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Accessibility
Association of Variants in *RETN* With Plasma Resistin Levels and Diabetes-Related Traits in the Framingham Offspring Study

Marie-France Hivert,1,2 Alisa K. Manning,3 Jarred B. McAteer,4,5 Josée Dupuis,3 Caroline S. Fox,6,7 L. Adrienne Cupples,3 James B. Meigs,1,2 and Jose C. Florez2,4,5

OBJECTIVE—The *RETN* gene encodes the adipokine resistin. Associations of *RETN* with plasma resistin levels, type 2 diabetes, and related metabolic traits have been inconsistent. Using comprehensive linkage disequilibrium mapping, we genotyped tag single nucleotide polymorphisms (SNPs) in *RETN* and tested associations with plasma resistin levels, risk of diabetes, and glycemic traits.

RESEARCH DESIGN AND METHODS—We examined 2,531 Framingham Offspring Study participants for resistin levels, glycemic phenotypes, and incident diabetes over 28 years of follow-up. We genotyped 21 tag SNPs that capture common (minor allele frequency >0.05) or previously reported SNPs at $r^2 > 0.8$ across *RETN* and its flanking regions. We used sex- and age-adjusted linear mixed-effects models (with/without BMI adjustment) to test additive associations of SNPs with traits, adjusted Cox proportional hazards models accounting for relatedness for incident diabetes, and generated empirical $P$ values ($P_e$) to control for type 1 error.

RESULTS—Four tag SNPs (rs1477341, rs4804765, rs1423096, and rs10401670) on the 3' side of *RETN* were strongly associated with resistin levels (all minor alleles associated with higher levels, $P_e < 0.05$ after multiple testing correction). rs10401670 was also associated with fasting plasma glucose ($P_e = 0.02$, BMI adjusted) and mean glucose over follow-up ($P_e = 0.01$; BMI adjusted). No significant association was observed for adiposity traits. On meta-analysis, the previously reported association of SNP −420C/G (rs1862513) with resistin levels remained significant ($P = 0.0009$) but with high heterogeneity across studies ($P < 0.0001$).

CONCLUSIONS—SNPs in the 3' region of *RETN* are associated with resistin levels, and one of them is also associated with glucose levels, although replication is needed. *Diabetes* 58: 750–756, 2009

From the 1General Medicine Division, Massachusetts General Hospital, Boston, Massachusetts; the 2Department of Medicine, Harvard Medical School, Boston, Massachusetts; the 3Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts; the 4Center for Human Genetic Research and Diabetes Research Center (Diabetes Unit), Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts; the 5Program in Medical and Population Genetics, Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, Massachusetts; the 6Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts; and the 7National Heart, Lung, and Blood Institute’s Framingham Heart Study, Framingham, Massachusetts.

Corresponding author: Jose C. Florez, jcflorez@partners.org.

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using comprehensive tag SNP linkage disequilibrium (LD) mapping.

**RESEARCH DESIGN AND METHODS**

The Framingham Offspring Study is a large community-based prospective cohort study designed to investigate cardiovascular disease risk factors. This analysis includes 2,531 participants (including 285 pedigrees and 1,445 unrelated individuals) who were followed over 28 years on a periodic basis (from exam 1 [1971–74] up to exam 7 [1998–2001]). Each exam cycle included anthropometric measurements, a physical exam, and blood samples related to cardiovascular risk factors. The study was approved by the institutional review boards of Massachusetts General Hospital, Boston University, and the Massachusetts Institute of Technology; written informed consent, including consent for genetic analyses, was obtained from all study participants.

