

# Influence of Adiponectin Gene Polymorphisms on Adiponectin Serum Level and Insulin Resistance Index in Taiwanese Metabolic Syndrome Patients

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## Abstract

Although mounting evidences have revealed an association between the gene coding for adiponectin and serum adiponectin levels, much controversy still surrounds the association of the adiponectin gene with metabolic traits such as insulin resistance in obesity and type 2 diabetes (T2DM). On the other hand, very few studies have looked into the relations between adiponectin genetic variants and risks of metabolic syndromes (Mets). The present study assessed the influence of two common adiponectin single-nucleotide polymorphisms (SNPs), rs266729 (C-11377G) and rs1501299 (G276T) in the risk of Mets. A community-based population of 137/110 case/control was genotyped by PCR-RFLP, and the levels of serum adiponectin, fasting serum glucose, fasting serum insulin, homeostasis model assessment of insulin resistance (HOMA-IR), uric acid and C-reactive protein of each subject were measured. The distribution of genotypic and allelic frequencies of C-11377G or G276T was not statistically different between the Mets and control groups. However, among the patients with Mets, those carrying GG at C-11377G had a lower level of serum adiponectin ( $P < 0.001$ ), higher levels of fasting serum glucose ( $P = 0.0142$ ), fasting serum insulin ( $P < 0.001$ ) and HOMA-IR ( $P < 0.001$ ) compared with those carrying the CC or CG genotype. Our data suggest that subjects who carry the homologous GG genotype at C-11377G of the adiponectin gene may be of higher risk of Mets and should be monitored more closely with other serum biochemical indexes.

**Key Words:** adiponectin, polymorphism, insulin resistance, metabolic syndrome

## Introduction

Evidences based on observation and intervention studies have indicated that the quantity and quality of dietary fats influence insulin resistance (19, 22). Adiponectin is an adipokine that is specifically and abundantly expressed in the adipose tissue and sensitizes the body to insulin (4, 33). Hypoadiponectinemia, caused by interactions of genetic and environmental factors, appears to play an important causal role in insulin resistance in type 2 diabetes (T2DM) and metabolic syndromes (Mets) (12, 14, 23, 25, 32).

Adiponectin, a protein produced and secreted by adipocytes, influences the body's response to insulin. The protein is encoded by the *ADIPOQ* gene located on chromosome 3q27, which has been reported to be linked to T2DM and Mets (27). Several single-nucleotide polymorphisms (SNPs) in the *ADIPOQ* gene have been shown in Japanese and European populations to be associated with diabetes (9, 10, 27) or insulin resistance syndrome (17). In French subjects, 2 SNPs in the promoter region of the *ADIPOQ* gene, C-11377G (rs266729) and SNP G-11391A (rs17300539), were significantly associated with hypoadiponectinemia and T2DM (27). In Korean subjects without diabetes, SNPs G276T and T45G have been associated with various levels of adiponectin as well as body mass index (BMI), fasting insulin concentration and homeostasis model assessment of insulin resistance (HOMA-IR) (13, 27). Taken together, the experimental and observational data support the hypothesis that adiponectin plays an important role in the pathogenesis of Mets. However, a previous work on *ADIPOQ* SNPs showed that the SNPs associated with Mets or circulating adiponectin levels differed according to both the study cited and the ethnic population studied (18). In this study, we investigated the effects of two well characterized SNPs of *ADIPOQ* on circulating adiponectin levels and insulin resistance indexes in a Taiwanese population. Based on the data obtained, a mechanism for adiponectin-associated metabolic syndromes is proposed.

## Materials and Methods

### *Study Population and Sampling Method*

This was a community-based case-control study that included subjects selected from a random sample of Taichung population, Taiwan. There were a total of 363,543 residents aged 40 or over 40 in this area and about 4.09% of the national population was of the same age. A two-stage sampling design was used to select the participants, with the

sampling rate proportional to size within each stage. A total of 4,280 individuals were selected for further recruitment for the present study. During household visits, a total of 3,530 subjects were found eligible for the study, and 2,359 agreed to participate and to provide complete information. The overall response rate was 66.83%. Among them, 137 cases with Mets and 110 subjects without any component of Mets had their genotypes of polymorphisms analyzed.

