associated with fasting plasma glucose concentrations 5.2 and 11.1 mg/dL higher, respectively, than those of the GG genotype.


KEY WORDS Adiponectin, genetics, metabolic syndrome, obesity, diabetes, elderly

INTRODUCTION

Adiponectin is a plasma glycoprotein of adipose tissue origin (1–4). The plasma concentration of adiponectin was reported to be lower in subjects with the phenotypes of the metabolic syndrome (MetS) (5), including obesity (6–8), type 2 diabetes (9, 10), dyslipidemia (8, 11), and hypertension (12, 13). Lower plasma concentrations of adiponectin also were associated with insulin resistance (7, 8, 10), which is generally taken as the core biological defect of the MetS (14).

Genetic studies in humans also provide strong evidence for the association between adiponectin gene and the MetS (15). Various single-nucleotide polymorphisms (SNPs) of the human adiponectin gene were reported to be associated with obesity (16, 17), type 2 diabetes (18, 19), and insulin sensitivity (16, 20). The association of adiponectin SNPs and dyslipidemia or hypertension was less well explored (15).

Previous genetic studies did not examine these associations specifically in the elderly, who are expected to have more extensive environmental exposure. Whether the genetic effects of adiponectin observed in younger groups can be replicated in an elderly population is an interesting question. In this study, we investigated the association of 4 adiponectin SNPs with the MetS phenotypes among 1438 subjects with a mean age of 71.9 yr.

SUBJECTS AND METHODS

Subjects

The study was performed in Tainan City, located in southern Taiwan, which has a population of ~700,000. The sampling scheme was a stratified systemic cluster sampling of households throughout the city. First, 1 or 2 subdivisions from each of the 7 administration areas of the city were randomly selected. Second, households within each selected subdivision were systematically selected. Third, the selected households were informed about the survey by telephone and letter from the medical center of National Cheng Kung University. Subjects who were ≥65 yr old according to the government population registration in 1999 were included. The study was conducted from November 2000

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through May 2001. Of the 2146 eligible persons, 1438 (801 M, 637 F) agreed to participate.

Written informed consent was obtained from all subjects. The study was approved by the institutional review board of National Cheng-Kung University Hospital.

Biochemical and anthropometric measurements

The subjects were interviewed by trained assistants with the use of a structured questionnaire, which included demographic information and medical history. The questionnaire also included physical activity (type, duration, and weekly frequency of leisure-time physical activity in the past year), smoking (the number of cigarettes smoked and the duration of smoking), and alcohol consumption (types of alcoholic beverage, frequency of consumption per week, and usual amount consumed each time). Total physical activity was calculated by summing the average metabolic equivalents h/wk for each activity during the past year. Cigarette smoking was dichotomized by a cutoff of 10 pack-years. To estimate alcohol consumption, we calculated the average weekly absolute alcohol intake (in g of ethanol). Alcohol consumption was dichotomized by using a cutoff of 60 g/wk for 5 y.

While each subject was sitting, 2 readings of systolic (SBP) and diastolic (DBP) blood pressure were measured after a 15-min rest with the use of a DINAMAP vital sign monitor (model 1846SX; Critikon Inc, Irvine, CA). Height, weight, and waist circumference were measured. Biochemical measurements of glucose and lipids were obtained by using standard protocols as previously described (21).

The World Health Organization’s definition of obesity in Asians as body mass index (BMI; in kg/m2) previously described (21).

Alcohol consumption was dichotomized by using a cutoff of 510 g/wk for 5 y.

Dyslipidemia was defined by fasting triacylglycerol concentrations ≥ 200 mg/dL or history. Hypertension was defined as SBP > 140 mm Hg or DBP > 90 mm Hg or history. The MetS, according to modified National Cholesterol Education Program (NCEP) criteria (23), included any 3 of the following: 1) waist circumference > 90 cm in men and >80 cm in women were used (22, 23). Diabetes was defined by fasting plasma glucose concentrations ≥ 126 mg/dL or by history. The MetS, according to modified National Cholesterol Education Program (NCEP) criteria (23), included any 3 of the following: 1) waist circumference > 90 cm in men and >80 cm in women; 2) triacylglycerol ≥ 150 mg/dL; 3) HDL cholesterol < 40 mg/dL in men and < 50 mg/dL in women. Hypertension was defined by SBP > 140 mm Hg or DBP > 90 mm Hg or history. The MetS, according to modified National Cholesterol Education Program (NCEP) criteria (23), included any 3 of the following: 1) waist circumference > 90 cm in men and >80 cm in women; 2) triacylglycerol ≥ 150 mg/dL; 3) HDL cholesterol < 40 mg/dL in men and < 50 mg/dL in women; 4) SBP ≥ 130 mm Hg or DBP ≥ 85 mm Hg; and 5) fasting plasma glucose ≥ 100 mg/dL. The International Diabetes Federation (IDF) definition of the MetS includes central obesity (waist circumference > 90 cm for men, >80 cm for women) plus any other 2 criteria in the modified NCEP criteria as recently proposed (Internet: http://www.idf.org/home/).

