

The +299(G>A) Resistin Gene Polymorphism and Susceptibility to Type 2 Diabetes in Thais

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Summary The prevalence of type 2 diabetes has been increased in Thais. Resistin is an adipokine that involve in glucose homeostasis and is a candidate gene for type 2 diabetes. We performed a case-control study in representative sample of 200 Thai volunteers, 105 controls and 95 type 2 diabetes subjects. The purposes of the present study were to investigate the association between two SNPs (single nucleotide polymorphisms) in the resistin gene, at positions +299(G>A) and –420(C>G), and biochemical parameters; to determine whether these polymorphisms are linked to increased risk of type 2 diabetes. At position +299(G>A) of the resistin gene, the resistin concentration among type 2 diabetes subjects was significantly higher in GA/AA genotypes (3.40 ng/ml) than the GG genotype (1.99 ng/ml). Resistin gene polymorphism at position +299(G>A) in type 2 diabetes patients was significantly more frequent than in the control group ($p = 0.004$). Polymorphism at position –420(C>G) showed no significant relationship with type 2 diabetes ($p = 0.095$). Logistic regression analysis was shown that +299(G>A) gene polymorphism was increased risk factors for type 2 diabetes ($p = 0.013$). In conclusion, these finding suggest that resistin gene polymorphism at position +299(G>A) has impact on the increased resistin concentrations and may influence susceptibility to type 2 diabetes in Thais.

Key Words: +299(G>A) resistin gene polymorphism, type 2 diabetes

Introduction

The type 2 diabetes is increasing rapidly, and is becoming a significant global health problem. White adipose tissue

secretes a variety of adipokines, which may provide the link between insulin resistance and obesity, and resistin is one such molecule [1, 2]. The human resistin gene is localized to chromosome 19p13.3. Resistin suppressed adipocyte differentiation by 80% in 3T3-L1 cells [3]; failure of adipocyte differentiation has been suggested as a cause of type 2 diabetes, possibly through an ectopic overload of fatty acids and lipotoxicity of nonadipose tissues, such as muscles and liver [4, 5]. The function of resistin related to glucose

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homeostasis and insulin resistance in type 2 diabetes patients, is not consistent. Some studies have reported increased resistin expression levels in type 2 diabetes, insulin resistance or obesity [6, 7], whereas some failed to detect any change in resistin levels in these conditions [8–10]. The results of studies investigating genetic variations in resistin, including single nucleotide polymorphisms (SNPs), are controversial. The SNP on the resistin gene promoter –420(C>G) has been associated with a prevalence of type 2 diabetes [11], but not all reports have consistently produced these findings [1]. Genetic variant at nucleotide +299 (IVS2 +181(G>A) and obesity have been reported as risk factors for type 2 diabetes in Caucasians [12]. However, the findings for a Japanese type 2 diabetes population did not concur [13]. Overall, some, but not all, studies have found associations between resistin gene polymorphism, and type 2 diabetes. Therefore, the genetic variations of the resistin gene in humans require clarification. The purposes of the present study were to investigate the association between two SNPs in the resistin gene, one promoter variant at position –420(C>G) and one intron 2 variants at position +299(G>A) from a translation start site, and the levels of resistin, glucose, insulin, total cholesterol, triglycerides, blood urea nitrogen, creatinine, blood pressure, and anthropometric variables; to determine whether these polymorphisms are linked to increased risk of type 2 diabetes.

Materials and Methods

Study population

The case-control study comprised of 200 subjects allocated into 2 groups. The first group was controls without type 2 diabetes ($n = 105$); the second group was type 2 diabetes patients ($n = 95$). Diabetes was diagnosed based on the American Diabetes Association criteria, reported in 2008 [14]. Subjects were excluded if they had severe hypertension, liver, kidney, thyroid, cardiovascular or any active inflammatory diseases, as well as receiving insulin. The same medical doctor conducted the physical examinations throughout the study and measured blood pressure (BP). The type 2 diabetes subjects were volunteers from a diabetes mellitus outpatient clinic of Burapha University Hospital, Chonburi and Rajvithi Hospital, Bangkok, Thailand. The protocol was approved by the Ethics Committee of Burapha University and all participants agreeing to participate by signing informed consent form.