### *The Metabolic Syndrome*

Mets was defined clinically, based on the presence of three or more of the following American Heart Association (AHA) Mets criteria with some modifications specific for oriental people (modified NCEP ATP III): [1] fasting plasma glucose  $\geq$  100 mg/dl, [2] serum triglycerides  $\geq$  150 mg/dl, [3] serum high-density lipoprotein cholesterol (HDL-C)  $<$ 40 mg/dl in men and  $<$ 50 mg/dl in women, [4] blood pressure  $\geq$  130/85 mmHg and [5] waist circumference  $>$ 90 cm in men and  $>$ 80 cm in women.

### *The Serum Insulin, HOMA-IR, and Adiponectin*

The serum insulin level was measured by a commercial enzyme-linked immunosorbent assay kit (Diagnostic Products, Los Angeles, CA, USA). Insulin sensitivity was estimated with a HOMA-IR equation, which times the fasting serum insulin (mU/ml) with the fasting plasma glucose (mM), and then divided by 22.5 (5, 16). The serum adiponectin was measured by the common method Human Adiponectin RIA Kit HADP-61HK (LICAO Ltd, Hong Kong) as previously described (8, 31).

### *Genotyping Assays*

Genomic DNA was prepared from peripheral blood leucocytes using a QIAmp Blood Kit (Qiagen, Chatsworth, CA, USA). The standard PCR-RFLP genotyping procedure was performed as previously published (1-3, 7, 11, 15, 30). Briefly, the following primers were used for *ADIPOQ* rs266729 (C-11377G): 5'-GCTCTGTGTGGACTGTGGAG-3' as the forward and 5'-AGAAGCAGCCTGGAGAACTG-3' as the reverse primers; and *ADIPOQ* rs1501299 (G276T): 5'-TGGTCCTTTGGTGCTGAGTT-3' as the forward and 5'-TACCCAAGCTTTGCTTTCT-3' as the reverse primers. The following cycling conditions were performed: one cycle at 95°C for 5 min; 35 cycles at 95°C for 30 s, 62°C for 30 s and 72°C for 30 s, and a final extension at 72°C for 10 min. The PCR products were then digested with *BsmI* and further identified in 3% agarose gel electrophoresis.

**Table 1. Demographic data of subjects with (cases) and without metabolic syndrome (controls)**

	Cases n = 137	Controls n = 110	P-value
Age (year)	56.6 ± 10.2	55.2 ± 10.7	0.3218
Male/Female	62/75	52/58	0.7165
Weight (kg)	72.29 ± 7.75	57.63 ± 5.74	< 0.001*
BMI (kg/m <sup>2</sup> )	27.3 ± 2.3	22.1 ± 1.4	< 0.001*
Waist Circumference (cm)	90.8 ± 6.7	74.6 ± 5.8	< 0.001*
Fasting Blood Glucose (mg/dl)	125.0 ± 4.7	91.8 ± 3.1	< 0.001*
HDL Cholesterol (mg/dl)	37.2 ± 3.8	56.9 ± 1.3	< 0.001*
Triglycerides (mg/dl)	194.8 ± 47.4	68.8 ± 26.4	< 0.001*
Systolic Blood Pressure (mmHg)	148.5 ± 19.9	116.9 ± 7.5	< 0.001*
Diastolic Blood Pressure (mmHg)	87.4 ± 11.5	69.8 ± 7.0	< 0.001*
Fasting Insulin (μU/ml)	13.9 ± 2.1	6.1 ± 0.9	< 0.001*
HOMA-IR	4.3 ± 0.7	1.4 ± 0.3	< 0.001*
Adiponectin (μg/ml)	6.8 ± 0.6	11.8 ± 1.0	< 0.001*
Uric Acid (mg/dl)	6.3 ± 0.6	5.3 ± 0.4	< 0.001*
C-Reactive Protein (mg/dl)	0.31 ± 0.30	0.17 ± 0.16	0.0062*
Smoking	47	27	0.0843
Drinking	36	33	0.1814
Areca Chewing	8	7	0.4627

Abbreviations: BMI, Body mass index; HDL, High-density lipoprotein; HOMA-IR, Homeostasis model assessment of insulin resistance; \* indicates statistically significance with  $P$ -value < 0.05.

