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Absence of Evidence for an Association Between Resistin Gene Variants and Insulin Resistance in an Asian Population with Low and High Blood Pressure

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Abstract

Aims—Although the function of resistin in human biology is unclear, some evidence suggests resistin gene variants influence insulin resistance, and insulin resistance-related hypertension. We searched for associations between common resistin gene variants and factors related to insulin resistance in Asian individuals with high or low blood pressure.

Methods—Non-diabetic Chinese or Japanese sibling pairs were included if one had extreme hypertension and the other was either hypertensive or hypotensive. Four common, non-coding single nucleotide polymorphisms (SNPs) were identified by sequencing the resistin gene in 24 hypertensive probands. Generalized estimating equations-based regressions were then performed to test for SNP associations using the entire study population (n=1556).

Results—Of 72 tests, only one was significant at the 0.05 level; 3.5 significant tests were expected by chance alone. High variability in insulin and triglyceride levels created wide confidence intervals, thus the negative results are not conclusive for these phenotypes. However, the large sample size resulted in narrow confidence intervals for BMI, fasting and 120 minute post-load glucose, and high and low density lipoprotein cholesterol.

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Conflict of interest statement

The authors declare that they have no conflict of interest

Conclusion—Factors associated with insulin resistance are not likely influenced by the resistin gene in non-diabetic Asian individuals with high and low blood pressure.

Keywords

resistin; hypertension; Asian population; insulin resistance; single nucleotide polymorphisms

Introduction

Insulin resistance syndrome (IRS) is a metabolic disorder characterized by the co-occurrence of obesity, dyslipidemia, impaired glucose tolerance, hyperinsulinemia, and hypertension [1]. These factors are associated with Type 2 diabetes mellitus and atherosclerotic cardiovascular disease. IRS is influenced by both environmental and genetic factors; however, the genetic determinants of insulin resistance are poorly characterized.

Resistin has been promoted as a candidate gene for insulin resistance since its first description in mice [2]. This first study identified the cysteine-rich resistin protein as a cytokine secreted by adipocytes that acts as an antagonist to insulin. While more recent studies have largely supported a physiological role for resistin in glucose metabolism in rodent models, the primary role for resistin in human biology is unclear [3].

Conflicting findings have made it difficult to determine a role for resistin in IRS. Some genetic association analyses suggest a link between resistin gene variants and insulin resistance [4–7], while others report no such association [8,9]. Studies have found an association between certain resistin gene variants and increases in blood pressure in subjects with Type 2 diabetes mellitus, but not in controls without diabetes [4], while another reported an association between a resistin variant and hypertension only in non-diabetic controls, but not in subjects with diabetes [10]. Two large studies in Japan reported an association between a genetic variant in the resistin gene promoter with BMI and metabolic syndrome variables [11, 12], but no association with either coronary heart disease or renal dysfunction [12].

A goal of the Stanford Asia and Pacific Program for Hypertension and Insulin Resistance (SAPPHIRE) is to use a candidate gene approach to identify genes associated with insulin resistance in hypertensive individuals. Subjects enrolled in the study were from Chinese and Japanese families having siblings with hypertension (concordant siblings), or at least one hypertensive and one hypotensive sibling (discordant siblings). A previous study of this group reported that hypertensive subjects had significantly different plasma lipid levels and insulin sensitivities than their hypotensive siblings [13]. The present study used the SAPPHIRE population to investigate the role of the resistin gene as a potential determinant of IRS in individuals with high or low blood pressure. By protocol, the subjects were not obese and did not have diabetes.

