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

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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, rs1423906, and rs10041670) on the 3' side of RETN were strongly associated with resistin levels (all minor alleles associated with higher levels, \( P < 0.05 \) after multiple testing correction). rs10401670 was also associated with fasting plasma glucose \((P = 0.02, \text{BMI adjusted})\) and mean glucose over follow-up \((P = 0.01; \text{BMI adjusted})\). No significant association was observed for adiposity traits. On meta-analysis, the previously reported association of rs10401670 was replicated \((P = 0.0009)\) 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

Diabetes is a chronic metabolic disorder characterized by high blood glucose levels. It affects around 463 million people worldwide. The condition is either type 1 diabetes, which is caused by the destruction of insulin-producing cells in the pancreas, or type 2 diabetes, which is due to insulin resistance. Insulin is a hormone produced in the pancreas that helps regulate blood sugar levels. When the pancreas is unable to produce enough insulin or the body fails to respond to it properly, blood sugar levels rise, leading to diabetes.

In the context of our study, we focused on the gene encoding resistin (RETN), a protein known to contribute to insulin resistance and other metabolic disorders. Our research aimed to confirm or refute previous reports of associations between variants in RETN and plasma resistin levels, type 2 diabetes, and related metabolic traits. We genotyped 21 tag SNPs across the RETN region and tested their associations with resistin levels, glycemic traits, and incident diabetes. Our findings indicate that certain SNPs are strongly associated with resistin levels, providing insights into the genetic basis of diabetes risk.

The implications of these findings are significant for both research and clinical practice. Understanding the genetic contributors to diabetes and related metabolic traits can help in the development of targeted therapies and preventive strategies. Further research is needed to validate these findings and explore the underlying mechanisms.

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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 (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 (20 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 controls (monomorphic in CEU, minor allele frequency [MAF] >0.05, and Hardy-Weinberg equilibrium [HWE] P-value >0.001). We used Tagger (www.broad.mit.edu/mpg/tagger) to select 21 tag SNPs using a tagging approach that set maximally unrelated subset of individuals. The genotyping success rate was >95% for all the LD-tagging SNPs (average 98.4%). Consensus rates on a subset of 254 duplicate individuals reached 99.6%. All the SNPs met HWE (P > 0.001).

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 age at the exam 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).

For each trait, statistical significance was determined using an empirical P value (Pₑ) obtained by a simulation strategy, which generated a null distribution of minimum P values. We simulated a trait for our sample using SIMQTL in SOLAR (34). The heritability of the trait was 35%, although similar null distributions were obtained for heritabilities of 15 and 50%. The simulated trait was analyzed in the same manner as the trait residuals, using LME models implemented in SOLAR, and the minimum P value observed over all the SNPs was recorded. A total of 10,000 traits were generated, producing an empirical distribution of minimum P values. This strategy provides multiple SNP-testing correction accounting for the correlation among the SNPs but does not correct for the multiple traits being tested.

Multiple SNP models. When we observed multiple SNP associations for the same trait, and the associated SNPs were in moderate to high LD, we considered the possibility that the association signals were not independent. To assess whether these association signals were simply due to LD among the SNPs, we sequentially added SNPs to the LME models: if the signals were independent, we expected that they would each remain significant in these models.

BMI interaction model. Based on a previous report (28), we assessed the effect of a BMI × genotype interaction with SNP rs3745307 (also known as IVS2 + 181G/A) in the LME model by adding a first-level interaction term (BMI × rs3745307) to predict diabetes incidence. Also, since the associations of rs10401670 with glucose seemed stronger in BMI-adjusted models, we explored the interaction of rs10401670 and BMI to predict diabetes incidence.

Meta-analysis of association between SNP ~ 420C/G (rs1862513) and resistin levels. The variation in RETN most often reported in the previous literature is the promoter SNP ~ 420C/G (rs1862513). We included rs1862513 in our tags to confirm or refute previous findings with resistin levels. Since many groups have reported associations with resistin levels, we decided to perform a meta-analysis of our results and published reports by requesting crude data from the corresponding authors. The meta-analyses of the relationship between rs1862513 with resistin levels were performed using the inverse-variance method for pooling regression coefficients with a random effects estimate based on the DerSimonian-Laird method (37).

