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ASSOCIATIONS OF RESISTIN LEVELS WITH RESISTIN GENE POLYMORPHISM AND METABOLIC SYNDROME IN THAIS

ASOCIJACIJA NIVOA REZISTINA SA POLIMORFIZMOM GENA ZA REZISTIN I METABOLIČKIM SINDROMOM KOD TAJLANĐANA

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Summary

Background: Metabolic syndrome (MS) is a clinical constellation comprising risk factors associated with developing cardiovascular disease and type 2 diabetes. Resistin has been suggested as a linkage between obesity, inflammation and type 2 diabetes. This study aimed to investigate resistin concentrations and hematological-biochemical parameters in MS subjects and controls, and to determine whether two resistin gene (*RETN)* polymorphisms (–420C>G & +299G>A) are linked to resistin levels and MS among Thais.

Methods: This case-control study was performed with 322 Thai volunteers: 160 MS subjects and 162 controls. Anthropometric parameters and hematological-biochemical variables were determined. The *RETN* –420C>G (rs1862513) and +299G>A (rs3745367) polymorphisms were genotyped by PCR-RFLP technique.

Results: The resistin levels of the MS group were significantly higher than those of the control group. Resistin levels were positively correlated with anthropometric parameters and WBC count in the MS group. According to *RETN* –420C>G polymorphism, MS subjects with the G allele (CG/GG) (3.9 μg/L) had significantly higher resistin con-

Kratak sadržaj

Uvod: Metabolički sindrom (MS) predstavlja kliničko stanje koje obuhvata faktore rizika povezane s razvojem kardiovaskularne bolesti i dijabetesa tipa 2. Rezistin je naveden kao moguća spona između gojaznosti, inflamacije i dijabetesa tipa 2. Cilj ove studije bio je da se istraže koncentracije rezistina i hematoloških-biohemijskih parametara kod obolelih od MS-a i kontrolnih subjekata i da se utvrdi da li su dva polimorfizma gena za rezistin (*RETN*) (–420C>G i +299G>A) povezana s nivoima rezistina i MS-om kod Tajlanđana.

Metode: Ova anamnestička studija obuhvatila je 322 tajlandska dobrovoljca: 160 obolelih od MS-a i 162 kontrolna subjekta. Određeni su antropometrijski parametri i hematološko-biohemijske varijable. Genotipizacija *RETN* i polimorfizama izvršena je tehnikom PCR-RFLP.

Rezultati: U grupi sa MS-om, nivoi rezistina bili su značajno viši nego u kontrolnoj grupi. Nivoi rezistina bili su u pozitivnoj korelaciji sa antropometrijskim parametrima i brojem leukocita u grupi sa MS-om. Prema polimorfizmu *RETN* –420C>G, oboleli od MS-a sa G alelom (CG/GG) (3,9 μg/L) imali su značajno više koncentracije rezistina nego subjekti sa CC genotipom (2,4 μg/L); što se tiče

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centrations than in subjects with the CC genotype (2.4 μg/L); with regard to *RETN* +299G>A polymorphism, carriers with the A allele (GA/AA) (3.8 μg/L) had significantly higher resistin levels than subjects with the GG genotype (2.7 μg/L), after adjusting for potential covariates. However, the *RETN* –420C>G and +299G>A polymorphisms were not found to be associated with MS, hematological-biochemical parameters and anthropometric variables.

Conclusions: These findings suggest resistin levels are linked with MS and the *RETN* –420C>G and +299G>A polymorphisms have impacted the circulating resistin concentrations. However, these two *RETN* polymorphisms probably do not influence susceptibility to MS among Thais.

Keywords: resistin, gene polymorphisms, metabolic syndrome, Thai

polimorfizma *RETN* +299G>A, nosioci sa A alelom (GA/AA) (3,8 μg/L) imali su značajno više nivoe rezistina nego subjekti sa GG genotipom (2,7 μg/L), posle prilagođavanja za potencijalne kovarijable. Međutim, polimorfizmi *RETN* –420C>G i +299G>A nisu bili u asocijaciji sa MS-om, hematološko-biohemijskim parametrima i antropometrijskim varijablama.