Biological measurements

The body weight of each subjects was measured using a carefully calibrated beam balance (Detecto®, Detecto Scale Manufacturing, MO). 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 and Waist and hip ratio (WHR) was calculated.

About 10 ml of venous blood were taken from the subjects in the morning, after overnight fast. Resistin concentrations were determined by sandwich enzyme-linked immunosorbent assay (sandwich ELISA) and insulin concentrations by radioimmunoassay (Linco Research, Inc., MO.). Glucose, blood urea nitrogen (BUN), creatinine, total cholesterol (TC), and triglycerides (TG) were measured using enzymatic methods by DADE Dimension®AR.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique

DNA was extracted from EDTA-treated whole blood by Flexi Gene DNA kit (QIAGEN, Hilden, Germany). DNA-fragments of the SNPs –420 and +299 were amplified by PCR (PE Applied Biosystems) with the following primers—(5'-3'): SNP –420 forward TGTCATTCTCACCCAGAGACA, reverse TGGGCTCAGCTAACCAAATC, SNP + 299 forward GAGAGGATCCAGGAGGTC, reverse GTGAGACCAAACGGTCCCTG, as described by Kunnari *et al.* [15]. The PCR products were digested overnight with different restriction enzymes. For SNP-420, 5 U of *Bpi I*, and for SNP + 299 the *Alu I* restriction endonuclease, were used. The digestion products were separated by 2% agarose gels stained with ethidium bromide.

Statistical analysis

Statistical analyses were carried out using SPSS for Windows version 11.5 (SPSS, Chicago, IL). The median, range and 95% confidence interval (C.I.) were calculated. The difference between groups was compared by using the Mann-Whitney U-Wilcoxon Rank Sum W test. The difference in genotypic frequencies of the two groups was assessed by Chi-square test. The Minitab statistical computer program was used to calculate the odds ratio (OR). To assess the associated between type 2 diabetes as dependent variable and potential factors, logistic regression was applied. A p value<0.05 was considered statistically significant.

Results

The median and 95% confidence interval (C.I.) for age, anthropometric variables, blood pressure, glucose, BUN, creatinine, total cholesterol, triglycerides, insulin, and resistin levels were shown in Table 1. Age, BMI, BUN and blood pressure of type 2 diabetes group were not statistically significantly different from those of control group. The medians of waist/hip ratio, glucose, insulin, resistin, creatinine and lipids were significantly higher in the type 2 diabetes than control subjects ($p<0.050$). The anthropometric variables, blood pressure, and biochemical parameters of the type 2 diabetes subjects, for resistin poly-

Table 1. Median and 95% confidence interval (C.I.) for age, anthropometric variables, blood pressure, and biochemical parameters in control and type 2 diabetes

	Control	Type 2 diabetes	<i>p</i> value
	<i>n</i> = 105	<i>n</i> = 95	
	Median (95% C.I.)	Median (95% C.I.)	
Age (years)	55.0 (53.0–58.0)	57.0 (54.0–59.5)	0.099
BMI (kg/m ²)	24.7 (24.0–25.5)	26.2 (24.5–27.3)	0.437
Waist/hip ratio	0.87 (0.86–0.88)	0.92 (0.89–0.93)	0.000*
Glucose (mg/dL)	84 (78–86)	135 (128–144)	0.000*
Insulin (μ U/dL)	14.6 (12.5–15.1)	18.3 (13.8–19.7)	0.002*
Resistin (ng/mL)	1.9 (1.2–2.1)	3.3 (2.0–3.4)	0.000*
BUN (mg/dL)	13 (11–13)	13 (11–13)	0.293
Creatinine (mg/dL)	0.8 (0.7–0.8)	1.0 (0.8–1.0)	0.001*
TG (mg/dL)	124 (89–130)	164 (120–180)	0.000*
TC (mg/dL)	207 (191–210)	242 (216–247)	0.000*
Systolic BP (mmHg)	129 (120–130)	134 (130–138)	0.260
Diastolic BP (mmHg)	80 (80–84)	80 (80–82)	0.734