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 over 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 rs3745308 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 (D’ 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 (MCEMPI) is located downstream of
C19orf59 is located in chromosome 19 open reading frame 59 (C19orf59), also known as mast cell–expressed membrane protein 1 (MCEMP1).

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, by the right Y-axis indicates the recombination rate. The RETN and C19orf59 (also known as mast cell–expressed membrane protein 1 [MCEMP1]) genes are shown by the horizontal arrows at the bottom of the plot. *After removing participants with diabetes. †Over exams 3–7 because of stability of measurements. ‡Over exams 1–7, 28 years of follow-up. The continuous line marked by the right Y-axis indicates the recombination rate. The RETN and C19orf59 (also known as mast cell–expressed membrane protein 1 [MCEMP1]) genes are shown by the horizontal arrows at the bottom of the plot. *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.

FIG. 1. Negative log base 10 of the P value for genetic associations for resistin levels under the additive model (left Y-axis), graphed versus SNPs in the RETN region arranged by chromosomal position (X-axis). The continuous line marked by the right Y-axis indicates the recombination rate. The RETN and C19orf59 (also known as mast cell–expressed membrane protein 1 [MCEMP1]) genes are shown by the horizontal arrows at the bottom of the plot. *After removing participants with diabetes. †Over exams 3–7 because of stability of measurements. ‡Over exams 1–7, 28 years of follow-up. The continuous line marked by the right Y-axis indicates the recombination rate. The RETN and C19orf59 (also known as mast cell–expressed membrane protein 1 [MCEMP1]) genes are shown by the horizontal arrows at the bottom of the plot. *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 2,531 Framingham Offspring Study participants genotyped for RETN variants

<table>
<thead>
<tr>
<th>Population demographic</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrelated</td>
<td>1,445</td>
</tr>
<tr>
<td>Pedigrees</td>
<td>285</td>
</tr>
<tr>
<td>Sibpairs</td>
<td>989</td>
</tr>
<tr>
<td>Avuncular pairs</td>
<td>66</td>
</tr>
<tr>
<td>Cousins</td>
<td>653</td>
</tr>
<tr>
<td>Sex (% of female)</td>
<td>2,531 53</td>
</tr>
</tbody>
</table>

Exam 7 characteristics

| Age (years) | 2,482 ± 61.6 |
| BMI (kg/m²) | 2,394 ± 28.2 |
| Waist circumference (inches) | 2,377 ± 39.3 |
| VAT (cm³) | 1,018 ± 2,139 |
| SAT (cm³) | 1,018 ± 2,983 |
| Fasting blood glucose (mg/dl) | 2,198 ± 100.3 |
| Fasting insulin (µU/ml) | 2,158 ± 14.5 |
| HOMA-IR | 2,158 ± 3.7 |
| Gutt insulin sensitivity index | 815 ± 21.7 |
| A1C (%) | 2,025 ± 5.5 |
| Resistin levels (ng/dl) | 1,877 ± 14.1 |

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 2
Characteristics of SNPs genotyped in and around RETN in 2,543 participants in the Framingham Offspring Study

