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Serum Resistin and Kidney Function: A Family-Based Study in Non-Diabetic, Untreated Individuals

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Abstract

**Background:** High serum resistin levels have been associated with kidney dysfunction. Most of these studies have been carried out in individuals with severe kidney impairment, diabetes, cardiovascular disease and related treatments. Thus, the observed association might have been influenced by these confounders. Our aim was to study the relationship between serum resistin, urinary albumin/creatinine ratio (ACR) and glomerular filtration rate (GFR) in a family-based sample, the Gargano Family Study (GFS) of 635 non diabetic, untreated Whites.

**Methods:** A linear mixed effects model and bivariate analyses were used to evaluate the phenotypic and genetic relations between serum resistin and both ACR and eGFR. All analyses were adjusted for sex, age, age squared, BMI, systolic blood pressure, smoking habits and physical exercise.

**Results:** After adjustments, resistin levels were slightly positively associated with ACR ($\beta \pm SE = 0.049 \pm 0.023$, $p = 0.035$) and inversely related to eGFR ($\beta \pm SE = -1.43 \pm 0.61$, $p = 0.018$) levels. These associations remained significant when either eGFR or ACR were, reciprocally, added as covariates. A genetic correlation ($\rho_g = -0.31 \pm 0.12$; adjusted $p = 0.013$) was observed between resistin and eGFR (but not ACR) levels.

**Conclusion:** Serum resistin levels are independently associated with ACR and eGFR in untreated non-diabetic individuals. Serum resistin and eGFR share also some common genetic background. Our data strongly suggest that resistin plays a role in modulating kidney function.


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Introduction

Kidney dysfunction is a worldwide public health concern and a major risk factor for end-stage renal disease, cardiovascular events and premature death [1]. Identifying and treating risk factors for this abnormality may be a valuable approach to prevent such devastating clinical outcomes [1]. Insulin resistance, inflammation and endothelial dysfunction have been recognized, among other factors, as prime movers of kidney dysfunction [2,3,4]. Recently, new molecules, secreted by adipose tissue and known as adipokines, have been linked to all the above mentioned conditions [5]. Among these is resistin, a 12.5 kDa cysteine-rich protein which is also abundantly secreted by macrophages [6]. High serum resistin levels have been associated with kidney dysfunction, including low glomerular filtration rate (GFR) and increased albuminuria, in several studies [7,8,9,10,11,12,13]. However, most of these data were obtained in cohorts with severe kidney impairment and cardiovascular disease and with a high proportion of diabetic patients [7,8,9,10,11,12]. Of note, the only study in the general population was carried out in Japanese individuals among whom, however, hypertension was highly prevalent (i.e. almost 50%) [13]. Thus, the observed associations might have been influenced by any of these abnormalities as well as their related treatments (i.e. thiazolidinediones, statins and anti-hypertensive drugs), which are known to affect circulating resistin levels [14,15,16].

The aim of the present study was to investigate the relationship between circulating resistin levels and renal function, as assessed by urinary albumin/creatinine ratio (ACR) and GFR in the absence of the above mentioned confounders. To pursue this aim a family-based sample, the Gargano Family Study (GFS) of 635
non-diabetic Whites individuals, not treated with medications known to interfere with glucose homeostasis, lipid profile, blood pressure and/or to modulate resistin and ACR levels were studied. We also addressed the issue of whether or not circulating resistin shares a common genetic background with either trait and, if so, if this was explained at least in part by two SNPs in the resistin gene RETN (i.e. rs1862513 and rs3745367) that have been previously associated with resistin levels [17,18,19].

Materials and Methods

Subjects

The GFS comprises a total of 635 non-diabetic White individuals, from 218 families recruited in the Gargano area (an homogeneous geographical area in Center-East Italy) examined as previously described [18,20,21]. Briefly, subjects were examined between 08:00 and 09:00 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.

All study subjects were not treated with medications known to interfere with glucose homeostasis, lipid profile, blood pressure and known to modulate resistin and ACR levels.

Ethics

The study and the informed consent procedures were approved by the local Institutional Ethic Committee IRCCS (Istituto di Ricovero e Cura a Carattere Scientifico) “Casa Sollievo della Sofferenza”. All participants gave written consent.

Measurements

Plasma glucose was measured by the glucose oxidase method on a Beckman Glucose Analyzer 2 (Beckman Coulter, Inc., Fullerton, CA), serum insulin was measured by microparticle enzyme immunoassay (Abbott IMx Insulin Assay, Abbott Laboratories, Abbott Park, IL), and lipid profile (total serum cholesterol, HDL cholesterol and serum triglycerides) were measured by enzymatic method, Cobas, Roche Diagnostic, Welwin Garden City, Herts, UK.