Subjects and Methodology

Study population

The study population (n = 1556) was enrolled in the SAPHIRE Network of the Family Blood Pressure Program, which has been described elsewhere [14]. Chinese and Japanese subjects were recruited at three field centers [Stanford (San Francisco Bay Area); Hawaii (Oahu); and Taiwan (three recruitment locations in Taipei and Taichung)]. The majority of subjects were recruited by identifying hypertensive probands through clinical record screening. Subjects were at least 35 years of age. Subjects older than 60 were included if they were hypotensive or if documentation of hypertensive status before age 60 could be provided. The consortium study protocol defined hypertension as 1) systolic blood pressure (SBP) >160 mmHg, diastolic blood pressure (DBP) of > 95 mmHg, 2) taking two medications for high blood pressure (beta blockers, calcium channel blockers, diuretics, or Angiotensin Converting Enzyme inhibitors), or 3) taking one medication for high blood pressure and still having SBP > 140 mmHg or DBP > 90 mmHg. Hypotension was defined as blood pressure (BP) in the bottom 30% of the age- and sex- adjusted distribution. For this population, hypotensive values were as follows: for males under age 45, SBP 115 mm Hg, DBP 76 mmHg; for females under age 45, SBP 107 mmHg, DBP < 70 mmHg; for males over 45, SBP 122 mmHg, DBP 78 mmHg; for females over 45, SBP 118 mmHg, DBP 75 mmHg. Families were excluded if both parents were treated for hypertension before 60 years of age. Subjects with a history of diagnosed diabetes, severe renal disease (creatinine < 1.5 mg/dL), or a body mass index (BMI) greater than 35 kg/m² were also excluded, as were individuals with cancer, a terminal illness, or individuals who were pregnant or fewer than 6 months post-partum.

Institutional review committees at all three field centers approved the study protocol. Written informed consent was obtained from all participants.

Blood pressure measurements

BP was measured according to a common protocol using the DINAMAP automated blood pressure reading device. Three readings were taken at 1 min intervals after subjects had rested in the sitting position for 10 min. The present analysis is based on the average of the second and third readings for SBP and DBP.

Laboratory

A 75-g oral glucose tolerance test (OGTT) was performed on each subject. Subjects with fasting plasma glucose level greater than 6.99 mmol/L were diagnosed with diabetes and excluded. The concentrations of plasma glucose, insulin, and lipids were measured as previously described [13–15].

SNP identification

A shotgun plasmid library was created using a bacterial artificial chromosomes (BAC) containing 150 Kb of human genomic DNA, including the entire coding region of the resistin gene and at least 20 Kb of upstream and downstream flanking DNA. Approximately 250 double-stranded, 3 Kb plasmid subclones were sequenced from both ends (ABI 3100

DNA Sequencer, Applied Biosystems, Inc., Foster City, CA, USA), and the contiguous sequences were assembled to obtain the full resistin gene sequence using the PHRED and PHRAP programs [16,17]. Thirty evenly-spaced sequence tagged sites were screened in 24 representative hypertensive study participants (50% Male, 50% Female; 50% Chinese, 50% Japanese). SNPs with allelic frequency > 5% were chosen for the association study using the POLYPHRED suite of programs [18].

SNP genotyping

Genotyping was performed on genomic DNA isolated from peripheral white blood cells using the Puregene DNA extraction kit (Minneapolis, MN., USA). SNPs were genotyped using the Taq Man® allelic discrimination assay (Applied Biosystems, Inc., Foster City, CA, USA). DNA was amplified by PCR using Taq Gold (Perkin-Elmer, Inc., Waltham, MA, USA) and detection of PCR products was accomplished by the Taq Man® assay using a 6-FAM-labeled FRET probe for one allele and a VIC-labeled probe for the other allele. PCR products were analyzed using an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Inc., Foster City, CA, USA).

