Common Variants in the Adiponectin Gene (*ADIPOQ*) Associated With Plasma Adiponectin Levels, Type 2 Diabetes, and Diabetes-Related Quantitative Traits

The Framingham Offspring Study

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OBJECTIVE—Variants in *ADIPOQ* have been inconsistently associated with adiponectin levels or diabetes. Using comprehensive linkage disequilibrium mapping, we genotyped single nucleotide polymorphisms (SNPs) in *ADIPOQ* to evaluate the association of common variants with adiponectin levels and risk of diabetes.

RESEARCH DESIGN AND METHODS—Participants in the Framingham Offspring Study ($n = 2,543$, 53% women) were measured for glycemic phenotypes and incident diabetes over 28 years of follow-up; adiponectin levels were quantified at exam 7. We genotyped 22 tag SNPs that captured common (minor allele frequency >0.05) variation at $r^2 > 0.8$ across *ADIPOQ* plus 20 kb 5′ and 10 kb 3′ of the gene. We used linear mixed effects models to test additive associations of each SNP with adiponectin levels and glycemic phenotypes. Hazard ratios (HRs) for incident diabetes were estimated using an adjusted Cox proportional hazards model.

RESULTS—Two promoter SNPs in strong linkage disequilibrium with each other ($r^2 = 0.80$) were associated with adiponectin levels (rs17300539; $P_{nominal}$ [P_n] $= 2.6 \times 10^{-8}$; $P_{empiric}$ [P_e] $= 0.0005$ and rs822387; $P_n = 3.8 \times 10^{-5}$; $P_e = 0.001$). A 3′-untranslated region (3′UTR) SNP (rs6773957) was associated with adiponectin levels ($P_n = 4.4 \times 10^{-4}$; $P_e = 0.005$). A nonsynonymous coding SNP (rs17366743, Y111H) was confirmed to be associated with diabetes incidence (HR 1.94 [95% CI 1.16–3.25] for the minor C allele; $P_n = 0.01$) and with higher mean fasting glucose over 28 years of follow-up ($P_n = 0.0004$;

$P_e = 0.004$). No other significant associations were found with other adiposity and metabolic phenotypes.

CONCLUSIONS—Adiponectin levels are associated with SNPs in two different regulatory regions (5′ promoter and 3′UTR), whereas diabetes incidence and time-averaged fasting glucose are associated with a missense SNP of *ADIPOQ*. *Diabetes* 57: 3353–3359, 2008

A diponectin is an adipokine produced by adipocytes that has drawn attention over the past few years for its potential role in diabetes physiology (1). Adiponectin is believed to have anti-inflammatory and insulin-sensitizing properties. High levels of circulating adiponectin have been associated with lower diabetes incidence in many prospective studies (1).

Adiponectin is encoded by the gene *ADIPOQ* located in the chromosomal region 3q27. *ADIPOQ* spans 16 kb and contains three exons. Previous genome-wide linkage scans have identified 3q27 as a susceptibility locus for diabetes (2,3). Various single nucleotide polymorphisms (SNPs) in *ADIPOQ* have been reported to be associated with adiponectin levels and/or diabetes but with inconsistent results. A recent comprehensive review (4) showed that a few *ADIPOQ* SNPs were associated with adiponectin levels and insulin resistance, but none was consistently associated with diabetes or with adiposity as measured by BMI. As underlined by Menzaghi et al. (4), the lack of consistent findings emphasizes the need for comprehensive characterization of the genetic variation in and around the *ADIPOQ* gene. They also emphasized the need to address the issue that some *ADIPOQ* SNPs seem to be associated with adiponectin levels, whereas others seem to be associated with insulin resistance and diabetes-related metabolic traits.

With this background in mind, we conducted a fine-mapping study of *ADIPOQ*, including regulatory regions upstream and downstream of the gene. We studied participants of the Framingham Offspring Study (FOS), a large representative community-based sample that has been followed prospectively for cardiovascular risk factors, including intermediate metabolic traits and diabetes. Our goals were to confirm the associations of SNPs reported previously, to identify SNPs that might have stronger adiponectin or diabetes association signals than those reported, and to seek new associations with adiponectin levels or diabetes incidence using comprehensive characterization of the gene and detailed metabolic phenotyping

over a long follow-up, in a large population sample to ensure adequate power.