Participants underwent standardized procedures for all anthropometric measurements (weight, height, and waist circumference [at the umbilicus]). BMI was calculated using measured weight (kg) and the square of height (m²). Diabetes was defined by 2003 American Diabetes Association clinical criteria, where case subjects were defined as those who used oral hypoglycemic or insulin therapy at any exam or had a fasting plasma glucose (FPG) ≥7.0 mmol/l at the index exam and FPG ≥7.0 mmol/l on at least one prior exam. Fasting resistin levels were measured once at exam 7. For diabetes, we were primarily interested in outcomes and metabolic traits measured over follow-up (time-averaged mean FPG over follow-up [exams 3–7, chosen for measurement stability]), and at the last follow-up (exam 7) including FPG, fasting insulin; homeostasis model assessment of insulin resistance (HOMA-IR) (30); A1C levels; the Gutt 0- to 120-min insulin sensitivity index (31), conducted in a subsample; BMI; waist circumference; visceral adipose tissue (VAT); and subcutaneous adipose tissue (SAT), measured by computed tomography (the latter two conducted in a subsample) (32). FPG was measured immediately with a hexokinase reagent kit (A-gent glucose test; Abbott, South Pasadena, CA), and A1C was measured by high-performance liquid chromatography (33). Other plasma analyses were frozen at ~80°C until assay: fasting plasma insulin was measured with a human-specific insulin assay (Linco, St. Louis, MO), and fasting total resistin levels were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). Intra-assay coefficients of variation were <3% for glucose, 6.1% for insulin, and 9.0% for resistin.

**SNP selection.** We downloaded SNPs from the region of interest (29 kb on the 5’ end plus 10 kb on the 3’ end of RETN) from the phase 2 HapMap database (www.hapmap.org) in January 2006. Due to sparse coverage of the region, we then mined the dbSNP database to choose additional SNPs across the region so as to ensure adequate coverage. We genotyped a set of 58 SNPs in the HapMap European-descent CEU plate, and 27 of them passed quality-control criteria (nonmonomorphic in CEU, minor allele frequency [MAF] >0.05, and Hardy-Weinberg equilibrium [HWE] P > 0.001). We used Tagger (www.broad.mit.edu/mpg/tagger) to select 21 tag SNPs using a pairwise correlation approach to capture (with an r² > 0.5) the 27 SNPs that passed quality control in the region of interest. Previously reported SNPs were forced in the region of interest at an r² of 0.8 and 100% at an r² of 0.7.

**Statistical analysis.** The quantitative traits were regressed against covariates in order to produce Studentized residuals, which were used as the dependent variable in the subsequent genetic models. Two covariate adjustment schemes were used: the first with sex, age, and age² adjustment and the second with BMI added to age and sex to examine the strength of the SNP associations when adjusted for overall adiposity. For resistin levels and glucose-related traits (mean glucose exam 3–7, FPG, fasting insulin, HOMA-IR, A1C, and Gutt 0- to 120-min insulin sensitivity index), we excluded participants with diabetes.

The association between each trait residual and each SNP was assessed using a linear mixed-effects (LME) model implemented in SOLAR (34) to correct for the covariance between family members; the covariance structure was determined by the degree of relatedness between each relative pair. To assess SNP associations with type 2 diabetes, we used Cox proportional hazards survival analysis, with diabetes as the outcome and the survival time as the variable at which diabetes was first determined. The survival time of individuals without diabetes was the age at their last exam. The model was implemented with the survival package in R (35), with the same adjustments as in the LME models, with covariates taken at the first exam. Trait correlation among siblings was modeled with a frailty term in the survival model (36).

**RESULTS**

Characteristics of the participants genotyped in the Framingham Offspring Study are presented in Table 1. Overall, 2,531 participants were included in this analysis, 53% were women, and 10% had a diagnosis of diabetes before the 28 years of follow-up. Mean resistin levels measured at exam 7 were 14.1 ± 7.2 ng/dl. The heritability of resistin levels in the Framingham Offspring Study was estimated to be 35% (adjusted for sex, age, age², and BMI). Other metabolic traits measured at exam 7 and the mean glucose levels over exams 3–7 are presented in Table 1. Resistin levels were modestly correlated with BMI (r = 0.16), waist circumference (r = 0.18), VAT (r = 0.15), and SAT (r = 0.13; all correlations age and sex adjusted; all P values < 0.001).