Statistical analysis

Two regression models were estimated for tests of association between the dependent variables, factors associated with insulin resistance [BMI, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglycerides, fasting glucose and insulin, 120 min post-load glucose and insulin, and HOMA index], and the independent resistin genotype variables. One model compared the mean values of the two homozygotes, using the common homozygote as the comparison group, which makes no assumption concerning the inheritance of the trait. The additive model estimated the average effect of a substitution of one copy of the rarer allele for the common allele (the variable being coded as 0, 0.5, or 1 for the proportion of the rarer allele in the genotype). Both models included variables for age, sex, the interaction of age with sex, ethnicity, hypertensive status (four types, with three the types of hypertension plus hypotension), and recruitment center (five centers in all, with three centers in Taiwan plus Honolulu and Stanford). Exponential regression models were used for the quantitative variables, which allowed expressing the effect sizes as a per cent difference in means between genotypes; the antilog of a genotype's regression coefficient, $\exp(b)$, is the amount by which a mean is multiplied when an independent variable increases by one unit. Standard errors were obtained by the method of generalized estimating equations (GEE) which allows valid estimates of standard errors despite the presence of heteroscedasticity and correlated response variable values within sibships [19]. For dependent variables with highly skewed distributions (fasting and post load insulin, triglyceride level, and HOMA index), comparison of several regression models showed that working weights taking the standard deviation of the error term being proportional to the mean generally resulted in slightly smaller standard errors than by assuming that the variance was proportional to the mean or that the error variance was constant. The study design for hypertension analyses used sibship-matched hypotensive controls; to be consistent with the study design, tests for association between hypertension status (hypertensive versus hypotensive) with resistin SNP

genotype used conditional regression models with discordant sibships, adjusted for age, sex, and the interaction between age and sex.

Results

Descriptive statistics

Clinical and demographic characteristics of the study population are shown in Table 1. All subjects were Asian, and three quarters were of Chinese decent. The remaining population was of Japanese descent. Twenty seven percent of the study population was hypotensive, and the majority of these were Chinese due to more efficient access to patient records for recruitment of hypotensive individuals in Taiwan. The mean age of hypertensive subjects was slightly higher than that of hypotensive subjects. Hypertensive subjects differed from hypotensive subjects for all clinical characteristics except for LDL-C levels. There was no indication of a physiological difference between the Chinese and Japanese subjects (from the Hawaii and California sites only; data not shown).

Identification of resistin SNPs

Seven non-coding SNPs were identified in the resistin gene from the sub-sample of 24 hypertensive individuals, and of these, four SNPs with allelic frequencies >5% were selected for the association study. These SNPs have been previously reported in the NCBI database, and are identified here as: RETN2 (+299G>A; rs 3745367), RETN4 (+739C>G; rs 3219178), RETN5 (−420C>G; rs 1862513), and RETN6 (−358G>A; rs 3219175). The frequencies of the minor alleles by ethnicity are as follows. For Japanese, the minor allele frequencies for RETN2 through RETN6 with their standard errors are 0.434 (0.024), 0.314 (0.022), 0.359 (0.027), and 0.234 (0.022) (the standard errors are based on generalized estimating equations methodology and account for siblings having correlated genotypes). For Chinese, the corresponding allele frequencies are 0.367 (0.013), 0.266 (0.012), 0.352 (0.027), and 0.186 (0.010). Tests for differences in allele frequencies between Chinese and Japanese for the four polymorphisms have these p-values for RETN2-RETN6, respectively: $p = 0.024, 0.058, 0.78, \text{ and } 0.031$.

Association Analyses

The entire sample of hypertensive and hypotensive subjects was used to test for associations between the resistin SNPs and BMI, HDL-C, LDL-C, and triglyceride concentrations, and fasting and post-load glucose and insulin levels. For each phenotype, the effects of each of the four resistin SNPs were assessed using both the homozygote and additive regression models (Table 2). The estimated effects of genetic variation on the examined phenotypes were small; the two homozygote genotypes for each SNP had mean values that differed by only a few percent for most phenotypes. Of the 72 regression analyses performed, just one comparison was found to be significant at the 0.05 level; the less frequent RETN4 (+739G/G) homozygote had a covariate-adjusted mean BMI that was 2.9% higher than the mean BMI of the more common homozygote (+739C/C).