RESEARCH DESIGN AND METHODS

Participants include 2,543 individuals from the FOS. This cohort, composed almost exclusively of individuals from European descent, was commenced in 1971 and has received periodic examinations, including fasting blood tests (5,6). This analysis includes 28 years of follow-up from exam 1 (1971–1974) through exam 7 (1998–2002) for diabetes and cross-sectional data from exam 7. The study was approved by the institutional review board at Boston University, and informed consent was obtained from all study participants.

Fasting total adiponectin levels were measured at exam 7, as were weight, height, and waist circumference with the participant standing. BMI was calculated as the weight in kilograms divided by the square of height in meters (kg/m^2). Diabetes was defined as use of diabetes therapy at any exam or fasting plasma glucose (FPG) $\geq$7.0 mmol/l at the index exam and FPG $\geq$7.0 mmol/l on at least one prior exam. Metabolic traits included time-averaged mean FPG over 28 years of follow-up (exams 1–7) and FPG, fasting insulin, insulin resistance by homeostasis model assessment (7), A1C, BMI, and waist circumference. Among diabetes-related quantitative traits that have been collected in the FOS, we also considered in subsidiary analyses *1*) at exam 5, results of a 75-g oral glucose tolerance test (OGTT) and, derived from OGTT results, the Gutt 0–120 min insulin sensitivity index (8); and *2*) at exam 7, visceral and subcutaneous adipose tissue volumes estimated by computerized tomography scanning (9). FPG and insulin assays have been described (10). Total adiponectin was measured by ELISA (R&D Systems, Minneapolis, MN). Assay coefficients of variation were <3% for glucose, 6.1% for insulin, and 5.8% for adiponectin.

SNP selection. Using *Tagger* (www.broad.mit.edu/mpg/tagger), we chose tag SNPs to cover the common variation (minor allele frequency [MAF] >0.05) in a genomic segment spanning from 20 kb upstream to 10 kb downstream of *ADIPOQ*. Arbitrary limits of the flanking regions were chosen based on current knowledge of the size of typical linkage disequilibrium regions and distances from the gene of previously reported variants influencing gene expression around coding sequences. Nineteen tags were initially chosen to capture all common variants in the CEU population from Phase 2 HapMap, accessed January 2006 (www.hapmap.org) at an $r^2 \geq 0.8$ using a pairwise tagging approach. While genotyping was being completed, a new release of HapMap became available. Reiteration of the tagging procedure with the same settings on the newer dataset yielded a previously ungenotyped tag SNP (rs864265), whereas rs13085499 from the original tag set was not needed to capture all variation found in HapMap v21; the remaining tag SNPs did not change. We included rs13085499 in our analysis for thoroughness as well as two additional SNPs, rs2241766 (+45T/G) and rs1501299 (+276G/T), that had been previously genotyped in the FOS.

Genotyping. We genotyped using iPLEX Sequenom, except rs2241766 and rs1501299, which were genotyped by mass spectroscopy (Sequenom, San Diego, CA). The genotyping success rate was >95% (average 98.2%), and the consensus rate on a subset of 254 duplicate individuals was 99.4%. To correctly estimate MAF and HWE in the SNPs, we obtained these estimates in a maximally unrelated subset of 1,491 individuals selected by choosing one person per pedigree. All of the SNPs were in HWE ($P > 0.01$ for all but rs182052, whose HWE P value was 0.0002).

Statistical analysis. Quantitative traits were regressed against covariates to produce Studentized residuals that were used as the dependent variable in the subsequent genetic models. To examine the strength of the SNP associations accounting for overall adiposity, we used two covariate adjustment schemes: sex, age (per year), and age^2 and sex, age, age^2, and BMI (per kg/m^2) adjusted. The association between each trait residual and each SNP was assessed using a linear mixed effects (LME) model implemented in SOLAR (11) to account for within-family correlations. Each SNP was included in a model as a fixed effect with additive coding. Subsidiary analyses were performed with recessive and dominant models with the main traits of interest (adiponectin levels, mean glucose, and diabetes survival). The models included random effects to account 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 the diabetes phenotype, 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 (12), 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 (13). We provide power calculation for diabetes incidence in supplementary Table 9, available in an online appendix at http://dx.doi.org/10.2337/db08-0700. We conducted subsidiary analysis using incidence of