* $p < 0.05$ by using Mann-Whitney U-Wilcoxon Rank Sum *W* test (Two-Tailed).
C.I., Confidence Interval.

morphism genotypes at positions +299 and –420, were shown in Table 2. With regard to +299(G>A) polymorphism in type 2 diabetes subjects, there were no differences in anthropometric variables, blood pressure or biochemical parameters between the different genotypes, except for resistin. Resistin concentrations were higher in the GA/AA genotypes, heterozygous combined with homozygous mutant types, than the GG genotype (wild type) ($p < 0.05$). Regarding to the –420(C>G) polymorphism in type 2 diabetes subjects, the data revealed no significant differences in any variable between the CG/GG genotypes (heterozygous combined with homozygous mutant types) and the CC genotype (wild type). The frequency of resistin gene polymorphism at +299(G>A) and –420(C>G) in all subjects were shown in Table 3. At +299(G>A) polymorphism, 31% of GA/AA genotype had type 2 diabetes, and resistin gene polymorphism at this position were

significantly more frequent than in the control group ($p = 0.004$). Polymorphism at position –420(C>G) showed no significant relationship with type 2 diabetes ($p = 0.095$). Logistic regression and odds ratios (OR) for possible associations between type 2 diabetes and age, waist/hip ratio and two resistin gene polymorphism at positions +299(G>A) as well as –420(C>G) were shown in Table 4. Three variables, age (OR = 1.1, $p = 0.014$), waist/hip ratio (OR = 3.5, $p = 0.020$) and gene polymorphism at positions +299(G>A) (OR = 2.3, $p = 0.013$), showed statistically significant association. Table 5 showed the relationship between hyperglycemia at cut off point > 126 mg/dl and gene polymorphisms. There was a statistically significant association between hyperglycemia and resistin gene polymorphism at positions +299(G>A) ($p < 0.05$), except for the position –420(C>G).

Table 2. Median and 95% confidence interval (C.I.) for age, anthropometric variables, blood pressure, and biochemical parameters in type 2 diabetes.

	+299(G>A) Polymorphism		<i>p</i> value	-420(C>G) Polymorphism		<i>p</i> value
	GG	GA/AA		CC	CG/GG	
	(<i>n</i> = 33) (95% C.I.)	(<i>n</i> = 62) (95% C.I.)		(<i>n</i> = 35) (95% C.I.)	(<i>n</i> = 60) (95% C.I.)	
Age (years)	57.0 (53.5–64.5)	57.5 (53.8–59.2)	0.531	57.0 (53.3–62.0)	57.5 (53.9–60.1)	0.820
BMI (kg/m ²)	26.9 (23.8–27.8)	24.9 (24.1–27.3)	0.772	26.9 (24.6–28.5)	24.8 (23.7–27.3)	0.471
Waist/hipratio	0.93 (0.88–0.95)	0.92 (0.89–0.93)	0.353	0.91 (0.88–0.93)	0.92 (0.89–0.93)	0.598
Glucose (mg/dL)	130 (126–145)	136 (122–153)	0.239	131 (122–139)	143 (126–156)	0.349
Insulin (μU/dL)	19.4 (16.7–24.5)	17.3 (12.5–19.1)	0.194	19.5 (16.0–20.9)	17.3 (12.9–20.0)	0.378
Resistin (ng/mL)	2.0 (1.8–3.7)	3.4 (2.2–3.7)	0.035*	3.1 (1.9–3.8)	3.4 (2.0–3.7)	0.716
BUN (mg/dL)	14 (11–16)	12 (10–13)	0.484	15 (10–17)	12 (11–13)	0.059
Creatinine (mg/dL)	1.0 (0.8–1.0)	1.0 (0.8–1.1)	0.220	0.9 (0.8–1.1)	1.0 (0.8–1.1)	0.818
TG (mg/dL)	177 (116–225)	159 (107–171)	0.667	164 (111–194)	164 (119–187)	0.590
TC (mg/dL)	246 (220–279)	228 (197–244)	0.299	226 (197–270)	244 (216–253)	0.855
Systolic BP (mmHg)	130 (130–140)	136 (126–141)	0.861	139 (130–140)	130 (126–138)	0.273
Diastolic BP (mmHg)	80 (79–80)	82 (80–85)	0.329	80 (75–83)	80 (80–84)	0.976

