

BRIEF REPORT

Heritability of Serum Resistin and Its Genetic Correlation with Insulin Resistance-Related Features in Nondiabetic Caucasians

Claudia Menzaghi, Angelo Coco, Lucia Salvemini, Ryan Thompson, Salvatore De Cosmo, Alessandro Doria, and Vincenzo Trischitta

Research Unit of Diabetology and Endocrinology (C.M., A.C., L.S., S.D.C., V.T.), Scientific Institute Casa Sollievo della Sofferenza, 71013 San Giovanni Rotondo (FG), Italy; Research Division (R.T., A.D.), Joslin Diabetes Center, and Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115; and Department of Clinical Sciences (V.T.), University “La Sapienza”, 00161 Rome, Italy

Context: Serum levels of resistin are believed to modulate insulin resistance in humans.

Objective: The aim of this study was to investigate whether serum resistin levels are genetically controlled and whether this control is shared with other insulin resistance traits.

Design and Methods: The study cohort included 264 nondiabetic probands, Caucasian from Italy, and their 473 adult family members. Phenotypic characterization included anthropometric variables, blood pressure, fasting glucose and insulin, lipid profile, and resistin levels. Genotypes were determined at position g.-420C→G (rs1862513), IVS2+181G→A (rs3745367), and GAT_(n) polymorphisms of the resistin (*RETN*) gene.

Results: In the 264 unrelated probands, resistin levels were significantly ($P < 0.01$) correlated with adiposity, blood pressure, C-reactive

protein, and the metabolic syndrome score. In a variance component analysis of the 264 probands and their 473 relatives, about 70% of the observed variation of serum resistin levels was heritable ($P < 0.0001$). A small, but significant ($P = 0.004$) proportion of this variance was explained by the G→A variation at position IVS2+181 of the *RETN* gene. Significant genetic correlations ($P < 0.05$) were observed between resistin and body mass index ($\rho_g = 0.30$), waist circumference ($\rho_g = 0.32$), the insulin resistance index HOMA_{IR} ($\rho_g = 0.28$), and the metabolic syndrome score ($\rho_g = 0.35$).

Conclusions: These data indicate that serum resistin is highly heritable and has some common genetic background with traits related to insulin resistance, reinforcing the hypothesis that this adipokine may play a pathogenic role in insulin resistance-related abnormalities, including type 2 diabetes and cardiovascular disease. (*J Clin Endocrinol Metab* 91: 2792–2795, 2006)

RESISTIN, A CYSTEINE-RICH peptide also known as ADSF (adipocyte secreted factor) or FIZZ-3 (found in inflammatory zone 3), is believed to play a role in several metabolic pathways (1, 2) and inflammatory responses (3, 4). Many, although not all, studies have reported an association between elevated circulating resistin levels and insulin resistance-related abnormalities in humans (5–8). In addition, several single-nucleotide polymorphisms (SNPs) have been identified in the human resistin gene (*RETN*) and reported to be associated with insulin resistance, obesity, and type 2 diabetes (9–14). Of note, one of these (a C to G substitution at position –420 in the 5' flanking region of the gene) alters transcriptional activity and is associated with increased resistin mRNA levels in abdominal fat (14) and elevated serum resistin levels (13, 14). Based on these data, resistin has been

proposed as a novel mediator of insulin resistance in humans.

The aim of our study was to investigate whether serum resistin levels are genetically determined and, if so, to assess whether this trait shares a common genetic background with metabolic features related to insulin resistance. To this end, we studied 737 nondiabetic individuals from 264 nuclear families, for whom resistin levels, several variables related to insulin resistance, and the genotypes of the g.-420C→G (rs1862513), IVS2+181G→A (rs3745367), and GAT_(n) polymorphisms of the *RETN* gene were determined. These polymorphisms were chosen because of the previously described associations either with insulin resistance and obesity in our population (*i.e.* IVS2+181G→A and GAT_(n)) (10, 12) or with circulating resistin levels in other studies (*i.e.* –420C→G) (13, 14).

Subjects and Methods

Subjects

A total of 264 unrelated nondiabetic subjects recruited among the employees of the Scientific Institute “Casa Sollievo della Sofferenza” (San Giovanni Rotondo, Italy) and their 473 first-degree relatives volunteered to participate and were recruited as previously described (15).

First Published Online May 2, 2006

Abbreviations: BMI, Body mass index; CRP, C-reactive protein; HDL, high-density lipoprotein; HOMA_{IR}, insulin resistance index homeostasis model assessment; PAI-1, plasminogen activator inhibitor type 1; SNP, single-nucleotide polymorphism.