TABLE 1
Characteristics at entry and at exam 7 of 2,543 FOS participants genotyped for *ADIPOQ* variants

	n	Mean $\pm$ SD
Total individuals genotyped (n)	2,543	
Unrelated (n)	1,448	
Pedigrees (n)	282	
Sibpairs (n)	994	
Avuncular pairs (n)	66	
Cousins (n)	653	
Sex (% women)	2,543	53
Age at entry (exam 1) (years)	2,543	34.6 ± 9.8
BMI at entry (exam 1) (kg/m^2)	2543	24.9 ± 4.3
Cases of diabetes over 28 years (n)	244	
Mean glucose from exams 1–7 (mg/dl)	2,543	99.3 ± 16.9
Exam 7		
BMI (kg/m^2)	2,405	28.2 ± 5.4
Waist circumference (inches)	2,388	39.3 ± 5.6
Fasting glucose (mg/dl)	2,371	104.3 ± 26.6
Fasting insulin (μU/ml)	2,292	14.8 ± 8.7
HOMA-IR	2,292	4.0 ± 3.2
A1C (%)	2,189	5.7 ± 1.0
Plasma adiponectin (μg/ml)	2,018	10.0 ± 6.1

HOMA-IR, homeostasis model insulin resistance calculated by [fasting glucose $\times$ fasting insulin]/22.5.

hyperglycemia (defined as fasting blood glucose >100 mg/dl on two exams during follow-up).

Statistical significance was determined using an empirical P value 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 (11). 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 of the SNPs was recorded. Ten thousand traits were generated to create an empirical distribution of minimum P values. This strategy provides correction for correlation among the SNPs but does not correct for the multiple traits being tested. We report both nominal (P_n) and empiric (P_e) P values.

Multiple SNP models. To assess whether association signals were due to linkage disequilibrium among the SNPs or were independent, we sequentially added SNPs to LME models. If the signals were independent, we expected that they would each remain significant in these models. Alternatively, if any became nonsignificant, we would conclude that the positive associations were due to the tag SNP remaining significant in the multi-SNP model.

BMI stratification and interaction models. As exploratory analyses, we assessed the effects of BMI on the associations between all SNPs and the main traits of interest (adiponectin levels, mean glucose over follow-up, and diabetes incidence) by testing the associations stratified by obesity status (nonobese, i.e., BMI <30 kg/m^2, and obese, i.e., BMI $\geq$30 kg/m^2). We also tested the presence of interactions between BMI and each SNP for the same traits.

RESULTS

The clinical characteristics of study participants are presented in Table 1. Mean adiponectin levels were 7.4 ± 4.5 μg/ml for men and 12.4 ± 6.4 μg/ml for women. Characteristics at exam 5, including the OGTT results, are presented in supplementary Table 1, and exam 7 adiposity measures by computed tomography are in supplementary Table 2, both of which are available in the online appendix.

Details of the genotyped SNPs are presented in Table 2. The tag SNPs captured 100% of SNPs passing quality control with an $r^2 \geq 0.80$ and an MAF >0.05. The details of all SNPs captured by our tagging approach are available in supplementary Table 3 in the online appendix. The SNPs span 45,832 bp, with an average inter-SNP distance of 1,706 bp. A nonsynonymous coding SNP located in exon 3 (rs17366743 [Y111H]) had an MAF of 0.036 in the FOS but

TABLE 2
Characteristics of 22 SNPs genotyped in and around *ADIPOQ* in 2,543 men and women in the FOS