The widths of the 95% confidence intervals depend on the frequencies of the homozygote genotypes and also on the variability of the phenotype (Table 2). Confidence intervals were

most narrow for RETN2 and RETN5 and most wide for RETN6, and relatively narrow for fasting and post-load glucose, BMI, HDL-C, and LDL-C, but much wider for fasting and post-load insulin and triglyceride levels.

Conditional logistic regression models were used to test for association between the four common resistin SNPs and hypertension versus hypotension within sibships. None of the SNPs were significantly associated with hypertensive status (data not shown).

Discussion

Four common genetic variants were identified as having allelic frequencies > 5% in the sub-sample of 24 hypertensive Chinese and Japanese participants from the SAPHIRE study population. All of these were located in non-coding regions of the resistin gene. Analyses for the association between each of these four SNPs and factors related to IRS revealed only one significant association between the RETN4 (+739C>G) polymorphism and BMI. Since 3.6 out of 72 tests would be expected to be significant at the 0.05 level by chance alone, this isolated result gives little evidence to support a role for resistin as a genetic determinant of insulin resistance related variables in this population of individuals with high and low blood pressure. The 95% confidence intervals for the genotype effects were quite small for some variables such as BMI and fasting glucose, making a sizeable effect unlikely in this population. A post hoc power computation showed that for 80% power and a 5% type 1 error rate, the large sample size provided good power to detect fairly subtle effects for some variables: for fasting glucose and BMI, there would be 80% power to detect a decrease of only 3.7% and 3.0%, respectively, for the best case (RETN2b) and a decrease of 4.6% and 5.7%, respectively, for the worse case (RETN6b). For fasting insulin, which is quite variable across individuals, the minimal detectable differences for these SNPs would be much higher at 14.8% and 26.7%. Clearly, detecting genetic effects on fasting insulin level is a difficult problem and will require a very large sample size or, perhaps, repeated measures on individuals.

Association studies have provided a wealth of contradictory evidence regarding the influence of resistin gene polymorphisms on factors related to IRS, but it is possible that many of these apparently conflicting findings can be explained by a lack of statistical power due to small sample sizes. For example, although many studies report a lack of association between resistin gene variants and type 2 diabetes [4–6,10,20–25], only one of these analyzed a sample population larger than 1000 subjects [4]. When data from three of the negative result studies [20–22] were analyzed together with new samples in a meta-analysis of 3536 subjects, the combined results showed an association between the –420C>G variant (RETN5) and diabetes [26].

The sample size for the present study, over 1500 genotyped subjects, was larger than many gene association studies, and the confidence intervals for fasting and post-load serum glucose, HDL-C, LDL-C, and BMI were narrow; we therefore can be relatively confident that no major association exists between these traits and the common resistin SNPs present in the SAPHIRE study population. However, the person-to-person variation in triglyceride

and fasting and post-load insulin levels was high, making the confidence intervals wide. Thus, the negative results are less conclusive for these phenotypes.

The study design limited testing for association between resistin genotype and BMI, fasting glucose level, etc. among people who were non-obese and non-diabetic. Despite the lack of association of RETN SNPs with BMI in this sample, it still remains possible that the risk of frank obesity is associated with resistin genotype in the populations from which the samples were drawn. This might help explain some of the differences between the results of this study of people of East Asian ancestry and the two large studies in Japan which reported associations of RETN2 with BMI [11,12].

In summary, common variants in the resistin gene are not strongly associated with several factors related to IRS, including hypertension, BMI, high and low density cholesterol, and serum glucose, in the SAPHIRE population of non-diabetic hypertensive and hypotensive siblings of Japanese and Chinese origin who are not obese.

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Table 1

Demographics and clinical characteristics of the study population.