**p*<0.05 by Mann-Whitney U-Wilcoxon Rank Sum W test (two-tailed).

C.I., Confidence Interval.

Table 3. Genotype frequencies of +299(G>A) and -420(C>G) polymorphisms in all subjects (*n* = 200)

SNP + 299	Genotype frequencies		<i>p</i> value ^a
	Type 2 diabetes	Control	
GA/AA	31% (<i>n</i> = 62)	23.5% (<i>n</i> = 47)	0.004*
GG	16.5% (<i>n</i> = 33)	29% (<i>n</i> = 58)	
SNP-420	Type 2 diabetes	Control	<i>p</i> value ^a
CG and GG	30% (<i>n</i> = 60)	27% (<i>n</i> = 54)	0.095
CC	17.5% (<i>n</i> = 35)	25.5% (<i>n</i> = 51)	

^a Pearson chi-square, **p*<0.05

Discussion

Many studies tried to elucidate the functions of resistin, and reported that resistin may impair glucose tolerance, insulin action, and decreased glucose uptake in skeletal muscle cells [16, 17]. In mice, neutralizing resistin with antibody improved insulin action and glucose uptake in diet-induced obesity [2]. Resistin was four times more highly expressed in human omental and abdominal subcutaneous white adipocytes than in adipocytes from the thigh, suggesting that human resistin could play a major role in obesity-related insulin resistance [18]. It was well known that most of type 2 diabetic patients were related to obesity and insulin resistance. Some authors reported that serum resistin levels were increased in type 2 diabetes subjects [7, 19]. Such findings have been re-confirmed by this study. Likewise, in Thai subjects, the type 2 diabetes group had

Table 4. Logistic regression analysis when type 2 diabetes was used as dependent variable and age, waist/hip ratio and resistin gene polymorphism at +299(G>A) as well as at -420 (C>G) were taken as independent variables.

Variables	β	Odds ratios Exp (β)	(95% C.I.)	<i>p</i> value
gene +299(G>A)	0.811	2.3	1.2–4.3	0.013*
gene -420(C>G)	0.175	1.2	0.7–2.3	0.593
age	0.045	1.1	1.0–1.2	0.014*
waist/hip ratio	1.241	3.5	1.2–9.9	0.020*

**p*<0.05

C.I., Confidence Interval

Table 5. The relationship between hyperglycemia and gene polymorphisms when hyperglycemia (cut off point>126 mg/dl) was used as dependent variable.

Variables	Odds ratios Exp (β)	<i>p</i> value
gene +299(G>A)	1.930	0.042*
gene -420(C>G)	1.609	0.142

**p*<0.05

higher waist/hip ratio and resistin levels than the control group so it could be indicated that type 2 diabetes mellitus was associated with increased central obesity. In contrast, other studies found no difference in plasma resistin levels between patients with type 1 and type 2 diabetes, and healthy controls [20]. Studies have reported no association between resistin levels and markers of insulin resistance in type 2 diabetes patients [8, 21]. Therefore, the true function of resistin requires further study.