SNP	Previous name in the literature	Location on chromosome 3 (NCBI 35)	Relation to gene	Call rate	HWE P value	Major allele	Minor allele	MAF
rs10937273		188032397	5′ of promoter	0.951	0.013	G	A	0.43
rs864265		188036994	5′ of promoter	0.967	0.071	G	T	0.14
rs822387		188038739	5′ of promoter	0.995	0.816	T	C	0.10
rs16861194	−11426A/G	188042127	Promoter	0.991	0.290	A	G	0.07
rs17300539	−11391G/A	188042162	Promoter	0.984	0.816	G	A	0.10
rs266729	−11377C/G	188042176	Promoter	0.980	0.055	C	G	0.27
rs182052		188043484	Intron 1	0.993	0.0002	G	A	0.33
rs822391		188046505	Intron 1	0.963	0.686	T	C	0.19
rs822396		188049579	Intron 1	0.995	0.784	A	G	0.19
rs12495941		188050882	Intron 1	0.951	0.781	G	T	0.35
rs7649121		188051487	Intron 1	0.994	0.893	A	T	0.19
rs17366568		188053155	Intron 1	0.977	1.000	G	A	0.11
rs2241766	+45T/G	188053594	Exon 2 coding synonymous	0.748	0.182	T	G	0.15
rs1501299	+276G/T	188053825	Intron 2	0.727	0.623	G	T	0.26
rs3821799		188054188	Intron 2	0.994	0.802	C	T	0.46
rs3774262		188054516	Intron 2	0.994	0.597	G	A	0.14
rs17366743	Y111H	188054791	Exon 3 coding nonsynonymous	0.996	1.000	T	C	0.04
rs6773957		188056407	3′UTR	0.988	0.796	G	A	0.41
rs6444175		188062446	3′ of 3′UTR	0.982	0.459	G	A	0.27
rs13085499		188063542	3′ of 3′UTR	0.982	0.933	A	G	0.47
rs7628649		188068083	3′ of 3′UTR	0.990	0.113	C	T	0.14
rs17373414		188068229	3′ of 3′UTR	0.979	0.186	C	T	0.13

Call rate, success rate of genotyping each SNP in the FOS sample; HWE P value, Hardy-Weinberg equilibrium test P values, with significance set at ≤0.0001. MAF, MAF in the FOS, calculated from a subsample of 1,491 unrelated participants.

was reported to have an MAF of 0.075 in the HapMap CEU sample. Allele frequencies were otherwise similar to HapMap frequencies.

Supplementary Fig. 1, available in the online appendix, illustrates the linkage disequilibrium map of the 35 SNPs. As previously reported, *ADIPOQ* is composed of two regions of high linkage disequilibrium, one covering the 5′-promoter region, and the second including intron 2, exon 3, and the 3′-untranslated region (3′UTR), separated by a segment of high recombination rate.

Adiponectin levels were available in 2,018 genotyped participants. Figure 1 illustrates the association of SNPs with adiponectin levels and the strength of their association (expressed as $-\log P$) adjusted for sex, age, and age^2 or for sex, age, age^2, and BMI. The minor A allele (MAF = 0.10) at SNP rs17300539 (−11391G/A), located in the promoter region, showed the strongest association with higher adiponectin levels under an additive model ($P_n = 2.6 \times 10^{-8}$; $P_e = 0.0005$). Also in the 5′–linkage disequilibrium region, the minor C allele at rs822387 (MAF = 0.10) was strongly associated with higher adiponectin levels ($P_n = 3.8 \times 10^{-5}$; $P_e = 0.001$) and was in strong linkage disequilibrium ($r^2 = 0.794$ in unrelated members of the FOS) with rs17300539.

In the 3′UTR, the minor A allele at rs6773957 (MAF = 0.41) showed the strongest association with higher adiponectin levels ($P_n = 4.4 \times 10^{-4}$; $P_e = 0.005$). Located further downstream in the 3′ region, rs6444175 showed a nominal trend of association with adiponectin levels that did not remain significant after multiple testing correction ($P_n = 0.017$; $P_e = 0.2$); however, further adjustment for BMI seemed to strengthen the association ($P_n = 0.003$; $P_e = 0.04$). Also, in the second linkage disequilibrium region, rs1501299 (+276G/T; located in intron 2) showed a nominal association with adiponectin levels once adjusted

for BMI ($P_n = 0.007$), but the empiric P value was not significant ($P_e = 0.10$). The two latter SNPs (rs6444175 and rs1501299) are in strong linkage disequilibrium with each other ($r^2 = 0.92$) and in moderate linkage disequilibrium with rs6773957 ($r^2 \cong 0.5$). A few other SNPs had nominal associations with adiponectin levels; all of the details for each SNP association with adiponectin levels (without and with adjustment for BMI) are shown in Table 3.