	Hypertensives (n=1137)	Hypotensives (n=419)
Chinese/Japanese	806/331	361/58
Male/Female	556/581	168/251
Age	52.23 ($\pm$ 8.29) *	47.52 ($\pm$ 7.88)
BMI (kg/m ²)	26.29 ($\pm$ 3.4)	23.75 ($\pm$ 3.09)
Glucose, fasting (mg/dL)	95.93 ($\pm$ 18.61)	89.14 ($\pm$ 10.74)
Glucose, 120 min post OGTT (mg/dL)	149.78 ($\pm$ 53.28)	125.79 ($\pm$ 39.93)
Insulin, fasting (mg/dL)	8.15 ($\pm$ 5.14)	6.05 ($\pm$ 4.49)
Insulin, 120 min post OGTT (mg/dL)	74.15 ($\pm$ 62.09)	48.86 ($\pm$ 47.99)
HDL-C (mg/dL)	44.19 ($\pm$ 12.36)	47.45 ($\pm$ 13.45)
LDL-C (mg/dL)	121.28 ($\pm$ 34.38)	116.42 ($\pm$ 36.11)
Triglycerides (mg/dL)	154.53 ($\pm$ 95.34)	107.21 ($\pm$ 64.2)
HOMA index	1.99 ($\pm$ 1.43)	1.37 ($\pm$ 1.19)

*
Standard deviation in parentheses.

Table 2

Effect of resistin genotypes on variables related to insulin resistance. Genotype effects are presented as percent difference between the means of the comparison genotypes.

Response Variable	Resistin SNP	Genotype Comparison	Effect Size in Percent (95% confidence interval)	p-Value
BMI	RETN2	homozygotes*	-0.7% (-2.8 – 1.4)	0.52
		additive†	-1.1% (-3.1 – 0.9)	0.26
	RETN4	homozygotes	2.9% (0.3 – 5.4)	0.03
		additive	1.8% (-0.3 – 4.0)	0.10
	RETN5	homozygotes	-0.7% (-3.0 – 1.6)	0.56
		additive	-1.1% (-3.1 – 1.1)	0.33
	RETN6	homozygotes	0.0% (-4.0 – 4.2)	0.98
		additive	-1.5% (-4.0 – 1.1)	0.26
HDL-C	RETN2	homozygotes	2.5% (-1.8 – 7.0)	0.26
		additive	2.4% (-1.8 – 6.8)	0.27
	RETN4	homozygotes	-1.6% (-5.7 – 2.7)	0.46
		additive	-3.3% (-7.8 – 1.5)	0.18
	RETN5	homozygotes	1.6% (-2.4 – 5.8)	0.43
		additive	2.0% (-1.8 – 6.0)	0.31
	RETN6	homozygotes	1.1% (-6.2 – 8.9)	0.77
		additive	0.9% (-3.9 – 6.0)	0.72
LDL-C	RETN2	homozygotes	-2.5% (-7.0 – 2.3)	0.30
		additive	-3.0% (-7.4 – 1.5)	0.19
	RETN4	homozygotes	-0.7% (-6.2 – 5.0)	0.79
		additive	-1.4% (-5.9 – 3.3)	0.55
	RETN5	homozygotes	0.0% (-4.3 – 4.5)	0.99
		additive	0.9% (-3.3 – 5.2)	0.69
	RETN6	homozygotes	0.0% (-7.6 – 8.3)	1.00
		additive	0.4% (-4.8 – 5.9)	0.88
Triglycerides	RETN2	homozygotes	-3.4% (-13.7 – 8.2)	0.55
		additive	-4.7% (-14.4 – 6.1)	0.38
	RETN4	homozygotes	0.4% (-10.4 – 12.5)	0.95
		additive	2.6% (-7.3 – 13.4)	0.62
	RETN5	homozygotes	-7.0% (15.9 – 2.9)	0.16
		additive	-1.9% (-9.4 – 7.4)	0.68
	RETN6	homozygotes	-10.8% (-26.8 – 8.7)	0.26
		additive	-6.2% (-17.0 – 6.0)	0.30
Fasting Glucose	RETN2	homozygotes	-1.1% (-3.6 – 1.6)	0.43
		additive	-0.4% (-3.1 – 2.3)	0.76
	RETN4	homozygotes	-1.8% (-4.9 – 1.5)	0.29
		additive	-1.0% (-4.1 – 2.1)	0.52

