INTRODUCTION
The occurrence of the metabolic syndrome (MS) is increasing rapidly. It is becoming more prevalent worldwide, particularly visceral obesity, with a corresponding impact on global incidence rates of type 2 diabetes and cardiovascular disease. Thailand has gone through a period of epidemic transition, and many metabolic risk factors, including obesity, dyslipidemia, insulin resistance and hypertension, are now common among the Thai population. Adiponectin is an adipocyte-specific secreted protein that has been shown to have anti-inflammatory, anti-diabetic, and anti-atherogenic properties. Adiponectin is likely to reduce obesity, type 2 diabetes and cardiovascular disease. It has been estimated that between 30-70% variability in adiponectin levels is determined by genetic factors. However, evidence for the association of adiponectin gene (ADIPOQ) polymorphism, including -11377C>G (rs266729) in the promoter region, and +45T>G (rs2241766) in exon 2, with adiponectin concentrations and MS is inconsistent. Moreover, the relationship between ADIPOQ -11377C>G & +45T>G polymorphisms and MS among Thais has not been investigated to date. The present study investigates the association between ADIPOQ polymorphisms with adiponectin levels that may represent one of the several mechanisms connecting ADIPOQ and MS. Therefore, this study aimed to evaluate adiponectin levels and biochemical parameters in MS subjects and healthy controls, and to determine whether two ADIPOQ polymorphisms (-11377C>G and +45T>G), are linked to plasma adiponectin levels and MS among Thais.

MATERIALS AND METHODS

Study subjects
The protocol was approved by the Ethics Committee of Rangsit University. All subjects agreeing to participate signed a consent form. The study was performed with 332 Thai volunteers from suburban and urban residential areas in Bangkok, Thailand, aged between 24 to 64 years old. Among them, 164 were MS subjects and 168 were healthy controls. The study also sought to identify links between two polymorphisms, -11377C>G (rs266729) and +45T>G (rs2241766) of the adiponectin gene, in relation to adiponectin levels and the metabolic syndrome. Three hundred and thirty-two Thai volunteers: 164 metabolic-syndrome subjects and 168 healthy control subjects were investigated. The adiponectin and HDL-C levels of the metabolic-syndrome group were significantly lower than the control group ($p<0.001$). Decreased concentration of adiponectin was associated with -11377C>G polymorphism ($p<0.001$); this polymorphism was significantly more frequent in the metabolic-syndrome group than in the control group ($p<0.001$). However, +45T>G polymorphism of the adiponectin gene was found not to be related to adiponectin level or metabolic syndrome. Therefore, -11377C>G polymorphism was related to the metabolic syndrome susceptibility, and this polymorphism impacted on circulating adiponectin concentrations among Thais.

Key Words: metabolic syndrome, visceral obesity, adiponectin, gene polymorphism, Thais
healthy controls. A physical examination and medical history check on all subjects was performed; those with a history of liver, kidney and cardiovascular diseases, were excluded from the study. The metabolic syndrome was defined using the modified NCEP/ATP III criteria.10 The new cut-off on waist circumference in the Asia and Pacific Region was used, instead of the original cut-off for waist circumference in the ATP III criteria. Adoption of the new cut-off point for fasting plasma glucose criterion has already been reported (>100 mg/dL). The modified NCEP/ATP III definition required at least three of the following:

- Raised waist circumference: >90 cm in Asian men and >80 cm in Asian women
- Raised triglyceride (TG) levels: >150 mg/dL
- Reduced high-density lipoprotein cholesterol (HDL-C): <40 mg/dL in men and <50 mg/dL in women
- Raised blood pressure: systolic blood pressure >130 mmHg or diastolic blood pressure >85 mmHg, or current antihypertensive medication
- Raised fasting plasma glucose: >100 mg/dL

Measurement of biochemical markers

Ten milliliters of overnight fasting venous blood were taken from each subject. Plasma adiponectin and insulin levels were measured using a radioimmunoassay kit from Linco Research (St Louis, USA). The sensitivity of the adiponectin assay was 0.8 ng/mL. Glucose, total cholesterol (TC), HDL-C, and TG were measured using enzymatic methods by DADE Dimension®.

Anthropometric and blood pressure measurements

Anthropometric measurements comprising weight, height, waist circumference, and hip circumference, were recorded. The body weight of each individual dressed in light clothing was measured using a carefully calibrated beam balance (Detecto®). The height of each individual was measured using a vertical measuring rod. The body mass index (BMI) was expressed as weight (kg) / height (m²). Blood pressure (BP) was measured by a nurse after 5 to 10 minutes’ rest in the sitting position.