Diabetes incidence was associated with only one SNP, rs17366743 (Y111H), which is a nonsynonymous SNP
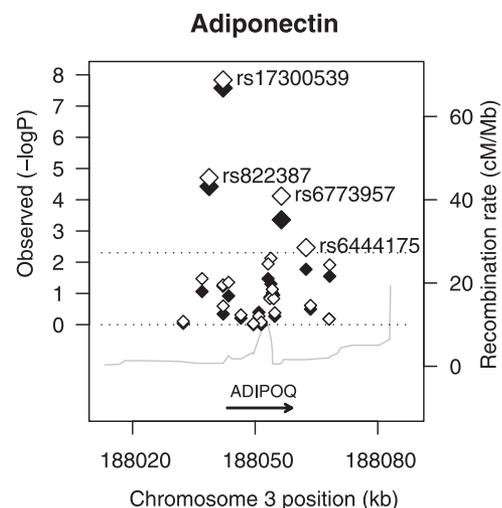
FIG. 1. Negative log base 10 of the P value for genetic associations for adiponectin levels under the additive model (left y-axis), graphed versus SNPs in the *ADIPOQ* region arranged by chromosomal position (x-axis). The continuous line marked by the right y-axis indicates the recombination rate. The *ADIPOQ* gene is shown by the horizontal arrow at the bottom of the plot. ◇, Traits adjusted for sex and age; ◆, additional adjustment for BMI.

TABLE 3
Adiponectin levels for each SNP in and around *ADIPOQ* in the FOS

SNP	Position on chromosome 3 (NCBI 35)	n	Alleles M/m	M/M	M/m	m/m	P value (adjusted for sex, age, and age^2)	Empiric P value*	P value (adjusted for sex, age, age^2, and BMI)	Empiric P value†
rs10937273	188032397	1,870	G/A	10.07 ± 6.39	10.11 ± 6.1	9.53 ± 5.53	0.91	1.0	0.79	1.0
rs864265	188036994	1,885	G/T	10.14 ± 6.17	9.74 ± 6.03	9.05 ± 4.83	0.09	0.71	0.03	0.38
rs822387	188038739	1,949	T/C	9.81 ± 6.09	10.9 ± 6.1	11.52 ± 4.97	3.77×10^{-5}	0.001	1.96×10^{-5}	0.001
rs16861194	188042127	1,942	A/G	10.02 ± 5.98	9.8 ± 6.76	7.7 ± 5.08	0.06	0.57	0.05	0.53
rs17300539	188042162	1,929	G/A	9.71 ± 6.06	11.5 ± 6.21	11.56 ± 5.25	2.64×10^{-8}	0.0005	1.46×10^{-8}	0.0005
rs266729	188042176	1,921	C/G	9.96 ± 5.9	9.96 ± 6.28	10.43 ± 6.58	0.46	1.0	0.25	0.98
rs182052	188043484	1,949	G/A	10.01 ± 5.69	9.95 ± 6.38	10.2 ± 6.64	0.12	0.83	0.04	0.47
rs822391	188046505	1,898	T/C	9.98 ± 6.09	9.99 ± 6.14	9.24 ± 5.84	0.62	1.0	0.49	1.0
rs822396	188049579	1,947	A/G	10 ± 6.08	10.07 ± 6.11	9.17 ± 6.1	1.0	1.0	0.92	1.0
rs12495941	188050882	1,876	G/T	10.09 ± 6.28	10.1 ± 6.16	9.35 ± 5.51	0.40	1.0	0.53	1.0
rs7649121	188051487	1,947	A/T	9.9 ± 5.94	10.16 ± 6.24	9.99 ± 6.89	0.99	1.0	0.83	1.0
rs17366568	188053155	1,917	G/A	10.07 ± 6.02	9.61 ± 6.41	10.6 ± 5.8	0.03	0.38	0.01	0.15
rs2241766	188053594	1,499	T/G	9.9 ± 6.07	10.4 ± 6.45	9.88 ± 4.66	0.13	0.85	0.14	0.88
rs1501299	188053825	1,459	G/T	9.93 ± 6.42	10.01 ± 5.69	10.39 ± 5.49	0.05	0.50	0.007	0.10
rs3821799	188054188	1,947	C/T	9.79 ± 6.29	10.11 ± 6.15	10.05 ± 5.55	0.10	0.75	0.07	0.66
rs3774262	188054516	1,947	G/A	9.86 ± 6.03	10.49 ± 6.29	10.35 ± 6.14	0.11	0.81	0.15	0.88
rs17366743	188054791	1,952	T/C	9.99 ± 6.07	10.23 ± 6.32	$NA \pm NA$	0.54	1.0	0.42	1.0
rs6773957	188056407	1,933	G/A	9.58 ± 6.23	10.24 ± 6.16	10.21 ± 5.35	0.0004	0.005	0.0001	0.002
rs6444175	188062446	1,926	G/A	9.84 ± 6.34	10.19 ± 5.88	10.12 ± 5.7	0.03	0.21	0.003	0.04
rs13085499	188063542	1,928	A/G	9.91 ± 6.33	10.08 ± 6.12	9.93 ± 5.75	0.32	0.99	0.24	0.98
rs7628649	188068083	1,938	C/T	9.94 ± 6.09	10.2 ± 6.03	10.4 ± 6.26	0.65	1.0	0.66	1.0
rs17373414	188068229	1,924	C/T	9.84 ± 6.01	10.64 ± 6.51	8.99 ± 5.25	0.03	0.32	0.01	0.16