Zaključak: Ovakvi nalazi pokazuju da su nivoi rezistina povezani sa MS-om i da su polimorfizmi *RETN* –420C>G i +299G>A uticali na koncentracije rezistina u cirkulaciji. Međutim, ova dva polimorfizma *RETN* verovatno ne utiču na podložnost MS-u kod Tajlanđana.

Ključne reči: rezistin, genski polimorfizmi, metabolički sindrom, Tajlanđanin

Introduction

Metabolic syndrome (MS) is a public health problem that has reached epidemic proportions with a rapidly increasing worldwide prevalence (1). According to data from the fourth National Health Examination Survey in Thailand, MS is diagnosed in more than 23% of adults aged ≥20 years (2). MS is an interesting topic due to it being a constellation of type 2 diabetes and proatherogenic risk factors (1). Obesity remains a primary diagnostic criterion for MS. Resistin, a pro-inflammatory adipokine, has been suggested to be correlated with obesity and to be predictive of coronary atherosclerosis and type 2 diabetes in humans (3). However, the role of resistin in MS is still controversial (1, 4–5). Up to two-thirds of plasma resistin variation may be attributable to heritable influences (6). Several studies with conflicting results have examined the relationship of resistin gene (*RETN*) variation with MS and circulating resistin levels (3, 7–8). In the present study, *RETN* –420C>G (rs1862513) and +299G>A (rs3745367) polymorphisms were chosen as tags because the G allele of single nucleotide polymorphism (SNP) –420C>G and the A allele of SNP +299G>A, in haplotype and separately, were related with increased risk of impaired beta-cell function and insulin sensitivity (9); SNP –420C>G effect on resistin expression may be due to its effect on transcription factor binding to *RETN* promoter (10). Thus, the present study has been interested in investigating the knowledge on the association between *RETN* polymorphisms and resistin levels that may represent one of the several etiological mechanisms connecting *RETN* and MS. This study aimed to investigate resistin concentrations and hematological-biochemical parameters in MS subjects and healthy controls, and to determine whether two *RETN* polymorphisms (–420C>G & +299G>A) are linked to plasma resistin levels and MS among Thais.

Materials and Methods

Study subjects

The present study enrolled 322 Thai subjects living in suburban and urban residential areas of Bangkok, Thailand. Among them, 162 healthy controls (86 male, 76 female) and 160 MS subjects (73 male, 87 female) were chosen during the health screening program check-up among subjects. This research used a case-control design. The statistical power in our sample size calculation was 0.80 at alpha = 0.05, suggesting an adequate number of samples. The subjects were aged from 24 to 64 years old. A physical examination and medical history check were performed in all subjects and those with a history of liver, kidney, inflammatory, respiratory, and cardiovascular diseases were excluded from the study. Metabolic syndrome was defined using the modified NCEP/ATP III criteria (11). Adoption of the new cut-off point for fasting plasma glucose has already been reported (>5.5 mmol/L). The new cut-off for waist circumference in Asia and the Pacific Region was used, instead of the original cut-off for waist circumference in the ATP III criteria. MS as an entity defined by the modified NCEP/ATP III includes at least three or more of the following abnormalities: central obesity (waist circumference >90 cm in Asian men and >80 cm in Asian women), triglyceride (TG) levels >1.69 mmol/L, high-density lipoprotein cholesterol (HDL-C) <1.04 mmol/L in men and <1.30 mmol/L in women, fasting plasma glucose >5.5 mmol/L and systolic and/or diastolic blood pressure ≥130/≥85 mmHg.

This study was conduced under the principles of the Declaration of Helsinki and the protocol was approved by the Ethics Committee of Rangsit University (RSEC No.016/53). All subjects agreeing to participate signed a consent form.

Measurement of biochemical parameters

Blood samples for biochemical parameters were collected from subjects in the morning after a 12 h fast. Ten milliliters were taken from each subject. Resistin concentrations were determined by sandwich enzyme-linked immunosorbent assay (sandwich ELISA) and insulin concentrations by radioimmunoassay (Linco Research, Inc, USA). Glucose, blood urea nitrogen (BUN), creatinine, TG, HDL-C, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and uric acid were measured using enzymatic methods by DADE Dimension®AR. EDTA blood was used to determine hematological variables e.g. hemoglobin, hematocrit, and platelet count and white blood cell (WBC) count were determined by the Coulter Counter.