JCEM is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

Inclusion criteria were a fasting plasma glucose less than 7 mmol/liter and the lack of treatment with any medications. Briefly, subjects were examined between 0800 and 0900 h after an overnight fast. Height, weight, waist and hip circumferences, and blood pressure were measured in duplicate, and a blood sample was drawn for biochemical measurements and DNA extraction. Plasma glucose (mmol/liter), serum insulin (pmol/liter), and lipid profile [total serum cholesterol, high-density lipoprotein (HDL) cholesterol, and serum triglycerides] were measured using commercially available enzymatic kits. The insulin resistance index homeostasis model assessment (HOMA_{IR}) was calculated as fasting serum insulin (pmol/liter) × fasting plasma glucose (mmol/liter)/135. Serum C-reactive protein (CRP) concentrations were measured using a high-sensitivity automated nephelometric method (Dade Behring Marburg GmbH, Marburg, Germany), which had a detection limit of 0.05 mg/liter and a maximum coefficient of variation of 6%. Circulating levels of resistin were measured in serum samples obtained after an overnight fast. The concentration of serum resistin was measured using a commercial ELISA kit (Bio Vendor, Brno, Czech Republic). Inter- and intraassay coefficients of variation were 3.2–4% and 6.3–7.2%, respectively. Metabolic syndrome scores were calculated according to the Adult Treatment Panel III criteria (16). Clinical characteristics of study subjects are reported in Table 1. The study and the informed consent procedures were approved by the local research committee.

SNP genotyping

Genotypes at position g.-420 (rs1862513) and IVS2+181 (rs3745367) of the *RETN* gene were determined by means of PCR followed by single-base extension/fluorescence polarization (AcyloPrime-FP SNP Detection System) using a Wallac VICTOR² Multilabel Plate Reader (Perkin-Elmer, Boston, MA). The GAT_(n) of the *RETN* gene was genotyped by means of electrophoresis of fluorescence-labeled PCR products using an ABI 3100 Genetic Analyzer (Applied Biosystems, Monza, Italy) and standard protocols.

Data analysis

Data are summarized as the mean ± SD. Because of their skewed distribution, serum resistin, insulin, HOMA_{IR}, triglycerides, CRP, and plasminogen activator inhibitor type 1 (PAI-1) were analyzed after logarithmic transformation. To determine the contribution of genetic factors to serum resistin, the SOLAR software package (version 2.0) (17) was used. SOLAR performs a variance component analysis of family data that decomposes the total variance of the phenotype (serum resistin) into components that are a result of genetic effects (*i.e.* polygenic, additive genetic variance), measured covariates, and random environmental effects (*i.e.* measured en-

vironmental factors and random unmeasured factors). The relative contribution of genetic factors to serum resistin is then estimated by heritability (*h*²), defined as the ratio of the genetic variance component to the residual (after removal of covariates) phenotypic variance. All variance component analyses were performed with age and gender included as covariates in the model. To evaluate the contribution of the *RETN* genotypes to resistin variance, analyses were repeated after introducing a covariate coded as 1 for homozygous individuals for the major allele, 0 for heterozygotes, and −1 for homozygous individuals for the minor allele. Secondary analyses were also performed assuming dominant and recessive models. Bivariate analyses were conducted to partition the phenotypic correlation between two traits (ρ_p) into genetic (ρ_g) and environmental (ρ_e) correlations according to the equation $\rho_p = \rho_g \sqrt{h_1^2 h_2^2} + \rho_e \sqrt{(1 - h_1^2)(1 - h_2^2)}$ where h_1^2 and h_2^2 correspond to the heritability of traits 1 and 2, respectively (18). Evidence of pleiotropy (a common set of genes influencing more than one trait) is indicated by a genetic correlation significantly different from zero. A shared environmental effect is implied by a significant environmental correlation.

Results and Discussion

Salient clinical characteristics of the 264 unrelated subjects and their 473 first-degree relatives are shown in Table 1. Among the 264 unrelated individuals, resistin levels were positively correlated, after adjusting for age and gender, with body mass index (BMI) ($r = 0.19$; $P = 0.003$), waist circumference ($r = 0.19$; $P = 0.007$), mean blood pressure ($r = 0.16$; $P = 0.0012$), CRP ($r = 0.27$; $P < 0.001$), and the metabolic syndrome score ($r = 0.17$; $P = 0.011$). The overall effect of genetic factors on serum resistin was investigated by variance component analysis. After adjusting for age and gender, serum resistin was found to be highly heritable, with almost 70% of its variability explained by genetic factors ($h^2 = 0.68 \pm 0.078$; $P < 0.0001$). Of the three polymorphisms that we analyzed, only IVS2+181G→A explained a small proportion of resistin variance (1.5%; $P = 0.004$) when considered under an additive model. Similar results were obtained under a recessive model, whereas none of the polymorphisms had a significant effect using a dominant model. Table 2 shows the genetic (ρ_g) and environmental (ρ_e) correlations between serum resistin levels and insulin resis-