Data are means ± SD. M, Major allele; m, minor allele. *Empiric P value calculated by simulation strategy form the P values adjusted for sex, age, and age^2. †Empiric P value calculated by simulation strategy form the P values adjusted for sex, age, age^2, and BMI.

coding for a Y→H change at codon 111 in exon 3 of *ADIPOQ*. In age-sex adjusted analysis, those carrying the minor C allele (MAF = 0.036) were associated with a hazard ratio (HR) of 1.94 for incident diabetes (95% CI 1.16–3.25; $P_n = 0.01$). rs17366743 also was associated with 28-year time-averaged mean FPG ($P_n = 0.0004$; $P_e = 0.004$); consistent with our diabetes survival analysis, the minor C allele was associated with higher glucose levels. We had limited power to see smaller effects (HR 1.1–1.4) on diabetes incidence, even with higher MAF (supplementary Table 9). Figure 2 summarizes the associations of SNPs in *ADIPOQ* with diabetes incidence and mean FPG. A few SNPs also showed trends of associations with other glycemic quantitative traits, but none remained statistically significant after empiric correction (supplementary Tables 4 and 5, available in the online appendix). Subsidiary analysis did not show any SNP to be significantly associated with incidence of hyperglycemia (affecting 52.7% of the FOS) (supplementary Table 10, available in the online appendix).

Concerning adiposity, we did not find an association between any SNPs in *ADIPOQ* and adiposity measurements, including BMI, waist circumference, or visceral and subcutaneous adipose tissue volumes (supplementary Tables 4–6). In the obesity-stratified analyses, our main findings seemed to have a similar effect on adiponectin levels in both nonobese and obese strata; whereas two SNPs (rs17366568 and rs864265) appeared to have a stronger effect in the obese population. There was no significant interaction between any of the SNPs and BMI on diabetes incidence. All of the details for each SNP association with adiponectin levels, mean glucose, and diabetes incidence stratified by obesity status and tested for interaction with BMI are presented in supplementary Table 8, available in the online appendix.

In the 5′–linkage disequilibrium region–multiple SNPs model, both rs17300539 and rs822387 remained significantly associated with adiponectin levels, indicating that despite their strong linkage disequilibrium, they may represent independent signals. In the 3′–linkage disequilibrium region, including rs6773957, rs6444175,

Mean Glucose and Diabetes Survival
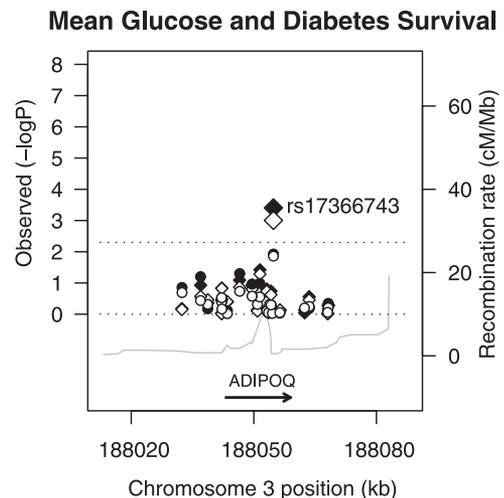
FIG. 2. Negative log base 10 of the *P* value for genetic associations with diabetes survival (circles) and mean glucose over 29 years of follow-up (diamonds) under the additive model (left *y*-axis), graphed versus SNPs in the *ADIPOQ* region arranged by chromosomal position (*x*-axis). The continuous line marked by the right *y*-axis indicates the recombination rate. The *ADIPOQ* gene is shown by the horizontal arrow at the bottom of the plot. Open symbols indicate traits adjusted for sex and age; closed symbols indicate additional adjustment for BMI.

and rs1501299 in the same model, there was a loss of significance for all three SNPs, indicating that they probably represent the same signal, with rs6773957 having the strongest individual effect. Finally, we examined a model with all SNPs associated with adiponectin levels. Only the two SNPs in the promoter region (rs17300539 and rs822387) remained significant in the model.