### Statistical Analyses

Continuous variables were reported as means ± standard deviation (SD) and categorical variables were reported as percentage (95% confidence intervals, CI). Differences in proportions and means were assessed using a  $\chi^2$  test or unpaired  $t$ -test. Hardy-Weinberg equilibrium statistic was also calculated. Using multiple logistic regression models, the relationship of *ADIPOQ* polymorphisms separately with Mets risks after adjusting for all the other covariates was determined. The covariates included age, education, cigarette smoking and alcohol consumption. General linear model was used to compare adjusted mean values of insulin and HOMA-IR between wild-type and variant types. All outcome  $P$ -values were of two-sided tests, and the data considered statistical significant was set at  $P < 0.05$ . All analyses were performed using SAS version 9.1 (SAS Institute Inc, Cary, NC, USA).

### Results

The demographic data of the subjects with Mets (cases) and without Mets (controls) are shown in Table 1. The average age and gender ratios in the subjects with and without Mets were 56.6 ± 10.2 and 55.2 ± 10.7 years old, and the male/female ratio was 62/75 and 52/58, respectively, which were not sig-

nificantly different ( $P > 0.05$ ). Individuals with Mets had significantly higher levels of weight, BMI, waist circumference, fasting blood glucose, triglycerides, systolic blood pressure, diastolic blood pressure, fasting serum insulin, HOMA-IR, uric acid, C-reactive protein and lower levels of HDL cholesterol and adiponectin ( $P < 0.05$ ) than those without Mets. As for the smoking, drinking and areca chewing habits, there was also not statistically significant ( $P > 0.05$ ).

The genetic and allelic frequencies of *ADIPOQ* rs266729 (C-11377G) and rs1501299 (G276T) among the cases and the controls are shown in Table 2. There was no differential distribution of genetic or allelic frequencies in either the *ADIPOQ* rs266729 (C-11377G) or rs1501299 (G276T) polymorphic sites between the cases and the control group ( $P > 0.05$ ).

Associations of these two SNPs with the levels of Mets related parameters, such as serum adiponectin, fasting blood glucose, fasting blood insulin, HOMA-IR, uric acid and C-reactive protein were further examined. The results stratified with the genotypic types of *ADIPOQ* rs266729 (C-11377G) in the cases are shown in Table 3. It is found that there was an obvious decreased level of adiponectin in subjects with the homologous GG genotype compared with subjects with CC or CG genotypes ( $P < 0.001$ ). At the same time, increased levels of fasting blood glucose ( $P = 0.0142$ ), fasting serum insulin ( $P < 0.001$ ) and HOMA-IR ( $P < 0.001$ ) in subjects with the GG genotype

**Table 2. Genetic analysis of the *ADIPOQ* gene between case and control groups**

<i>ADIPOQ</i> SNP	Cases n = 137 (%)	Controls n = 110 (%)	<i>P</i> -value
<i>ADIPOQ</i> -11377			
Genetic Frequency			
CC	77 (56.2)	64 (58.2)	0.8342
CG	50 (36.5)	40 (36.4)	
GG	10 (7.3)	6 (5.5)	
Allelic Frequency			
C	204 (74.5)	168 (76.4)	0.6245
G	70 (25.5)	52 (23.6)	
<i>ADIPOQ</i> 276			
Genetic Frequency			
TT	60 (43.8)	44 (40.0)	0.5245
TG	49 (35.8)	47 (42.7)	
GG	28 (20.4)	19 (17.3)	
Allelic Frequency			
T	169 (61.7)	135 (61.4)	0.9429
G	105 (38.3)	85 (38.6)	