TABLE 1. Clinical characteristics of the study subjects

	Nondiabetic unrelated subjects			Nondiabetic first-degree relatives		
	Mean ± SD	Median	Range	Mean ± SD	Median	Range
Male/female	90/174			191/282		
Age (yr)	32.5 ± 10.6	31.5	16–63	45.1 ± 14.6	46.0	16–82
BMI (kg/m ²)	24.9 ± 4.6	24.2	17–47.6	27.0 ± 4.7	26.4	17.2–48.2
% Overweight	13.3			36.2		
% Obese	24.3			23.3		
Waist (cm)	81.1 ± 12.3	80.0	60–133	86.7 ± 12.3	87.0	50–126
MBP (mm Hg)	87.2 ± 8.5	86.7	64–113	92.3 ± 10.5	93.3	63–135
FBG (mg/dl)	88.6 ± 8.9	88.5	60–125	91.6 ± 10	90.0	58–125
Serum insulin (μU/ml)	8.0 ± 4.3	7.1	1.9–32.7	8.0 ± 4.8	7.0	0.5–48.0
HOMA _{IR}	1.7 ± 1.0	1.5	0.36–8.4	1.8 ± 1.2	1.5	0.10–10.0
Cholesterol (mg/dl)	188.7 ± 38.6	184.0	109–314	207 ± 40.9	204.5	85–346
HDL-cholesterol (mg/dl)	53 ± 13.2	52.0	20–97	53.2 ± 13.5	52	21–119
Triglycerides (mg/dl)	92.9 ± 86.9	71.0	28–875	110.6 ± 86.6	88.0	28–1214
MS score	0.66 ± 0.87	0.5	0–4	1.07 ± 1.08	1.0	0–5
% affected	4.2			11.2		
CRP (mg/liter)	0.4 ± 0.2	0.35	0.3–3.7	0.4 ± 0.2	0.35	0.3–3.7
PAI-1 (ng/ml)	21.2 ± 12.9	19.0	3.0–70	26.2 ± 16.4	23.8	3.0–165
Fibrinogen (mg/dl)	287.5 ± 54.0	282.0	163–463	297.0 ± 68.6	289.5	149–744
Serum resistin (ng/ml)	5.6 ± 2.3	5.2	1.7–17.5	6.1 ± 3.3	5.4	1.2–26.8

FBG, Fasting blood glucose; MS score, metabolic syndrome score; MBP, mean blood pressure.

TABLE 2. Genetic (ρ_g) and environmental (ρ_e) correlations between resistin serum levels and insulin resistance-related features

	ρ_g	P	ρ_e	P
BMI	0.30 \pm 0.11	0.0063	−0.13 \pm 0.14	0.37
Waist	0.32 \pm 0.11	0.0071	−0.08 \pm 0.14	0.53
MBP	0.07 \pm 0.14	0.63	0.17 \pm 0.13	0.19
Triglycerides	0.10 \pm 0.11	0.37	0.02 \pm 0.14	0.64
HDL-cholesterol	−0.08 \pm 0.1	0.49	−0.03 \pm 0.15	0.82
FBG	0.26 \pm 0.14	0.06	−0.36 \pm 0.12	0.004
Serum insulin	0.24 \pm 0.14	0.09	−0.11 \pm 0.13	0.19
HOMA _{IR}	0.28 \pm 0.14	0.05	−0.16 \pm 0.13	0.2
MS score	0.35 \pm 0.15	0.017	−0.17 \pm 0.11	0.16
Fibrinogen	−0.16 \pm 0.13	0.21	0.29 \pm 0.12	0.02
CRP	0.28 \pm 0.17	0.08	0.17 \pm 0.11	0.12
PAI-1	0.28 \pm 0.16	0.09	−0.07 \pm 0.13	0.56

Analyses are adjusted for age and gender. MBP, Mean blood pressure; FBG, fasting blood glucose; MS score, metabolic syndrome score.

tance-related traits. Significant genetic correlations were observed with adiposity indices ($\rho_g = 0.30$ and 0.32 for BMI and waist circumference, respectively), the insulin resistance index HOMA_{IR} ($\rho_g = 0.28$), and the metabolic syndrome score ($\rho_g = 0.35$), indicating some degree of sharing of genetic background between resistin and insulin-resistance traits. However, addition of these variables to the variance component model did not substantially decrease the resistin heritability estimate ($h^2 = 0.68 \pm 0.078$, adjusted for HOMA_{IR} and metabolic syndrome score; $h^2 = 0.66 \pm 0.08$ adjusted for measures of adiposity), suggesting that genes unrelated to insulin-resistance pathways are also involved and might play a greater role than insulin-resistance genes in the modulation of resistin levels.