Genotyping

The genomic DNA extraction was performed from peripheral leukocytes by using proteinase K digestion and then phenol-chloroform extraction and alcohol precipitation. Genotyping of the SNPs was performed by using the SEQUENOM genotyping system (Sequenom Inc, San Diego, CA) at the National Genotyping Core Facility of Academia Sinica (Taipei, Taiwan). Briefly, the region harboring the SNP of interest was amplified by using polymerase chain reaction (PCR). The PCR product was used as the template for the following primer extension reaction. Primer for the primer extension reaction was so designed that the 3’ nucleotide was next to or a few nucleotides away from the SNP of interest. The primer extension was performed by using the homogeneous MassEXTEND mix (Sequenom Inc) with the appropriate terminator deoxyribonucleotide (complementary to the SNP of interest) and the other 3 deoxyribonucleotides. The addition of DNA polymerase allowed the extension of the primers to or through the polymorphic site and produced allele-specific extension products. The extension products were then subjected to matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF-MS) to resolve the alleles. Four SNPs [ie, -11426 (rs18681194), -11377 (rs226729), 45 (rs2241766), and 276 (rs1501299)] were arbitrarily chosen for genotyping on the basis of a literature review (15) and a higher allele frequency in the SNP database (Internet: http://www.ncbi.nlm.nih.gov/SNP/).

The success rate of all genotyping was >95%.

Statistical analyses

The data are presented as means ± SDs unless indicated otherwise. The tests of Hardy-Weinberg equilibrium and linkage disequilibrium (LD) were performed with the use of HAPLOVIEW software [version 3.2; Broad Institute, Cambridge, MA (24)]. D’, a statistic that measures the degree of LD between 2 polymorphic markers, was also calculated with the use of HAPLOVIEW software. If both alleles of a polymorphism are randomly associated with any allele of a neighboring polymorphism in a study population, these 2 markers are in complete linkage equilibrium (D’ = 0.0). If one allele of a marker shows up only with a specific allele of the neighboring marker, and the alternative allele shows up only with the second allele of the neighboring marker, these 2 markers are in complete LD (D’ = 1.0). A chi-square test was performed to test the distribution of genotypes. Logistic regression analyses were performed by using SNP276 genotypes for the odds ratios (ORs) of obesity and the MetS with adjustment for age and sex and the ORs for diabetes with adjustment for age, sex, and BMI. Linear regression using BMI as the dependent variable and age and sex as independent variables was performed to estimate the effects of genotypes on BMI. Likewise, linear regression using fasting plasma glucose as the dependent variable and age, sex, and BMI as independent variables was performed to estimate the effect of genotype on fasting plasma glucose.

Further adjustment with lifestyle factors, including smoking, alcohol consumption, and physical activity, in linear regression to estimate the effect of genotype on fasting plasma glucose was also performed. Trends across levels of the SNP276 genotypes were assessed by testing the statistical significance of the trend of a single variable, coded as the category of exposure (1, 2, etc) to estimate the dose-response effect. The difference between the means of 2 groups was tested by using Student’s t test and that among the means of 3 groups was tested by using analysis of variance. P < 0.05 was considered significant, and a P value between 0.05 and 0.1 was considered to be borderline significant. All statistical analyses were performed with STATA software (version 8.0; STATA Corp, College Station, TX).

RESULTS

The basic characteristics of these subjects were shown in Table 1. The subjects ranged in age from 65 to 88 y old. Approximately 56% of the subjects were male. Obesity, central
obesity, diabetes, dyslipidemia, hypertension, and MetS were present in 44%, 69%, 31%, 51%, 80%, and 51% of subjects, respectively. By the recent IDF criteria, the incidence of MetS present in 44%, 69%, 31%, 51%, 80%, and 51% of subjects, respectively. By the recent IDF criteria, the incidence of MetS

TABLE 1
Demographic characteristics of the study subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>71.9 ± 5.0†</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>637</td>
</tr>
<tr>
<td>Women</td>
<td>801</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157.7 ± 8.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.3 ± 11.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.6 ± 3.5</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>91.1 ± 10.4</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>107.8 ± 40.0 (6.0 ± 2.2)†</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>133.4 ± 75.4 (1.5 ± 0.9)†</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>49.7 ± 14.9 (1.2 ± 0.4)†</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>207.6 ± 40.7 (5.2 ± 1.0)†</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>146.4 ± 25.2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>78.2 ± 12.8</td>
</tr>
<tr>
<td>Obesity (%)</td>
<td>44</td>
</tr>
<tr>
<td>Central obesity (%)</td>
<td>69</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>31</td>
</tr>
<tr>
<td>Dyslipidemia (%)</td>
<td>51</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>80</td>
</tr>
<tr>
<td>Metabolic syndrome (%)</td>
<td>51, 46</td>
</tr>
</tbody>
</table>

† ± SD (all such values).
2 Values in parentheses are SI units (mmol/L).
3 Values are according to the criteria of the National Cholesterol Education Program (modified) and the International Diabetes Federation, respectively.