**Table 3. Differences in the genetic variants of *ADIPOQ* -11377 and related biochemistry characteristics in the case groups**

<i>ADIPOQ</i> -11377	CC+CG n = 127	GG n = 10	<i>P</i> -value
Serum Adiponectin ( $\mu\text{g/ml}$ )	7.88 $\pm$ 1.85	5.17 $\pm$ 0.51	< 0.001*
Fasting Blood Glucose (mg/dl)	122.04 $\pm$ 9.04	129.20 $\pm$ 2.78	0.0142*
Fasting Insulin ( $\mu\text{U/ml}$ )	12.48 $\pm$ 1.20	15.91 $\pm$ 1.03	< 0.001*
HOMA-IR	4.10 $\pm$ .023	4.72 $\pm$ 0.21	< 0.001*
Uric Acid (mg/dl)	6.18 $\pm$ 1.28	6.45 $\pm$ 0.13	0.5131
C-Reactive Protein (mg/dl)	0.29 $\pm$ 0.06	0.32 $\pm$ 0.02	0.1133

Abbreviations: HOMA-IR, Homeostasis model assessment of insulin resistance; \*: Statistically significance with *P*-value < 0.05.

than subjects with the CC or CG genotype were also observed. As for *ADIPOQ* rs1501299 (G276T), the differences detected among the patients with Mets were not statistically significant (data not shown).

### Discussion

To our knowledge, this is a pilot study concerning the relationship between genetic variants of *ADIPOQ* and clinical and biochemical parameters performed between cases with Mets and control groups and among the target group patients. No differential distribution of either genetic or allelic frequency of *ADIPOQ* C-11377G or *ADIPOQ* G276T between subjects with Mets and the control groups was observed. Interesting, only in the Mets patients, but not in all the subjects investigated, have we observed that

the *ADIPOQ* C-11377G promoter region variants were significantly associated with insulin sensitivity, and that the GG genotype of *ADIPOQ* C-11377G was associated with lower serum levels of adiponectin, resulting in higher blood fasting glucose and insulin levels, leading to higher risks of Mets. Similar results were not found in the *ADIPOQ* G276T SNP which is located in the intron region. Our working hypothesis is shown in Fig. 1.

The minor allele at the C-11377G SNP has previously been associated with higher BMI values in patients with T2DM than with C/C T2DM subjects, suggesting that this SNP may contribute to higher genetic risk for obesity in T2DM (9). Another study in a French population reported that the GG genotype was associated with low adiponectin concentrations and contributed to the genetic risk for T2DM, even

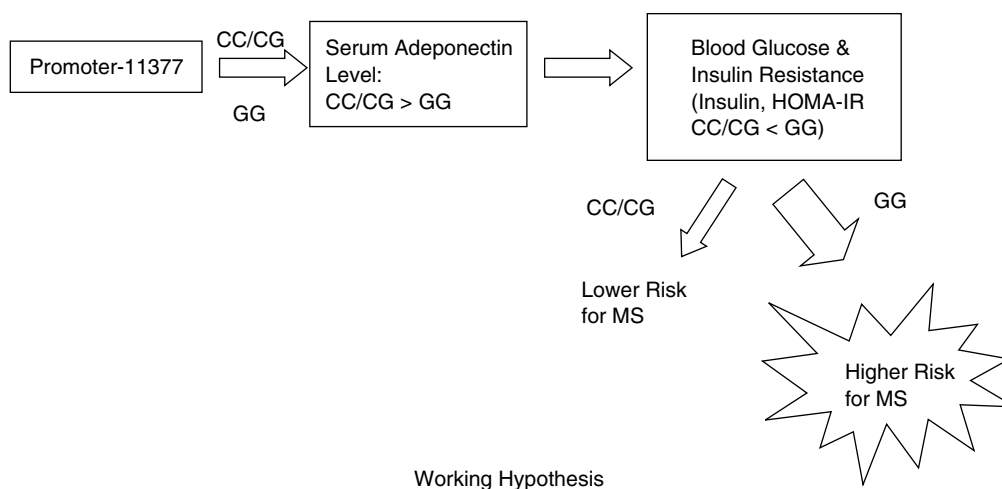


Fig. 1. A model of working hypothesis. This model exhibits the hypothesis that subjects of the GG genotype at promoter -11377 of the adiponectin *ADIPOQ* gene may have lower level of serum adiponectin, which leads to higher glucose in blood and higher insulin resistance than in subjects with the CC or CG genotypes. All these characteristics may be associated with higher risks for metabolic syndromes.