Anthropometric and blood pressure measurements

The body weight of each subject dressed in light clothing was measured using a carefully calibrated beam balance (Detecto®, Detecto Scale Manufacturing, USA). Height was measured using a vertical measuring rod. Body Mass Index (BMI) was conventionally calculated as weight in kg/height in meters^2. Waist and hip circumferences were assessed; waist to hip ratio was calculated. Blood pressure (BP) was measured by a nurse after 5 to 10 minutes' rest in the sitting position.

Genotyping of resistin gene polymorphisms

Genotyping was performed using the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) technique. DNA was extracted from peripheral leukocytes in EDTA-treated whole blood using a Flexi Gene DNA Kit (Qiagen, Hilden, Germany). DNA fragments of the SNPs –420 and +299 were amplified by PCR (PE Applied Biosystems, USA) with the following primers—(5'-3'):

SNP–420 forward – 5'-TGTCATTCTCACCCA-GAGACA-3'

reverse – 5'-TGGGCTCAGCTAACCAAATC-3'

SNP +299 forward – 5'-GAGAGGATCCAGG-AGGTC-3'

reverse – 5'-GTGAGACCAAACGGTCCCTG-3'.

A 50 µL PCR reaction was conducted, according to the protocol described by Kunnari et al. (12). The PCR products were digested overnight with different restriction enzymes. We used 5 U of *Bpi I* as well as *Alu I* restriction endonuclease for SNP –420 and for SNP +299, respectively. The digestion products were separated by 2% agarose gels and stained with ethidium bromide. The RFLP method was validated by using quality control DNA samples, which contained the –420C>G and +299G>A polymorphisms. About 25% of the samples were randomly selected to perform the repeated assay and the results were 100% concordant.

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. The median and 95% confidence interval (CI) were calculated. These two groups were compared using the Mann-Whitney U Test (two-tailed). Statistical differences between the groups, in terms of genotypic frequency, were assessed by chi-square test.

Results

The median and 95% confidence interval for biochemical-hematological parameters and anthropometric variables between MS subjects and controls are shown in *Table I*. Age, creatinine, BUN, hemoglobin, hematocrit and platelet count of the MS group were not significantly different from those of the control group. The medians of resistin, glucose, insulin, TG, liver enzymes, uric acid, WBC count, blood pressures and anthropometric parameters were significantly higher in the MS subjects than in the control subjects ($p<0.05$). Meanwhile, HDL-C levels of the MS group were significantly lower than in the control group ($p<0.001$). There was no obvious difference in the percentage of sex between the controls (male 53.1%, female 46.9%) and MS subjects (male 45.6%, female 54.4%) after using the chi-square test ($p>0.05$). Participants were separated into MS and control groups, and Spearman's Rank correlation test results are shown in *Table II*. In the MS group, resistin level was positively correlated with BMI, waist circumference and WBC count but negatively correlated with hematocrit ($p<0.05$). In the control group, resistin level was significantly correlated with BMI, hemoglobin and hematocrit. The biochemical-hematological parameters and anthropometric variables of the MS subjects, for the *RETN* polymorphism genotypes, –420C>G and +299G>A, are shown in *Table III*. After adjusting the variable to the covariates age, gender and BMI, the results showed that increased levels of resistin and WBC count were significantly associated with the *RETN* –420C>G polymorphism. Carriers with the G allele had higher resistin levels and WBC count than subjects with the CC genotype. With regard to *RETN* +299G>A polymorphism in MS subjects after adjusting the variable to the covariates, there were no differences in biochemical-hematological parameters, anthropometric variables and blood pressure between the different genotypes, except for resistin. The subjects with the A allele of *RETN* +299G>A polymorphism were associated with increased resistin levels, compared to subjects with GG genotypes (wild type). The distribution of SNPs *RETN*

Table I Biochemical-hematological parameters and anthropometric variables between control and MS groups.