TABLE 2
The genotype frequency of SNP276 by BMI and the odds ratio (OR) for obesity relative to the GG genotype by age- and sex-adjusted logistic regression analysis

<table>
<thead>
<tr>
<th>BMI</th>
<th>n (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25</td>
<td>405 (51.2)</td>
<td>283 (45.7)</td>
</tr>
<tr>
<td>≥25</td>
<td>21 (40.5)</td>
<td>294 (47.5)</td>
</tr>
</tbody>
</table>

† P = 0.029 (chi-square test for genotype distribution).
2 P = 0.014.
TABLE 3
The genotype frequency of SNP276 according to 2 sets of criteria for the metabolic syndrome (MetS) and the odds ratio (OR) of the MetS relative to the GG genotype by age-and sex-adjusted logistic regression analysis.1

<table>
<thead>
<tr>
<th>AGE/SEX</th>
<th>MNCEP</th>
<th>IDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>No (%)</td>
<td>Yes</td>
</tr>
<tr>
<td>Sex</td>
<td>No (%)</td>
<td>Yes</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>352 (51.8)</td>
<td>332 (45.7)</td>
</tr>
<tr>
<td>GT</td>
<td>275 (40.5)</td>
<td>339 (46.6)</td>
</tr>
<tr>
<td>TT</td>
<td>52 (7.7)</td>
<td>56 (7.7)</td>
</tr>
</tbody>
</table>

1 MNCEP, modified National Cholesterol Education Program; IDF, International Diabetes Federation. Genotype distribution: P = 0.056 for MNCEP definition, P = 0.004 for IDF definition (chi-square test).

2 P = 0.001.

3 P = 0.011.

4 P = 0.001.

DISCUSSION
The association of the genetic variations of adiponectin gene with various phenotypes of the MetS and diabetes has been examined in many ethnic populations (15–20), but, before the present study, it had not been investigated in subjects of older age. Subjects who are old are expected to have been more extensively exposed to environmental influences. It is not known whether the environmental influences would dilute the contribution of susceptible genotypes to the phenotypes or, rather, would make the phenotypes conferred by the susceptible genotypes more likely to show up. Therefore, it is interesting to investigate in an elderly group this genetic association that is repeatedly found in groups of younger age. In the present study, we found that, among 1438 subjects aged 65–88 y, the SNP276 in intron 2 was associated with the risk of obesity and MetS independent of age and sex. It was also associated with the risk of diabetes with an additive mode of inheritance, independent of age, sex, and BMI. These results suggest that adiponectin is still a significant genetic contributor to these phenotypes, even in older persons. One limitation of the present study was that, for reasons of cost-effectiveness, not all of the SNPs reported in the literature were genotyped. The SNPs were arbitrarily chosen on the basis of a literature review (15) and their higher allele frequency in SNP database. However, we were able to identify one SNP associated with obesity, MetS, and diabetes, a finding that should be adequate to implicate adiponectin as a genetic contributor to these phenotypes among elderly and thus to answer the question we raised.

In the present study, it was the G allele of SNP276 that was associated with a lower risk of obesity, MetS, and diabetes. In previous studies, both the T and G alleles were said to be associated with obesity or diabetes, even in the same ethnic population (15). This suggests that SNP276 may not have real biological effects and that it is most likely in LD with the responsible variants that harbor the biological effects on the reported metabolic phenotypes. So far, no SNP reported in the genetic association studies has been shown to affect metabolism through specific molecular mechanisms (15). However, some SNPs, including SNP276, were reported to be associated with the variation in adiponectin gene expression in either plasma protein or mRNA (15). Yet, different alleles of the same SNP were both reported to be associated with higher plasma adiponectin concentrations in different studies (15). Recently, among overweight or obese Koreans, SNP276 was associated with different responses in plasma adiponectin concentrations and in the insulin...
The metabolic phenotypes in older subjects may reflect their genetic makeup, unlike the metabolic phenotypes in the much younger control subjects who are commonly used in other genetic association studies. This use of elderly subjects would, in theory, increase the chances of detecting associations between genotypes and phenotypes. Subjects of this age group may therefore serve as the best subjects for population-based, case-control genetic studies of metabolic diseases. The present study also showed that the genetic variations these elderly subjects inherited from their parents at birth > 60 y ago still had significant effects on their metabolic phenotypes, despite extensive environmental exposure. At the same time, the use of an elderly population may have pitfalls. For example, subjects carrying certain genotypes in conjunction with the metabolic phenotypes may not survive to old age, which may render spurious any association of these genotypes with the metabolic phenotypes among elderly.

The authors’ responsibilities were as follows—Y-CY, F-HL, and C-JC: patient recruitment; I-LW and J-YL: genotyping and data collection; C-LC: statistical analysis; W-SY, C-LC, and C-JC: interpretation of the data and writing the manuscript; and W-SY, C-JC, and T-YT: obtaining research funding. None of the authors had a personal or financial conflict of interest.

REFERENCES