A tendency to share the same genetic background with resistin was also observed for fasting blood glucose, serum insulin, CRP, and PAI-1, although statistical significance was not reached for these traits with this sample size ($P = 0.06–0.09$). The inclusion of the IVS2+181G→A SNP in the model did not affect the genetic correlation observed between resistin and insulin resistance-related traits (data not shown). Significant environmental correlations (ρ_e) were observed with only two traits, namely fibrinogen and fasting plasma glucose (Table 2). The positive environmental correlation between resistin levels and fibrinogen, an inflammation marker, is consistent with our present understanding of the relationship between insulin resistance and inflammation. By contrast, the negative ρ_e coefficient between fasting plasma glucose and serum resistin, indicating an inverse environmental correlation between resistin and fasting plasma glucose levels, does not have a straightforward explanation. Similar apparently contradictory results have been reported in the literature for environmental correlations concerning features of the metabolic syndrome (18) or type 2 diabetes (19) and are difficult to interpret on the basis of the currently available data on the etiology of complex disorders.

To the best of our knowledge, this is the first study demonstrating significant heritability of serum resistin in humans. In the population that we studied, heritable factors account for approximately 70% of the observed variation in resistin levels, indicating a strong genetic control of this trait. This observation is consistent with the results of a previous

study reporting a significant heritability of resistin mRNA expression in the omental adipose tissue of baboons (20). Thus, heritability of resistin expression and/or circulating levels appears to be a generalized phenomenon in primates.

The g.−420 polymorphism in the promoter of the resistin gene was a strong candidate for such a genetic effect, given its impact on transcriptional activity and the previous reports of association with resistin levels, obesity, and type 2 diabetes (9, 14). However, in our population, this polymorphism did not contribute significantly to serum resistin variation. By contrast, we found a significant effect of the IVS2+181G→A SNP, which we previously reported to interact with obesity in modulating the risk of type 2 diabetes (10). The effect of this SNP, however, is rather small, accounting for only 1.5% of resistin variance. Although we cannot exclude that SNPs in as yet unscreened regulatory regions of *RETN* may play a greater role, our data suggest that serum resistin is mostly regulated by genes other than that coding for this molecule. Given the high degree of resistin heritability that we observed in this Caucasian population, it should be feasible to identify at least some of these other genes by means of genome-wide linkage screens.

In conclusion, our data indicate that circulating resistin levels are under a strong additive genetic control, which is shared in part with other traits related to insulin resistance. Although not a primary aim of our study, our results also confirm previously published data showing a moderate correlation between resistin levels and several features of the insulin resistance syndrome in the general population (5–8). Taken together, these data reinforce the hypothesis that abnormal serum resistin levels may play a pathogenic role in the development of insulin resistance-related abnormalities, including type 2 diabetes and cardiovascular disease.

Acknowledgments

Received December 15, 2005. Accepted April 21, 2006.

Address all correspondence and requests for reprints to: Claudia Menzaghi, Ph.D., Research Unit of Diabetology and Endocrinology, Scientific Institute “Casa Sollievo della Sofferenza”, 71013 San Giovanni Rotondo (FG) Italy. E-mail: c.menzaghi@operapadrepio.it; or Vincenzo Trischitta, M.D., Department of Clinical Sciences, University “La Sapienza”, Rome, Italy. E-mail: vincenzo.trischitta@uniroma1.it

Disclosure of potential conflicts of interest: C.M., A.C., L.S., R.T., S.D.C., A.D., and V.T. have nothing to declare.

This research was supported by Italian Ministry of Health Grant Ricerca Corrente 2005 (to C.M.), by Ministry of University and Scientific Research FIRB RBNE01C5S2_005 (to V.T.), by National Institutes of Health Grant HL73168 and a Grant-in-Aid from the American Heart Association (to A.D.), and by the Genetics Core of the Diabetes and Endocrinology Research Center at the Joslin Diabetes Center (DK36836).

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