Variables	Control (n=162)	MS (n=160)	*p*
	Median (95 % CI)	Median (95 % CI)	
Age (years)	46.0 (45.0–47.0)	48.0 (47.0–50.0)	0.241
Glucose (mmol/L)	4.66 (4.56–4.77)	5.30 (5.05–5.44)	<0.001**
BUN (mmol/L)	4.28 (3.93–4.64)	4.28 (4.28–4.64)	0.219
Creatinine (μmol/L)	68.63 (68.63–68.63)	68.63 (61.01–68.63)	0.424
TG (mmol/L)	0.95 (0.90–1.11)	1.91 (1.73–2.03)	<0.001**
HDL-C (mmol/L)	1.30 (1.24–1.35)	1.04 (1.01–1.11)	<0.001**
Uric acid (μmol/L)	274.0 (250.0–298.0)	357.0 (327.0–399.0)	<0.001**
AST (IU/L)	24.0 (23.0–25.6)	27.0 (25.5–30.0)	0.030*
ALT (IU/L)	26.0 (22.5–29.0)	33.0 (30.2–36.0)	<0.001**
ALP (IU/L)	68.0 (65.0–72.0)	74.0 (71.0–78.0)	<0.001**
Insulin (pmol/L)	70.20 (60.60–72.60)	94.80 (82.80–103.20)	<0.001**
Resistin (μg/L)	2.0 (1.6–2.3)	3.2 (2.6–3.9)	<0.001**
Hemoglobin (g/L)	145.0 (136.0–146.0)	146.0 (139.0–151.0)	0.153
Hematocrit (%)	42.0 (40.7–43.5)	42.9 (40.7–44.5)	0.341
WBC count (10^9/L)	6.4 (6.0–6.8)	7.5 (6.9–7.9)	<0.001**
Platelet count (10^9/L)	264.0 (255.0–279.7)	266.0 (251.2–280.8)	0.533
Diastolic BP (mmHg)	74.0 (72.0–76.0)	80.0 (80.0–85.0)	<0.001**
Systolic BP (mmHg)	120.0 (117.0–120.0)	130.0 (130.0–134.0)	<0.001**
BMI (kg/m^2)	22.8 (22.3–23.2)	26.5 (25.8–27.6)	<0.001**
Waist (m)	0.77 (0.75–0.79)	0.91 (0.87–0.93)	<0.001**
Waist/hip ratio	0.82 (0.81–0.84)	0.88 (0.87–0.89)	<0.001**

*p<0.05, **p<0.01 by Mann-Whitney U-Wilcoxon Rank Sum W test (2-tailed)

Table II Correlation coefficients of resistin levels with parameters in control and MS groups.

Variables	Correlation coefficients of resistin with parameters	
	MS (n=160)	Control (n=162)
Glucose	0.106	0.059
BUN	0.035	0.034
Creatinine	0.103	0.079
TG	0.031	0.083
HDL-C	–0.178	–0.155
Uric acid	0.128	0.104
AST	0.117	–0.186
ALT	0.144	–0.097
ALP	0.057	–0.125
Insulin	0.189	0.176
Hemoglobin	–0.221	–0.413**
Hematocrit	–0.292*	–0.447**
WBC count	0.261*	0.157
Platelet count	0.159	0.124
Diastolic BP	0.056	0.091
Systolic BP	0.043	0.078
BMI	0.215*	0.189*
Waist	0.199*	0.171
Waist/hip ratio	0.122	0.108

* = p< 0.05, ** = p< 0.01 by Spearman's rank correlation (2-tailed)

Table III Biochemical-hematological parameters and anthropometric variables, according to RETN –420C>G and +299G>A genotypes in MS subjects.