Response Variable	Resistin SNP	Genotype Comparison	Effect Size in Percent (95% confidence interval)	p-Value
120 min Post-OGTT Glucose	RETN5	homozygotes	0.4% (−2.4 – 3.3)	0.78
		additive	0.1% (−2.6 – 2.8)	0.94
	RETN6	homozygotes	−1.3% (−4.5 – 2.0)	0.43
		additive	−1.1% (−4.0 – 1.9)	0.47
	RETN2	homozygotes	0.3% (−5.2 – 6.1)	0.92
		additive	0.0% (−5.4 – 5.6)	0.99
	RETN4	homozygotes	−5.0% (−10.7 – 0.9)	0.10
		additive	−1.2% (−6.7 – 4.5)	0.66
	RETN5	homozygotes	−2.4% (−7.6 – 3.0)	0.37
		additive	−0.6% (−5.6 – 4.7)	0.82
	RETN6	homozygotes	−1.5% (−10.1 – 7.8)	0.74
		additive	−3.3% (−9.4 – 3.3)	0.32
Fasting Insulin	RETN2	homozygotes	4.0% (−8.0 – 17.6)	0.53
		additive	0.1% (−11.0 – 12.1)	0.98
	RETN4	homozygotes	8.6% (−5.8 – 25.3)	0.25
		additive	1.4% (−9.9 – 14.2)	0.82
	RETN5	homozygotes	−5.6% (−15.7 – 5.6)	0.31
		additive	−4.6% (−13.7 – 5.4)	0.35
	RETN6	homozygotes	4.5% (−16.0 – 29.9)	0.69
		additive	0.9% (−11.5 – 14.9)	0.90
	RETN2	homozygotes	0.0% (−13.5 – 15.5)	1.00
		additive	−5.1% (−17.9 – 9.7)	0.48
	RETN4	homozygotes	2.7% (−14.8 – 23.7)	0.78
		additive	−3.0% (−16.9 – 13.1)	0.69
120 min Post-OGTT Insulin	RETN5	homozygotes	−8.4% (−20.4 – 5.3)	0.22
		additive	−3.6% (−15.3 – 9.7)	0.58
	RETN6	homozygotes	−1.1% (−20.2 – 22.8)	0.92
		additive	−3.7% (−18.6 – 14.0)	0.66
	RETN2	homozygotes	−4.0% (−11.3 – 4.0)	0.32
		additive	−1.4% (−12.4 – 11.1)	0.82
	RETN4	homozygotes	−4.0% (−3.8 – 4.4)	0.35
		additive	1.8% (−11.2 – 16.8)	0.79
	RETN5	homozygotes	−3.3% (−11.3 – 5.3)	0.43
		additive	−6.5% (−16.5 – 4.8)	0.25
	RETN6	homozygotes	−2.8% (−10.4 – 5.3)	0.48
		additive	−3.0% (−15.9 – 11.8)	0.67
HOMA index	RETN2	homozygotes	−4.0% (−11.3 – 4.0)	0.32
		additive	−1.4% (−12.4 – 11.1)	0.82
	RETN4	homozygotes	−4.0% (−3.8 – 4.4)	0.35
		additive	1.8% (−11.2 – 16.8)	0.79
	RETN5	homozygotes	−3.3% (−11.3 – 5.3)	0.43
		additive	−6.5% (−16.5 – 4.8)	0.25
	RETN6	homozygotes	−2.8% (−10.4 – 5.3)	0.48
		additive	−3.0% (−15.9 – 11.8)	0.67

* Percent difference between the homozygote means.

† The independent variable was coded as 0, 0.5, 1 for common homozygote, heterozygote, and less common homozygote, respectively, so the estimated effect is the percent change per allele substitution.