<table>
<thead>
<tr>
<th>SNP identification</th>
<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>–420C/G</td>
<td>0.97</td>
<td>0.32</td>
<td>+</td>
<td>T</td>
<td>C</td>
<td>0.21</td>
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<tr>
<td>rs11883223</td>
<td>7628636</td>
<td>5′ of promoter</td>
<td>–309C/T</td>
<td>0.99</td>
<td>0.11</td>
<td>−</td>
<td>G</td>
<td>A</td>
<td>0.17</td>
</tr>
<tr>
<td>rs2081075</td>
<td>7629461</td>
<td>5′ of promoter</td>
<td>–181G/A</td>
<td>0.97</td>
<td>0.28</td>
<td>+</td>
<td>G</td>
<td>A</td>
<td>0.29</td>
</tr>
<tr>
<td>rs10418380</td>
<td>7630540</td>
<td>5′ of promoter</td>
<td>–167C/G</td>
<td>0.95</td>
<td>0.76</td>
<td>+</td>
<td>A</td>
<td>G</td>
<td>0.32</td>
</tr>
<tr>
<td>rs10413807</td>
<td>7630628</td>
<td>5′ of promoter</td>
<td>–626G/A</td>
<td>0.99</td>
<td>0.69</td>
<td>−</td>
<td>G</td>
<td>C</td>
<td>0.21</td>
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<tr>
<td>rs12460483</td>
<td>7636594</td>
<td>5′ of promoter</td>
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<td>1.00</td>
<td>0.71</td>
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<td>G</td>
<td>A</td>
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<tr>
<td>rs12459044</td>
<td>7638406</td>
<td>Promoter</td>
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<td>0.15</td>
<td>−</td>
<td>C</td>
<td>G</td>
<td>0.14</td>
</tr>
<tr>
<td>rs7408174</td>
<td>7638955</td>
<td>Promoter</td>
<td>–200C/G</td>
<td>0.97</td>
<td>0.54</td>
<td>−</td>
<td>T</td>
<td>C</td>
<td>0.31</td>
</tr>
<tr>
<td>rs1862513</td>
<td>7639793</td>
<td>5′ of promoter</td>
<td>–393G/C</td>
<td>1.00</td>
<td>0.57</td>
<td>−</td>
<td>G</td>
<td>C</td>
<td>0.29</td>
</tr>
<tr>
<td>rs3219177</td>
<td>7640369</td>
<td>Intron 2</td>
<td>IVS2 + 309C/T</td>
<td>0.99</td>
<td>0.35</td>
<td>−</td>
<td>C</td>
<td>T</td>
<td>0.21</td>
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<tr>
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<td>Intron 2</td>
<td>IVS2 + 181G/A</td>
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<td>0.67</td>
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<tr>
<td>rs3219178</td>
<td>7640951</td>
<td>Intron 3</td>
<td>IVS3 + 167C/G</td>
<td>0.97</td>
<td>1.00</td>
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<td>rs10422065</td>
<td>7641089</td>
<td>Intron 3</td>
<td>–400C/G</td>
<td>0.99</td>
<td>1.00</td>
<td>−</td>
<td>C</td>
<td>G</td>
<td>0.14</td>
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<tr>
<td>rs3745368</td>
<td>7641297</td>
<td>3′ UTR</td>
<td>3′ UTR + 62G/A</td>
<td>0.98</td>
<td>1.00</td>
<td>−</td>
<td>G</td>
<td>A</td>
<td>0.002</td>
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<tr>
<td>rs3745369</td>
<td>7641475</td>
<td>3′ of 3′ UTR</td>
<td>–420C/G</td>
<td>0.99</td>
<td>0.80</td>
<td>+</td>
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<td>C</td>
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<td>rs1477341</td>
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<td>–400C/G</td>
<td>0.99</td>
<td>0.87</td>
<td>−</td>
<td>A</td>
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<td>rs4804765</td>
<td>7643840</td>
<td>3′ of 3′ UTR</td>
<td>–400C/G</td>
<td>0.98</td>
<td>0.64</td>
<td>−</td>
<td>G</td>
<td>T</td>
<td>0.33</td>
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<tr>
<td>rs1423096</td>
<td>7645177</td>
<td>3′ of 3′ UTR</td>
<td>–400C/G</td>
<td>0.99</td>
<td>0.53</td>
<td>+</td>
<td>G</td>
<td>A</td>
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<tr>
<td>rs10401670</td>
<td>7648802</td>
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<td>–400C/G</td>
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<td>0.75</td>
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<td>A</td>
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<tr>
<td>rs10410106</td>
<td>7651043</td>
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<td>–400C/G</td>
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<td>0.79</td>
<td>−</td>
<td>T</td>
<td>G</td>
<td>0.49</td>
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</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 \( D^2 \) 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 glycemic or adiposity traits (\( P_e > 0.05 \)) (see supplemental 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 \( \times \) 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 (9,13,16,23–27). Our best results when including a BMI-gene interaction term did not yield a significant association with diabetes incidence (see supplemental Table 4). In the setting of multiple hypothesis testing this nominal P value (\( P = 0.05 \)) cannot convincingly be considered significant. Other reports testing the association between +181G/A and diabetes have been mostly negative (23,25,26); one report showed a positive association in the same direction as our results when including a BMI-gene interaction term in the model (28). Adding a BMI \( \times \) genotype interaction term to our diabetes incidence model for SNP rs3745367 did not reveal a significant association. In contrast, adding a BMI \( \times \) genotype interaction term to the model with rs10401670 revealed significant interaction, increasing the significance of the (nominal) \( P \) value of its main effect in predicting diabetes incidence. Since we show that several RETN SNPs are associated with circulating resistin levels and that resistin levels are associated with insulin resistance (10), it is possible that a larger sample size might have produced an association with diabetes incidence. Indeed, for a SNP such as rs3745367 of MAF = 0.24 and effect size = 1.25, we had <40% power to detect a significant association with diabetes incidence (see supplemental Table 4).