Variables	SNP –420C>G		*p*	SNP +299G>A		*p*
	CG/GG (n=95)	CC (n=65)		GA/AA (n=93)	GG (n=67)	
Glucose (mmol/L)	5.33 5.05–5.55	5.16 5.00–5.52	0.753	5.33 5.00–5.50	5.33 5.00–5.55	0.797
BUN (mmol/L)	4.28 4.28–4.64	4.64 4.07–5.21	0.384	4.28 4.28–4.64	4.28 4.28–4.64	0.970
Creatinine (µmol/L)	79.56 79.56–88.40	79.56 70.72–79.56	0.426	79.56 70.72–79.56	79.56 70.72–88.40	0.171
TG (mmol/L)	1.83 1.67–2.01	2.00 1.73–2.27	0.181	1.75 1.62–1.98	2.02 1.84–2.24	0.158
HDL-C (mmol/L)	1.06 1.01–1.14	1.04 0.98–1.14	0.123	1.09 1.01–1.17	1.04 0.98–1.09	0.831
Uric acid (µmol/L)	375.0 309.0–410.0	434.0 333.0–553.0	0.087	369.0 303.0–440.0	387.0 297.0–416.0	0.536
AST (IU/L)	25.0 24.0–29.0	29.0 26.0–34.0	0.837	27.0 25.0–29.0	27.0 23.0–33.0	0.549
ALT (IU/L)	33.0 29.0–38.0	36.0 31.0–41.0	0.430	37.0 31.0–39.0	32.0 28.0–38.0	0.769
ALP (IU/L)	73.0 70.0–77.0	78.0 70.0–82.0	0.156	74.0 71.0–78.0	75.0 70.0–81.0	0.297
Insulin (pmol/L)	109.8 83.4–119.4	90.0 79.2–98.4	0.484	99.0 85.8–115.8	88.8 77.4–112.8	0.553
Resistin (µg/L)	3.9 3.1–5.1	2.4 2.0–3.2	0.009**	3.8 2.7–4.5	2.7 2.0–3.4	0.014*
Hemoglobin (g/L)	144.0 133.0–152.0	147.0 139.0–153.0	0.310	146.0 134.0–153.0	145.0 133.0–152.0	0.752
Hematocrit (%)	42.0 40.0–44.2	44.2 40.5–45.3	0.097	42.0 40.0–45.0	43.1 39.8–44.5	0.232
WBC count (10^9/L)	7.8 7.3–8.1	6.0 5.7–8.4	0.013*	7.6 6.9–8.1	7.0 5.8–8.6	0.383
Platelet count (10^9/L)	274.5 248.8–294.2	259.0 241.8–279.0	0.364	270.0 246.0–298.5	260.5 246.7–283.1	0.307
Diastolic BP (mmHg)	81.0 80.0–86.0	80.0 80.0–88.0	0.966	81.0 80.0–85.0	80.0 80.0–88.0	0.882
Systolic BP (mmHg)	130.0 128.0–135.0	130.0 127.0–140.0	0.587	130.0 128.0–134.0	130.0 130.0–140.0	0.724
Waist (m)	0.91 0.87–0.92	0.90 0.87–0.92	0.114	0.90 0.86–0.91	0.91 0.88–0.92	0.883
Waist/hip ratio	0.89 0.87–0.91	0.88 0.87–0.90	0.152	0.88 0.86–0.89	0.89 0.87–0.90	0.204

Data are expressed as median (95% CI).
*= $p<0.05$, ** = $p<0.01$, after adjusting the variable to the covariates age, gender and BMI.

Table IV Genotypic and allelic distribution of *RETN* –420C>G and +299G>A polymorphisms in MS and control subjects.

	Control n (%)	HWE of MS p-value[a]	MS n (%)	HWE of control p-value[a]	Genotypic or allelic p-value[b]
RETN –420C>G					
Genotype					
C/C	60 (37.0)	0.565	65 (40.6)	0.293	
C/G	80 (49.4)	(df=1)	69 (43.1)	(df=1)	0.513
G/G	22 (13.6)	(χ^2=0.33)	26 (16.3)	(χ^2=1.10)	
Allele					
C	200 (0.617)		199 (0.622)		0.905
G	124 (0.383)		121 (0.378)		
RETN +299G>A					
Genotype					
G/G	79 (48.8)	0.283	67 (41.9)	0.104	
G/A	64 (39.5)	(df=1)	80 (50.0)	(df=1)	0.143
A/A	19 (11.7)	(χ^2=1.15)	13 (8.1)	(χ^2=2.64)	
Allele					
G	222 (0.685)		214 (0.669)		0.655
A	102 (0.315)		106 (0.331)		

[a] Based on results of the chi-square test.

[b] Based on results of the chi-square test for comparison between MS and control groups.