With 21 tag SNPs selected by a tagging approach that set an r² > 0.8, we were able to capture 96% (26 of 27 SNPs that passed quality control in the CEU plates) of SNPs in the region of interest at an r² > 0.8 and 100% at an r² > 0.7 (see supplementary Table 1 for details regarding coverage available at http://diabetes.diabetesjournals.org/cgi/content/full/db08-1339/DC1). Average distance between tag SNPs was 1.5 kb. The tag SNPs are shown in Table 2, with their location on chromosome 19 (NCBI B35 assembly), relation to RETN itself (in and around the gene), and other names given in prior publications. SNP rs3745367 was not followed further due to its low MAF (0.002) in our sample.

The LD map of the genotyped region is presented in supplementary Fig. 1 (D² statistics). RETN is a short gene spanning only 1,369 bp. A gene coding for an open reading frame (C19orf59) also known as mast cell–expressed membrane protein 1 (MCEMP1) is located downstream of
The 3' end of RETN and was fully captured by our tagging approach, with our last downstream SNP being on the 3' side of MCEMP1. The LD map for the tagging SNPs in the Framingham population is shown in supplementary Fig. 2. Circulating resistin levels were measured in 1,877 genotyped participants without diabetes. The associations for each tag SNP with resistin levels is illustrated in Fig. 1. The mean resistin level for each genotype and the nominal (uncorrected) and empirical (corrected for the number of SNPs) P values for each tag SNP are presented in Table 3.

Data are means ± SD or percent, unless otherwise indicated. *After removing participants with diabetes. †Over exams 3–7 because of stability of measurements. ‡Over exams 1–7, 28 years of follow-up on average.

### TABLE 1
Characteristics of SNPs genotyped in and around RETN variants

<table>
<thead>
<tr>
<th>Position (NCBI 35)</th>
<th>Relation to the RETN gene</th>
<th>Other name</th>
<th>Call rate</th>
<th>HWE P</th>
<th>Strand</th>
<th>Major allele</th>
<th>Minor allele</th>
<th>MAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs794070</td>
<td>7620814</td>
<td>5' of promoter</td>
<td>0.97</td>
<td>0.32</td>
<td>+</td>
<td>T</td>
<td>C</td>
<td>0.21</td>
</tr>
<tr>
<td>rs11883223</td>
<td>762836</td>
<td>5' of promoter</td>
<td>0.99</td>
<td>0.11</td>
<td>–</td>
<td>G</td>
<td>A</td>
<td>0.17</td>
</tr>
<tr>
<td>rs10401670</td>
<td>7648802</td>
<td>3' of 3' UTR</td>
<td>0.98</td>
<td>0.79</td>
<td>–</td>
<td>C</td>
<td>T</td>
<td>0.44</td>
</tr>
</tbody>
</table>

*Located in chromosome 19 open reading frame 59 (C19orf59), also known as mast cell–expressed membrane protein 1 (MCEMP1).
3’ region of RETN were associated with higher resistin levels (all $P_e < 0.05$). Since some of those SNPs were in moderate LD in the Framingham cohort (see supplementary Table 6 for specific D’ and $r^2$ values), we conducted multiple SNPs models. When models were examined with various combinations of these SNPs, rs4804765 and rs1423096 had independent associations with resistin levels and rs4804765 explained the association of the two other SNPs (rs1477341 and rs10401670). The best-fitting model included rs4804765 and rs1423096 and explained 1.5% of the variance in resistin levels.

One of these SNPs in the 3’ region, rs10401670, was also associated with mean glucose over follow-up ($P_e = 0.02$, after BMI adjustment $P_e = 0.01$) and FPG at exam 7 ($P_e = 0.10$, after BMI adjustment $P_e = 0.02$): its minor T allele was associated with higher glucose levels, concordant with a potential effect of its association with higher resistin levels. Two other SNPs showed associations with FPG at exam 7 (rs1423096, $P_e = 0.049$; and rs10413807, $P_e = 0.02$) but did not remain significant after adjustment for BMI. No other associations were observed in the glyceric or adiposity traits ($P_e > 0.05$) (see supplementary Table 3 for details).