The human resistin gene is localized on chromosome 19. Results of studies investigating genetic variations in the resistin gene were controversial. Our observations found an association between resistin gene polymorphism at +299(G>A) in type 2 diabetes and higher resistin concentration, but not in subjects carrying CG/GG resistin gene genotypes at -420(C>G). In a Japanese population, the -420 GG genotype was associated with type 2 diabetes; the -420 GG resistin genotype was related to increased promoter activity through specific Sp1/3 binding, which in turn, leads to increased resistin levels and increased risk of type 2 diabetes [11]. However, the current study found that polymorphism at -420(G>C) was not significantly associated with increased risk of type 2 diabetes. Conneely *et al.* failed to detect any correlation between resistin gene promoter polymorphism at -420(G>C) and type 2 diabetes [1]. No association between SNP of the resistin gene in the promoter region with obesity or insulin resistance was found [22]. Our study did not find a significantly increased risk of type 2 diabetes in resistin gene polymorphism at position -420 (C>G), and similar data were reported by Engert *et al.* [23]. In Caucasians, a resistin genotype at position +299(G>A) and obesity was a significant determinant of type 2 diabetes

risk [12] whereas resistin gene +299(G>A) was not associated with type 2 diabetes in Japanese subjects [13]. This polymorphism is in an intron, +299(G>A), 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 [24]. Intron polymorphism of *TFAP2B*, a susceptibility gene to type 2 diabetes, influenced adipocytokine gene expression transcriptional activity [25]. Therefore, resistin gene polymorphism at +299(G>A) may be a marker in linkage disequilibrium with an other polymorphism affecting gene expression. Resistin gene polymorphism at +299(G>A) in Thai diabetic subjects may contribute to increased resistin levels, which may be involved in the pathogenesis of type 2 diabetes by impaired insulin action. Logistic regression analysis of this study showed that +299(G>A) polymorphism, age and waist/hip ratio were linked to significantly increased risk of type 2 diabetes in Thais. The inconsistencies of resistin gene polymorphism among studies might be explained by the different genetic backgrounds or environmental conditions of the population studied. Furthermore, our study showed that there was a statistically significant association between hyperglycemia and resistin gene polymorphism at positions +299(G>A). Kiritoshi *et al.*, showed that hyperglycemia increased reactive oxygen species produced by mitochondria, resulting in activation of nuclear factor κ B, induction of cyclooxygenase-2 gene expression. These results might contribute to the pathogenesis of diabetic complications especially diabetic nephropathy [26]. Astaxanthin was a carotenoid that might scavenge the production of reactive oxygen species in mitochondria and has protective effects on diabetic nephropathy [27]. Therefore, type 2 diabetes should control the level of blood glucose and *Gymnema sylvestre* was one of alternative herbal medicines that might help to treat diabetes mellitus by preventing the sugar molecules absorption in the intestine [28].

In summary, we found that type 2 diabetic subjects displayed a significantly increased resistin concentration than controls and the levels of resistin in type 2 diabetes with GA/AA genotypes were significantly higher than those with

the GG genotype. The resistin gene polymorphism at +299(G>A) appeared to affect susceptibility to type 2 diabetes, especially among Thais, whereas -420(C>G) polymorphism was unlikely to play a major function in the etiology of type 2 diabetes among the Thai population. Moreover, we found that waist/hip ratio and age were also significantly associated with increased risk of type 2 diabetes. Further studies are needed to fully clarify the role of the resistin gene in type 2 diabetes, by investigation of other populations including large population-sample sizes, and how resistin polymorphism at +299(G>A) affects gene expression.

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Abbreviations

SNPs, single nucleotide polymorphisms; BP, blood pressures; BMI, Body Mass Index; WHR, Waist and hip ratio; BUN, blood urea nitrogen; TC, total cholesterol; TG, triglycerides.

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