Genotyping of adiponectin gene polymorphisms

Genotyping was performed using the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) technique. DNA was extracted from the peripheral leukocytes in EDTA-treated whole blood using a Flexi Gene DNA Kit (Qiagen, Hilden, Germany). Genotypes of single nucleotide polymorphisms (SNPs) +45T>G and -11377C>G were amplified using PCR (PF Applied Biosystems, USA). The -11377C>G polymorphic locus was amplified using the forward primer 5'-ACTTGCCCTGCCTGTCTG-3' and the reverse primer, 5'-CCTGGAGAATCGGAAGCTG-3'. The forward primer, 5'-GAAGTACCTCCTGCTGAGATG-3', and the reverse primer, 5'-TATCAGTGAGGGCTCTTGATG-3', were used to amplify a position of the +45T>G. Amplification was carried out in a total 50 μl reaction mix, containing: 100 ng of genomic DNA; 2 mM MgCl2, 0.2 mM deoxynucleotide triphosphates; 1 μM each primer; and 1 unit Taq DNA polymerase and 10 mM Tris-HCl (pH 8.3). PCR was performed by initial denaturation at 94°C for 5 min, then 32 cycles with 30 s of denaturation at 94°C, 30 s of annealing at 60°C, and extension at 72°C for 30 s, followed by a terminal extension for 7 min. PCR products were digested overnight with different restriction enzymes. We used 10 U of Smal as well as HhaI restriction endonuclease for SNP +45 and for SNP -11377, respectively. Digestion products were separated by 2.5% agarose gels stained with ethidium bromide.

Statistical analysis

The statistical software program SPSS 10.0 for Windows (SPSS Inc, Chicago, Illinois, USA) was used to analyze individual parameters detected in the healthy controls and MS subjects. These were then compared using the Mann-Whitney U Test (two-tailed). Statistical differences between groups, in terms of genotypic frequency, were assessed by chi-square test. The median and 95% confidence interval (CI) were calculated. The Minitab statistical computer program was used to calculate the odds ratio (OR). To assess links between MS as a dependent variable and other potential factors, logistic regression was

<table>
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<tr>
<th>Table 1. Median and 95% CI of age, anthropometric variables, and biochemical parameters between control and MS groups</th>
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<tbody>
<tr>
<td>Control (n=168)</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>Waist (cm)</td>
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<tr>
<td>Waist/hip ratio</td>
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<tr>
<td>Glucose (mg/dL)</td>
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<tr>
<td>TC (mg/dL)</td>
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<tr>
<td>TG (mg/dL)</td>
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<tr>
<td>HDL-C (mg/dL)</td>
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<tr>
<td>Insulin (µU/mL)</td>
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<tr>
<td>Adiponectin (µg/mL)</td>
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<tr>
<td>Systolic BP (mmHg)</td>
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<td>Diastolic BP (mmHg)</td>
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*p<0.01 by Mann-Whitney U test (2-tailed)
Adiponectin was related to adiponectin pkipnre shown in biochemical parameters with +45T>G polymorphism, were no significant to the covariates the SNP circumstance and waist/hip ratio were associated with analysis. When SNP biochemical parameters we performed logistic regression spearman’s rank correlation test results a control group. All of the anthropometric data differed cose, and HDL Medians (95% CI) for age, anthropometric parameters, in RESULTS was tested using the Hosmer goodness of fit test. A value <0.05 was considered statistically significant. Goodness-of-fit of the logistic regression models was tested using the Hosmer-Lemeshow test.

### RESULTS

Medians (95% CI) for age, anthropometric parameters, and biochemical markers in the MS and control subjects are shown in Table 1. The age difference between the MS and control groups was not significant. The adiponectin and HDL-C levels of the MS group were significantly lower than the control group (p<0.001). Meanwhile, glucose, insulin, TC, TG, systolic BP, and diastolic BP, increased significantly in the MS group, compared with the control group. All of the anthropometric data differed significantly between the MS and control subjects. Spearman’s Rank correlation test results are shown in Table 2. Adiponectin level positively correlated with HDL-C level, but correlated negatively with age, BMI, waist circumference, waist/hip ratio, glucose, TG, insulin, systolic BP, and diastolic BP.

To evaluate the associations of gene polymorphisms (-11377C>G and +45T>G) with anthropometric and biochemical parameters we performed logistic regression analysis. When SNP -11377C>G was used as a dependent variable, the results showed that decreased concentration of adiponectin and increased levels of TG, BMI, waist circumference and waist/hip ratio were associated with the SNP -11377C>G (p<0.05), after adjusting the variable to the covariates age, gender and smoking status. When SNP +45T>G was used as a dependent variable, there were no significant associations of anthropometric and biochemical parameters with +45T>G polymorphism, after adjusting the variable to the covariates; the results are shown in Table 3. The distribution of -11377C>G and +45T>G genotypes was in line with the Hardy-Weinberg principle (p=0.05). Test results for linkage disequilibrium found Lewontin’s coefficient [D’] and correlation coefficient [r²] between -11377C>G and +45T>G SNPs were 0.125 and 0.0061, respectively.