We performed subsidiary analyses using recessive and dominant genetic models for the main traits of interest. The results are presented in supplementary Table 7, available in the online appendix. In brief, dominant model results were very similar to the additive model, whereas recessive models showed similar direction of effect but no significant association. Some trends for SNPs other than the ones observed in the additive models were seen, but we consider those findings exploratory.

DISCUSSION

In the community-based FOS, we found that adiponectin levels were associated with SNPs located in two distinct regulatory regions around the *ADIPOQ* gene. SNPs in the promoter region associated with adiponectin levels confirm previous reports (rs822387 and rs17300539) (4,14). Our detailed mapping allowed us to detect that adiponectin levels were also associated with previously unreported SNPs in or near the 3′UTR (rs6773957 and rs6444175). Given their linkage disequilibrium pattern, one of these novel SNPs might account for the previously reported association between rs1501299 (+276G/T) and adiponectin levels (4). Diabetes incidence and time-averaged mean glucose were associated with rs17366743, a nonsynonymous coding SNP, confirming previous reports (15–17).

Adiponectin levels and SNPs in the 5′-promoter region. We found that the minor C allele rs822387 was associated with higher adiponectin levels, confirming the report of Heid et al. (14). As reported in both studies, rs822387 is in strong linkage disequilibrium with the promoter SNP rs17300539, but both SNPs remained statistically significant when considered jointly in the LME model. This suggests that they may both have an independent effect on adiponectin levels, but it would be difficult to affirm this with certitude because of their very strong mutual linkage disequilibrium.

Two SNPs in the promoter region have been frequently genotyped in *ADIPOQ* association studies: rs17300539 (−11391G/A) and rs266729 (−11377C/G). Our findings are consistent with Menzaghi et al. (4), by whom the minor A allele at rs17300539 was reported to be associated with higher adiponectin levels in a meta-analysis of >2,000 individuals. Furthermore, rs17300539 was shown to have a functional role in in vitro data: The A allele seemed to enhance the *ADIPOQ* promoter activity and increased transcriptional activity (18). rs266729 did not meet the inclusion criteria for the meta-analysis of Menzaghi et al. (4). Some studies have reported that rs266729 was associated with adiponectin levels (16), whereas others did not (19); it seemed that the level of obesity of the population studied might have played a role in revealing the association (17,19). Cauchi et al. (20) also suggest that obesity status contributes to the association between rs266729 and diabetes. We did not find a BMI × genotype interaction in the association between rs266729 and adiponectin levels or diabetes incidence. Our negative results are consistent with either false-positive findings in the initial reports or lack of power in the current study.

Adiponectin levels and SNPs in the 3′UTR region. Another SNP often genotyped in *ADIPOQ* studies was rs1501299 (+276G/T). In our analysis, the minor T allele was nominally associated with higher adiponectin levels, consistent with Menzaghi et al. (4). However, in vitro data has not supported a functional role for rs1501299 in adiponectin expression (18). It has been proposed that rs1501299 might be a marker, through linkage disequilibrium, of a functional variant in the 3′UTR region (4). A novel 3′UTR SNP, rs6773957, showed the strongest signal in this region of *ADIPOQ*. Others have reported other SNPs in the 3′UTR nominally associated with adiponectin levels (14,19). At this time, we could not find any published functional data directly addressing SNPs in the 3′UTR to confirm that any of these SNPs might be the cause of change in adiponectin levels. More in vitro studies are needed to discover the functional role of one or many of the variants in the 3′UTR.