Diabetes incidence was analyzed over the 28 years of follow-up. None of the SNPs offered convincing association with diabetes survival (all $P$ values $\geq 0.05$) (see supplementary Table 2). Because a previous study reported that IVS2 + 181G/A was associated with diabetes when an interaction with BMI was added to the model (28), we conducted diabetes incidence analysis with a BMI interaction term included in the model for this SNP, but even with this more refined replication attempt we did not detect a significant association. We also explored the effect of BMI on the association between rs10401670 and diabetes incidence: adding a BMI × rs10401670 term to the LME model revealed a significant interaction ($P = 0.02$), and the $P$ value for the main effect for rs10401670 reached nominal significance ($P = 0.01$).

The promoter SNP −420C/G (rs1862513) has been investigated by many groups, some examining its association with resistin levels (9,11,13,14,38) and a few with diabetes (29,39) or adiposity (15–18). The analysis of −420C/G (rs1862513) in the Framingham Offspring Study did not show an association with any of the traits measured, including resistin levels. To help attempt to discriminate low power from a true null association, we conducted a meta-analysis of the association of SNP −420C/G (rs1862513) with resistin levels. The details of each population included in the meta-analysis (9,11,13,14,38) and our results are presented in Table 4. The minor C allele seemed to be associated with higher resistin levels; this effect was mainly driven by the largest Japanese study. Heterogeneity was highly significant ($P < 0.0001$). The divergence between studies could be due to differences in ethnic background, age, sex distribution, diabetes status, or other characteristics. When we removed the diabetic subjects from the analysis, heterogeneity was still present.

**DISCUSSION**

We have demonstrated that circulating resistin levels are associated with SNPs in the 3’ region of RETN (rs1477341, rs4804765, rs1423096, and rs10401670) in a large, representative community sample. Among the four SNPs that were associated with resistin levels, rs4804765 and rs1423096 showed independent association according to multiple SNP models. One of those, rs10401670, was also associated with mean fasting glucose and FPG at exam 7. Moreover, rs10401670 was nominally associated with diabetes incidence when including a BMI interaction term in the model. No SNP showed significant association with adiposity traits.

**Association with resistin and glucose levels.** Previous reports of association of SNPs within the RETN gene region targeted specific known SNPS and thus achieved
only partial coverage; our extensive mapping in and around the RETN gene in a large sample has allowed us to reveal novel associations. The four SNPs associated with resistin levels are all located in the 3’ region, downstream of RETN. SNPs outside of the coding sequence can influence transcription or mRNA stability and thus affect transcript levels. We tried to explore the functional role of those SNPs located in the 3’ region of RETN by mining publicly available or private genome-wide expression quantitative trait loci datasets, including one obtained from subcutaneous and omental adipose tissue (E.E. Schadt, personal communication). Unfortunately, the fixed marker arrays utilized in these studies do not include our SNPs of interest or any SNPs in moderate to strong LD with fixed marker arrays utilized in these studies do not include our SNPs of interest or any SNPs in moderate to strong LD with those SNPs have very low LD with rs3745367 (r^2<0.10) or the other SNPs associated with resistin levels in our findings (all r^2<0.02).

### Adiposity traits

Reports of RETN associations with BMI or other measures of adiposity in populations of European descent have been inconsistent in the literature (15–20). Some have reported no association (19), while others did so only in subgroup analyses (15–17). The most commonly