The genotype frequencies of the study subjects are shown in Table 4. At the SNP +45T>G, 48.8% of subjects with TG/GG genotypes had MS. This SNP of the ADIPOQ showed no significant link to MS (p=0.080). However, SNP -11377C>G was significantly related to MS (p<0.001). The CG/GG genotype frequencies in the MS and control groups were 57.3% and 34.5%, respectively.

Logistic regression and odds ratios (OR) results, for possible associations between MS and age, sex, waist/hip ratio, hyperadiponectinemia (at cut-off point <7.5 μg/mL),1 SNPs +45T>G, and -11377C>G of the ADIPOQ, are shown in Table 5. Three variables, waist/hip ratio (OR=12.46, p=0.001), SNP -11377C>G (OR=2.10, p=0.042) and hyperadiponectinemia (OR=0.17, p<0.001) were significantly statistically related to MS. The Hosmer and Lemeshow goodness-of-fit test (χ²=10.2, p=0.251) was not statistically significant, meaning the fit between the predictive model and the data was acceptable.

### DISCUSSION

The current study found that MS subjects had lower adiponectin concentrations than the control group, and that -11377C>G polymorphism was related to adiponectin concentration and MS susceptibility among Thais. Our
results were consistent with previous studies, in Mexico City subjects, and a Korean prospective cohort study where MS subjects had lower adiponectin concentrations than non-MS subjects. The study of Calcatera et al., however, did not make this observation. In agreement with other reports in Croatian-origin subjects and Korean individuals, we found that hypoadiponectinemia was associated with MS; adiponectin levels were positively correlated with HDL-C but were negatively correlated with BMI, waist circumference and triglyceride. Adiponectin is an adipose-derived protein, with multivalent functions including anti-atherogenic, insulin-sensitizing and lipid-oxidation enhancing activities. Adiponectin levels have shown a significant inverse association with adiposity. Medina-Bravo et al found that obese children had lower adiponectin concentrations than non-obese subjects. Adiponectin has a down-regulation effect in relation to weight gain, and it is possible that an accumulation of visceral fat might produce inhibiting factors for adiponectin synthesis or secretion, such as TNF-α. However, the molecular mechanisms establishing the relationship between adiponectin and metabolic derangements observed in clinical and epidemiological studies have not been fully elucidated. One plausible explanation is that obesity, mainly visceral, is a condition in which there is an inflammatory state characterized by dysregulated production of adipokines such as decreased adiponectin. As such, it seems reasonable to observe the negative association between adiponectin concentration and obesity indices. BMI was the most widely used obesity indicator, but it seems that the best measure is waist circumference because it is used as a valid marker of central obesity, and subjects with central obesity have been suggested to be more prone to develop MS than lean subjects. There are other possible explanations for the association between adiponectin and HDL-C. It has been shown previously that the effects of adiponectin on HDL appear to be mediated by decreased HDL catabolism, rather than higher synthesis and inhibition of hepatic lipase activity. On this basis, it seems reasonable to observe a positive association between adiponectin concentrations and HDL-C in our study. Our study confirmed that plasma concentration of adiponectin may be a biomarker for MS.

This is the first report that has set out to determine the relationship between two SNPs, -11377C>G and +45T>G, and adiponectin levels and MS among Thais. The adiponectin gene is localized on chromosome 3q27. SNP +45T>G of ADIPOQ in exon 2, as well as SNP -11377C>G in the promoter region, have been studied intensively. Meta-analysis has shown that the links between these two polymorphisms, -11377C>G and +45T>G, and cardiovascular disease is significant. Fumeron et al reported the link between +45T>G and increased risk of conversion from normglycemia to diabetes or impaired fasting glucose. Previous studies also suggested that inherited factors play a role in determining adiponectin levels, and that adiponectin concentrations varied by race. Our results confirm that the SNP -11377C>G is associated with adiponectin as well as TG levels and obesity indices, consistent with results of previous studies in Taiwanese subjects, Italian children and Danish women. For example, the G allele of the SNP -11377 was found to be associated with low adiponectin and the G allele being associated with high triglyceride levels and the central to peripheral fat mass ratio. In contrast, studies of Croatian-origin subjects, European individuals, and in the Japanese population have been reported no association between the -11377C>G polymorphism and low adiponectin levels. However, few studies have investigated the relationship between ADIPOQ polymorphism and risk of MS. Regarding links between SNP -11377C>G and MS, early studies reported varying results. Our findings in Thais are consistent with previous reports of subjects of Croatian origin, specifically, that subjects with -11377C>G polymorphism had a higher risk of MS. On the other hand, there were no associations of -11377C>G polymorphism with MS in Taiwanese subjects, and with any clinical features of MS in the Japanese population. The results