–420C>G and +299G>A genotypes were in line with the Hardy-Weinberg equilibrium (HWE) ($p>0.05$) and the values of chi-square and degrees of freedom are shown in *Table IV*. Moreover, the results of genotypic and allelic frequencies of *RETN* –420C>G and +299G>A polymorphisms in MS and control subjects are also shown in *Table IV*. There were no significant differences in the genotypic and allelic frequencies of *RETN* –420C>G and +299G>A polymorphisms between the MS and control groups ($p>0.05$) and these two polymorphisms also showed no significant relationship with MS ($p>0.05$).

Discussion

Our findings suggest that MS subjects had higher resistin concentrations than the control group, and two SNPs in *RETN*, one promoter variant at –420C>G and one intron 2 variant at +299G >A from a translation start site, were related to the resistin concentration. By contrast, we did not find any relationships between these two SNPs and MS or any other metabolic feature including glucose, lipids, waist circumference and blood pressure among Thais. Resistin is involved in glucose homeostasis, lipid metabolism, and insulin action (13), and it has been linked to obesity, inflammation, type 2 diabetes and atherosclerosis, but the results of animal and human studies are still controversial (5, 13–15). Study of Sheng et al. (16) confirmed that human hepatic cells overexpressing resistin had impaired glucose uptake and glycogen synthesis. Resistin is four times more highly expressed in human omental and abdominal subcutaneous white adipocytes than in adipocytes from the thigh suggesting that resistin could play a major role in obesity-related insulin resistance (15). Obesity, especially central obesity, is a key feature for MS, and waist circumference is used as a valid marker of central obesity. Consistent with Mojtaba et al. (17), our study found an association between increased resistin levels and increased anthropometric parameters including waist circumference. These data suggest that high body weight or fat tissue may lead to increased circulating resistin concentrations. Moreover, a previous study suggested that subjects with central obesity are more prone to develop MS (18) and this finding is consistent with our study. MS is a clinical constellation comprising risk factors such as increased insulin resistance, low HDL-C and high TG. Insulin is known to up-regulate lipoprotein lipase, a critical factor for the production of HDL and the lipolysis of TG (19), and this relation could explain the dyslipidemia

in MS. However, the role of resistin in MS is controversial. Our findings, in agreement with previous data, revealed that plasma resistin levels are higher in subjects with MS compared to controls (1, 4), while others have not found an association between resistin levels and MS (5, 20). De Luis et al. (5) reported that resistin levels were not associated with the accumulation of MS factors or the diagnosis of MS in Spanish subjects. One explanation could be that the different genetic background among populations could account for the disagreements observed. Moreover, resistin is an interesting adipokine because it enhances the inflammation process which is associated with the development of atherosclerosis (13), and resistin also activates nuclear factor-kappa B (NF-κB) inflammatory signaling (21). Our results also indicated that increased plasma resistin levels were significantly correlated with increased WBC count, especially in the MS group. As metabolic syndrome by itself is associated with inflammation, there is the possibility that resistin may rather be related with inflammatory markers including WBC. Therefore, our study confirmed that the plasma resistin concentration may be a biomarker for the diagnosis of MS in Thais.