<table>
<thead>
<tr>
<th>Study</th>
<th>Ethnic background</th>
<th>Diabetes status</th>
<th>n</th>
<th>Effect (95% CI)</th>
<th>P</th>
<th>%W (random)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menzaghi et al. 2006</td>
<td>European (Italy)</td>
<td>Nondiabetic</td>
<td>616</td>
<td>-0.15 (-0.52 to 0.22)</td>
<td>0.69</td>
<td>14.09</td>
</tr>
<tr>
<td>Cho et al. 2004</td>
<td>Korean</td>
<td>Nondiabetic</td>
<td>173</td>
<td>3.29 (1.31–5.27)</td>
<td>0.001</td>
<td>12.54</td>
</tr>
<tr>
<td>Yasumato et al. 2008</td>
<td>Japanese</td>
<td>Nondiabetic</td>
<td>36</td>
<td>10.99 (4.07–17.9)</td>
<td>0.004</td>
<td>5.52</td>
</tr>
<tr>
<td>Osawa et al. 2005</td>
<td>Japanese</td>
<td>Nondiabetic</td>
<td>198</td>
<td>4.88 (3.08–6.68)</td>
<td>2.9×10^-7</td>
<td>12.80</td>
</tr>
<tr>
<td>Osawa et al. 2007</td>
<td>Japanese</td>
<td>Nondiabetic</td>
<td>1,927</td>
<td>4.97 (4.59–5.34)</td>
<td>2.3×10^-12</td>
<td>14.09</td>
</tr>
<tr>
<td>This study</td>
<td>European (USA)</td>
<td>Nondiabetic</td>
<td>2,020</td>
<td>0.49 (−0.3–1.01)</td>
<td>0.05</td>
<td>14.03</td>
</tr>
<tr>
<td>Overall random-effects model</td>
<td></td>
<td></td>
<td></td>
<td>3.51 (1.02–6.00)</td>
<td>0.0009</td>
<td></td>
</tr>
<tr>
<td>Overall heterogeneity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

*Resistin levels in ng/dl. %W, percentage of weight accorded to the study.

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TABLE 4

Results from meta-analysis of the association between SNP −420C/G (rs1862513) and resistin levels*
investigated variant (−420G allele) has been the subject of several conflicting reports (15–19). Our results are consistent with the notion that RETN is not associated with adiposity as assessed by BMI, waist circumference, or body fat composition measured by computed tomography scan. The correlation of adiposity measurements with resistin levels, but not RETN genetic variation, suggests that fat accumulation influences resistin levels, but RETN variants are not likely to cause weight gain and obesity.

**Strengths and limitations.** Our study represents a significant advance in its comprehensive coverage of RETN and its flanking regions, moderate to high statistical power with a large number of participants in a general community sample including a family-based component, and standardized phenotyping of anthropometric measurements, diabetes, and metabolic traits over 28 years of prospective follow-up. Nevertheless, this study has a few limitations. Power for diabetes incidence was limited (see supplemental Table 4), especially given our expectation of small effect sizes (hazard ratio <1.4). We had adequate power to detect a small proportion of the variance in quantitative traits explained by common SNPs (see supplementary Table 5); for example, we had 85% power to detect 1% of the variance explained (assuming $\alpha = 0.0001$ and an MAF $\geq 0.05$), but we may have missed smaller effect sizes in our genotype-phenotype correlations for the resistin levels or glycemic traits. Novel associations need independent replication before we can confidently claim they represent true findings. Currently, studies with large numbers of resistin levels measurements and custom genotyping for comprehensive coverage are uncommon. Also, our findings may need to be refined in populations with LD patterns that differ from those of European descent. Finally, genetic associations do not prove that the investigated variant (−420G allele) has been the subject of several conflicting reports (15–19). Our results are consistent with the notion that RETN is not associated with adiposity as assessed by BMI, waist circumference, or body fat composition measured by computed tomography scan. The correlation of adiposity measurements with resistin levels, but not RETN genetic variation, suggests that fat accumulation influences resistin levels, but RETN variants are not likely to cause weight gain and obesity.

**Conclusion.** We have found that SNPs in the 3’ region of RETN are associated with circulating resistin levels in the Framingham Offspring Study. One variant (rs10401670) located in the 3’ region of RETN, but in the second intron of MCEMP1, is associated with both resistin levels and fasting glucose. rs10401670 is also nominally associated with diabetes incidence once a putative interaction with BMI is taken into account. Functional studies are needed to investigate the role of MCEMP1 and to test whether these variants influence RETN expression, resistin production, and/or glucose regulation in appropriate tissues. Our new findings need to be replicated in independent data before we can claim that these associations are real: it appears that custom genotyping will be required.

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