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<th>Table 4. Genotype frequencies of SNPs +45T&gt;G and -11377C&gt;G (n=332)</th>
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<tr>
<td><strong>SNP +45T&gt;G</strong></td>
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<tr>
<td>MS (n=164)</td>
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<tr>
<td>TG and GG</td>
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<tr>
<td>TT</td>
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<tr>
<td>SNP -11377C&gt;G</td>
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<tr>
<td>CG and GG</td>
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<td>CC</td>
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1 Pearson chi-square, *p < 0.01

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<th>Table 5. Logistic regression analysis when MS was used as a dependent variable, and age, sex, waist/hip ratio, hypoadiponectinemia, SNPs +45T&gt;G and -11377C&gt;G, were taken as independent variables</th>
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<tr>
<td><strong>Variables</strong></td>
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<tr>
<td>Gene -11377C&gt;G</td>
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<tr>
<td>Hypoadiponectinemia</td>
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<td>Waist/hip ratio</td>
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*p<0.01
from our study relate to the SNP -11377C>G, and suggest that this SNP -11377C>G of the ADIPOQ in the promoter site may play a functional role in regulating adiponectin processing, possibly via changes in the DNA binding activity interfering with transcription-factor binding sites.28 This polymorphism may affect circulating adiponectin concentrations, as well as the development of MS in Thais.

Regarding the association between SNP +45T>G and plasma adiponectin, our study confirmed no significant association between this polymorphism and plasma adiponectin; this was similar to studies among blacks and whites, Europeans, and Japanese.6 On the other hand, there was a significant association between +45T>G polymorphism and plasma adiponectin values in healthy Caucasians.30 Regarding the relationship between SNP +45T>G and MS, some previous studies have reported inconsistent results.7,31-32 Our results are in line with studies that reported that the +45T>G polymorphism of the ADIPOQ is not associated with MS among Asian Indians5 or Malaysians.32 Furthermore, the study among Taiwanese subjects showed no conclusive evidence regarding a link between SNP +45T>G with those quantitative traits related to MS.31 In contrast, the study among Han Chinese in Sichuan Province, China, showed that SNP +45T>G was significantly linked to MS.33 The +45T>G located in exon 2 of the ADIPOQ is a silent mutation for Gly15 (GGT to GGG). The results from our study regarding SNP +45T>G therefore suggest this polymorphism is not related to adiponectin concentrations or MS among Thais. However, the reason for discrepant results between the present report and previous studies may result from the different genetic background of the study populations. The etiology of MS involves several factors, including genetics and lifestyle. However, the molecular mechanisms underlying the pathophysiology of MS are still far from being fully understood. Our study among Thais found associations between MS and adiponectin levels and SNP -11377C>G. It is possible that decreased plasma adiponectin concentrations may play a significant role in the development of MS, and screening for a common genetic background of hypoadiponectinemia may provide useful information concerning the management and assessment of MS.

In conclusion, this study among Thais found MS subjects had lower adiponectin levels than the controls, that SNP -11377C>G was linked to adiponectin concentration, and influences susceptibility to MS, However, our data failed to detect any significant association between SNP +45T>G and adiponectin level, or MS, among the Thais under study.

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AUTHOR DISCLOSURES

All authors have no conflicts of interest to declare.

REFERENCES


Original Article

Association of adiponectin gene -11377C>G polymorphism with adiponectin levels and the metabolic syndrome in Thais

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泰國人的脂聯素基因-11377C>G 多型性與脂聯素濃度和代謝症候群之關聯性

代謝症候群與增加心血管疾病及第二型糖尿病罹患風險具有相關性。脂聯素是一種由脂肪細胞分泌的蛋白質，具有促進胰島素敏感性及抗動脈粥狀硬化特性。本篇研究目的為評估代謝症候群患者與健康對照者的脂聯素濃度及生化參數。另外也探討兩個脂聯素基因 -11377C>G(rs266729) 與 +45T>C(rs2241766) 的多型性與脂聯素濃度和代謝症候群間的關聯性。332 名泰國志願者：164 名代謝症候群患者與 168 名健康對照者為研究對象。代謝症候群組的脂聯素與 HDL-C 濃度顯著低於對照組(p<0.001)。較低的脂聯素濃度與 -11377C>G 基因多型性有關(p<0.001)；此多型性在代謝症候群組比例顯著多於對照組(p<0.001)。然而，脂聯素基因+45T>C 的多型性與脂聯素濃度或是代謝症候群沒有相關性。因此，-11377C>G 多型性與代謝症候群的易感受性有關，而這個多型性影響泰國人血液循環中的脂聯素濃度。

關鍵字：代謝症候群、內臓型肥胖、脂聯素、基因多型性、泰國人