though they did not find a positive association between this SNP and insulin resistance (27). Moreover, Buzzetti *et al.* (6) observed that subjects carrying the -11377G variant had a lower insulin sensitivity and lower plasma adiponectin concentrations than non-carriers. However, other studies did not find any association between this *ADIPOQ* variant and insulin sensitivity as evaluated by hyperinsulinemic-euglycemic clamp (24, 29).

Small differences in adiponectin levels can have clinical significance. Our findings that a small increase in adiponectin along with a decrease in both fasting insulin and glucose levels in subjects with Mets carrying the GG genotype is similar with a previous report of the insulin-sensitizing effects of adiponectin focusing on *ADIPOQ* T45G (26). We proposed that the haplotype or an unknown *ADIPOQ* C-11377G locus nearby functional variant in linkage disequilibrium could decrease adiponectin levels and, consequently, insulin sensitivity in patients with metabolic syndrome. Results obtained on insulin-related phenotypes after adjusting for adiponectin levels supported this conclusion. Thus, the influence of the *ADIPOQ* SNPs on those insulin-related phenotypes may be fully explained by their influences on serum adiponectin levels. These data corroborate those obtained in adult obese subjects from different ethnic groups (10, 17, 27). In addition, the results for the *ADIPOQ* C-11377G analysis and the extent of decrease in systolic blood pressure and fasting plasma glucose was greater in the subjects with the CG or GG genotype than in those with the CC genotype (26). This finding is similar to our findings that genetic variants had differential effects on metabolic traits, such as glucose status in plasma. Other studies using

childhood populations support this hypothesis (21, 27, 28). Petron *et al.* (21) found that heterozygous and homozygous G alleles at the -11377 locus showed higher levels of fasting glucose, fasting insulin, homeostasis model assessment-IR index and triglyceride, and lower adiponectin levels compared with the C homozygotes in overweight/obese Italian children. Furthermore, Vasseur *et al.* (27, 28) found that the -11,391G/-11,377G haplotype was associated with low plasma adiponectin levels and T2DM, even though they failed to detect an association with the IR index.

Two major limitations of the present study include the small sample size for the heterogeneous and complex disease, Mets, and the weakness to present for the whole population with Mets. The small sample size may cause false positive findings, while the heterogeneity of the subjects may cause false negative findings. We have compared the proportion of the major allele of *ADIPOQ* C-11377G (74.5%) with the recorded data in the NCBI website ([http://www.ncbi.nlm.nih.gov/projects/SNP/snp\\_ref.cgi?rs=266729](http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=266729)) and found that there was no significant difference with the proportions of Asia studies (68.8~71.1%), although the allele frequency of the population we investigated was a little higher than other studies. Some conflicting findings among the present and previous studies can be due to several factors such as insufficient analyzing power, heterogeneity in the study population heterogeneity (*i.e.* the definition of lean or obese was not restricted enough) and subgrouping of the data with limited samples, or LD between investigated SNPs. The insufficiency to compare the distribution of genotypes among patients in different clinical conditions could be owed due to because of the limited samples with clinical

measurements. Lastly, the reason why we did not compare our findings with other studies was partly attributed to differences in allelic association with disease phenotype in various populations. It is worthwhile to study the relationship of *ADIPOQ* C-11377G and other SNPs with differential diet or drug treatment for metabolic syndromes (20).

In conclusion, our results suggested that the GG genotype of *ADIPOQ* C-11377G is related to a lower serum level of adiponectin leading to higher blood fasting glucose and insulin levels, and higher risks of Mets.

Declaration of Competing Interests: none declared.

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