Serum resistin concentrations were measured by a commercial ELISA kit (Bio Vendor, Brno Czech Republic). Inter- and intra-assay coefficients of variation were 3.2-4% and 6.3-7.2%, respectively [18].

Urinary albumin and creatinine concentrations were determined the same morning of the clinical examination on an early morning first void sterile urine sample by the nephelometric method (Behring Nephelometer Analyzer) and the Jaffè reaction-rate method (Hitachi 737 Autoanalyzer), respectively. Elevated urinary albumin excretion was diagnosed if the ACR was ≥2.5 mg/mmol in men and ≥3.5 mg/mmol in women.

GFR (eGFR) was estimated by CKD-EPI creatinine formula [22].

The insulin resistance index homeostasis model assessment (HOMAIR) was calculated as fasting serum insulin (pmol/liter) x fasting plasma glucose (mmol/liter)/135 [18,20,21].

Genotyping

SNPs rs1862513 and rs3745367 in the RETN gene, selected because of their previous association with resistin circulating levels [17,18,19] were genotyped as previously described [18]. In addition, for the RETN gene (i.e. 1,369 bp), rs3745367 is the only tag SNP described for CEU population (phase 2+3 HapMap database -www.hapmap.org- February 2009). Call rate and concordance rate were >98% and >99% respectively. Out of 635 study individuals, genotypes were available for 628 study subjects for rs1862513 and for 627 study subjects for rs3745367. Allele and genotype frequencies were as follows. For rs1862513, C: 68.1% and G: 31.9%; CC: 47.6%, CG: 41% and GG: 11.4%, respectively. For rs3745367, G: 68.6% and A: 31.4%; GG: 48.2%, GA: 40.8% and AA: 11%, respectively. Both SNPs were in Hardy–Weinberg Equilibrium (HWE) (p>0.05).
### Results

Clinical characteristics of study participants from the GFS are shown in Table 1. This study comprises 140 nuclear families, 75 sibships and 20 extended sibships (ranging 3–5 individuals). As previously reported [18] in this set, after adjusting for sex, age, age squared, smoking habits and physical exercise, serum resistin was highly correlated, ($r^2 = 0.73, \pm 0.08$ P = 6.05 × 10$^{-18}$).

As previously associated with serum resistin in the same setting [18] explains some phenotypic variation (15%) for serum resistin. By contrast SNP rs1862513 in our sample is not associated with resistin (p = 0.73). Neither SNP rs3745367 nor SNP rs1862513 were associated with ACR (p = 0.39 and p = 0.56, respectively) or eGFR (p = 0.41 and p = 0.53, respectively).

No significant environmental correlations were found between resistin and kidney function traits (Table S1).

Sixty-two out of 635 individuals (9.8% of the whole sample) were diagnosed as having hypertension (i.e. systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg). Exclusion of these individuals did not affect any of the results described above (data not shown).

### Discussion

The main finding of our study is that serum resistin is inversely associated with eGFR and shares with it a common genetic (but not environmental) background.

Importantly, of all the variables we had the opportunity to test in our study, resistin is by far the most important predictor of eGFR, accounting for 7% of its variance. Taken together, our data suggest that resistin is a strong modulator of kidney function.

We also observed a weaker association between resistin and ACR. However, the level of statistical significance of this association is marginal making the relevance of this finding uncertain.

Unfortunately we were unable to assess if the established notion of a common genetic background between serum resistin and GFR could be explained by some variation at the RETN locus. In fact, of the two SNPs we investigated, the only one which was associated with serum resistin (i.e. rs3745367), was not associated with eGFR.

It must be emphasized that the sample that we analyzed consists only of non-diabetic, untreated, relatively young individuals (mean age = 40 years) with normal kidney function and no clinical reports of cardiovascular disease. Thus, our results are not influenced by the possible confounders that may have heavily affected previous studies addressing the role of resistin on kidney function [7,8,9,10,11,12,13].
In conclusion, our study shows a strong and independent correlation between resistin levels and eGFR. The two variables share also some common genetic background. Dissection of the exact mechanisms underlying this relationship may help develop tailored interventions aimed at preventing kidney function loss in high risk individuals.

**Supporting Information**

**Table S1** Genetic, environmental and phenotypic correlations between serum resistin levels and kidney functions in the GFS. (DOC)

**Author Contributions**

Conceived and designed the experiments: CM VT. Performed the experiments: LS GF RT DM MG CDB. Analyzed the data: CM SDC AD VT. Contributed reagents/materials/analysis tools: CM RDP EM SDC AD VT. Wrote the paper: CM AD VT.

**References**