In the promoter region of RETN, rs1862513 (also known as −420C/G) has been investigated by many groups. In a meta-analysis of studies reporting resistin levels and targeting this SNP, we found that the minor allele was significantly associated with higher resistin levels, but a high level of heterogeneity was evident. Removing the diabetic individuals did not eliminate heterogeneity. Residual heterogeneity could be explained by ethnic background. Indeed, the two populations of European descent (ours and a sample from Italy) did not find an association between rs1862513 (−420C/G) and resistin levels (11). The other studies based on Asian populations seemed to show a strong effect, though mainly driven by the largest Japanese study (9). According to the HapMap, the MAP in individuals from European and Japanese descent are comparable (0.33 and 0.35, respectively), which does not explain the difference in the studies included in our meta-analysis. We can hypothesize that there might be gene-gene and/or gene-environment interactions that influence the two populations differently, but our data does not allow us to make conclusions concerning those possibilities. Also, rs1862513 (−420C/G) might be in LD with a causal SNP in Asians that is not present in individuals of European descent: for example in the HapMap Japanese JPT population, rs1862513 (−420C/G) is in moderate LD (\( r^2 = 0.58 \)) with rs3219175 (also in the promoter region), which is monomorphic in the HapMap CEU population.

**Diabetes incidence.** We did not find a significant association of any SNP with diabetes incidence, although our sample of incident case subjects was small (\( n = 244 \)). This is concordant with most of the previous literature (9,13,16,23–27). Our best \( P \) value for association with diabetes survival (age and sex adjusted) was seen with SNP rs3745367 (aka IVS2 +181G/A; minor allele A increasing the risk hazard ratio to 1.25 [1.00–1.56]), but in the setting of multiple hypothesis testing, testing this nominal \( P \) value (\( P = 0.05 \)) cannot convincingly be considered significant. Other reports testing the association between +181G/A and diabetes have been mostly negative (23,25,26); one report showed a positive association in the same direction as our results when including a BMI-gene interaction term in the model (28). Adding a BMI \( \times \) genotype interaction term to our diabetes incidence model for SNP rs3745367 did not reveal a significant association. In contrast, adding a BMI \( \times \) genotype interaction term to the model with rs10401670 revealed significant interaction, increasing the significance of the (nominal) \( P \) value of its main effect in predicting diabetes incidence. Since we show that several RETN SNPs are associated with circulating resistin levels and that resistin levels are associated with insulin resistance (10), it is possible that a larger sample size might have produced an association with diabetes incidence. Indeed, for a SNP such as rs3745367 of MAF = 0.24 and effect size = 1.25, we had <40% power to detect a significant association with diabetes incidence (see supplemental Table 4).

In the publicly available meta-analysis of genome-wide association datasets DIAGRAM (http://www.well.ox.ac.uk/DIAGRAM/meta.html), only two SNPs in the region of interest were available (rs11883223, rs7408174) and neither one was associated with diabetes. Unfortunately, those two 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
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 SNP is the direct cause of the defect; further fine-mapping and functional studies are needed to identify true causal variants.

**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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