This is the first report that has set out to determine the relationship between two SNPs, −420C>G and +299G>A, and resistin levels and MS among Thais. The human *RETN* is located on chromosome 19p13.2 and spans 1,369 bp with four exons and three introns (7). It was reported that a common SNP on the promoter of *RETN* –420C>G has been described as inducing resistin mRNA synthesis via the generation of an Sp1/Sp3 binding site (10), thus enhancing resistin transcription and plasma protein levels. Our study confirmed that the SNP –420C>G in the promoter of *RETN* was associated with increased resistin concentrations. Consistent with our results, previous studies have reported a relationship between the –420G allele and increased plasma resistin levels mainly in Asian populations including a Japanese sample (22), Malaysian men (7) and Finnish subjects (23). By contrast, studies in an Italian sample and Caucasians did not observe this relationship (3, 6). Furthermore, this study showed that there were no differences in metabolic features including glucose, lipids, blood pressure between CC carriers and G allele carriers, similar to the study of Norata et al. (3), whereas the study of Boumaiza et al. (24) found associations of SNP –420C>G with waist circumference and BMI. According to +299G>A polymorphism, this study investigated the relationships of this polymorphism and anthropometric-biochemical variables; the present results failed to detect any correlation of this SNP with those variables except resistin levels, while Ukkola et al. (23) found that subjects with the A allele of *RETN* +299G>A polymorphism had a protective effect against hypertension in a Finnish population-based cohort study (23). Moreover, our study also confirmed that *RETN* 299G>A polymorphism was significantly associated with increased plasma resistin levels, and similar results for SNP +299G>A have been reported in Malaysian men (7), Japanese (22) and Finnish subjects (23). On the other hand, the *RETN* +299G>A polymorphism analysis of the Framingham Offspring Study in the United States did not show an association with resistin levels (25). This polymorphism is in an intron, which generally has not been considered to have regulatory functions. However, it has been shown that SNPs in the non-coding region, such as the 3'-untranslated gene region, can affect gene expression (26). Intron polymorphism of TFAP2B, a susceptibility gene to type 2 diabetes, influenced adipo-cytokine gene expression transcriptional activity (27). Therefore, *RETN* +299G>A polymorphism may be a marker in a linkage disequilibrium with another polymorphism affecting gene expression. However, the variation of results among populations about the *RETN* variation, –420C>G and +299G>A, and resistin levels may contribute appreciably to differences in gene expression phenotypes by ethnicity. Therefore, our results in the present study imply that these two SNPs seem to have a role in the determination of circulating resistin concentrations. However, a previous study among Thais with type 2 diabetes found that resistin concentration was associated with the *RETN* +299G>A variation, but not –420C>G polymorphism, but this study among Thais with MS found an association of these two *RETN* polymorphisms and resistin concentrations. The divergent effects of *RETN* –420C>G and +299G>A polymorphisms in resistin concentrations between these two studies in Thais may be due to differences in the studied subjects (type 2 diabetes or MS subjects), disease status, sample numbers and gene–environment interactions. Menzaghi et al. (6) suggest that serum resistin is mostly regulated by genes other than that coding for this molecule. Moreover, type 2 diabetes is one part of the diagnostic criteria for MS; many components of MS are associated with a sedentary lifestyle (28).

For links between *RETN* variation and MS, the present study in Thais found that SNPs –420C>G and +299G>A were not significantly associated with an increased risk of MS. Regarding links between *RETN* –420C>G polymorphism and MS, early studies reported varying results (3, 7, 8). The presence of SNP –420C>G was associated with the increased incidence of metabolic syndrome in Italian (3) and Japanese subjects (8). By contrast, the subjects with SNP –420C>G at the *RETN* locus were not associated with MS susceptibility in Caucasians (29) as well as Malaysian men (7) and these results were confirmed by our study. Regarding links between the *RETN* +299G>A polymorphism and MS, in a Japanese cohort study subjects with the A allele of SNP +299G>A were associated with increased risk of MS (8), but this was not reflected in our study in Thais

and others conducted on Malaysian men (7). However, conflicting findings between these genetic association studies could be due to true differences in allelic association with the disease phenotype in different populations or interactions with other genes and environmental factors.

Many components of MS are related with a sedentary lifestyle, including increased adipose tissue (especially central obesity), decreased HDL-C, and a trend toward raised glucose, blood pressure, and triglycerides in the genetically susceptible. However, the molecular mechanisms underlying the pathophysiology of MS are still far from being fully understood. Our study found associations between MS and resistin levels and the present data also suggested that the influence of these two SNPs in *RETN* seemed to be factors influencing resistin levels among MS Thais. It is possible that increased plasma resistin concentrations may play a significant role in the development of MS, and screening for a common genetic background of reristin concentrations may provide useful information concerning the management and assessment of MS.

In conclusion, this study among Thais found that MS subjects had higher resistin levels than the controls; SNPs –420C>G and +299G>A were significantly associated with increased resistin levels but these two *RETN* polymorphisms were not associated with MS susceptibility in Thais.

Authors' contributions. KS participated in the study design, analysis as well as interpretation of data and manuscript preparation. RT participated in interpretation of data and manuscript preparation. PN participated in analysis of data. All authors read and approved the final manuscript.

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Conflict of interest statement

The authors stated that have no conflicts of interest regarding the publication of this article.

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