By extending our mapping up to 10 kb downstream to the *ADIPOQ* in the 3′ region, we found that minor A allele at rs6444175 was associated with higher adiponectin levels. This newly reported SNP is in moderate linkage disequilibrium with rs6773957 ($r^2 = 0.51$ in the FOS) and in strong linkage disequilibrium with rs1501299 ($r^2 = 0.92$). Using genetic association alone, we cannot distinguish which of the three SNPs (rs6773957, rs1501299 or rs6444175) is driving the association signal; the results of multiple regression analysis indicate that the effect of these three SNPs were not independent. Also, the multiple SNPs analysis including all SNPs associated with adiponectin levels in our data showed that only the promoter SNPs remained significantly associated in the model. This might be because the association signal in the 3′ region may be driven by the association stemming from the promoter SNPs, despite the high recombination rate and the low linkage disequilibrium between the two regions ($r^2 = 0.12$ for rs17300539 with rs6773957).

Diabetes and glycemic traits. Diabetes incidence over 28 years was nominally associated with rs17366743 (Y111H). This amino acid change from a Tyr (T allele) into a His (C allele) in position 111 of the adiponectin protein is located at the hinge between the collagen and the globular domains of adiponectin. It has been hypothesized that such a change might alter the spatial organization and the function of the protein by hindering the complexation of collageneous homotrimers in bundles (16). Directed functional changes in coding sequence have not shown that Y111H alters the high molecular weight formation of adiponectin, in contrast to other coding mutants (21); rather, Y111H is believed to disrupt the exonic splicing enhancer, which could influence alternative splicing of the message (http://fastsnp.ibms.sinica.edu.tw). Mutations in the exonic splicing enhancer have been shown in other examples of increased genetic risk for diseases, including the *BRCA1* gene in breast cancer (22). Therefore, even if rs17366743 does not influence the level of the circulating adiponectin, it could influence the functionality of the protein and so decrease the insulin-sensitizing role of adiponectin, putting carrier individuals at higher risk of diabetes. The minor C allele of rs17366743 is relatively rare in Framingham (3.6%) but somewhat more frequent in HapMap CEU subjects (7.5%). Our findings are consistent with the report by Vasseur et al. (16) and Ukkola et al. (15) but in contrast to the findings of Gu et al. (23). We accessed the DIAGRAM dataset (http://www.well.ox. ac.uk/DIAGRAM/) to look for other replications. Unfortu-

nately, because rs17366743 is a rare SNP, it was not genotyped directly, and its best proxy in HapMap European descent (rs7649121) offered low linkage disequilibrium ($r^2 \cong 0.17$), but the effect was in the same direction ($P = 0.009$).

The association of rs17366743 with time-averaged FPG in our data increases our confidence that this is a true finding and not a result of type 1 error. In the HERITAGE Family Study, the minor C allele was associated with a lower sensitivity index and a higher acute insulin response to glucose during an intravenous glucose tolerance test but not with the disposition index, suggesting higher insulin resistance and a compensatory response in individuals carrying the risk allele (15). In contrast, Heid et al. (14) did not find an association between Y111H and any of the parameters of the metabolic syndrome. In fact, despite extensive mapping and detailed phenotyping in their large sample, none of the SNPs genotyped by Heid et al. (14) was reported to be significantly associated with parameters of the metabolic syndrome. Similarly, very few SNPs other than rs17366743 were nominally associated with metabolic traits in our dataset. The reasons for such sparse findings might be related to the fact that glycemic and metabolic traits are influenced by so many genetic and environmental factors that the contribution of *ADIPOQ* variants is difficult to capture.

Strengths and limitations. Strengths of our study include high-quality genotyping, comprehensive coverage of *ADIPOQ* and its flanking regions, the statistical power afforded by a large number of participants in a general community sample including a family-based component, and standardized phenotyping over 28 years of follow-up. Nevertheless, this study has a few limitations. We measured total adiponectin and not the high molecular weight fraction, which has been proposed to have a stronger correlation with insulin resistance compared with total adiponectin (24). Also, adiponectin levels were measured at exam 7 (the last follow-up examination); results might have been different if adiponectin was measured earlier during the follow-up. If adiponectin truly lies on the causal pathway of diabetes development, measurements of adiponectin levels at the beginning of follow-up might have revealed associations between the relevant SNPs and both protein levels and diabetes incidence, in line with the concept of Mendelian randomization. We had limited power to test this concept: for example, for promoter SNP rs17300539 (our strongest signal associated with adiponectin levels), according to an additive model, each minor allele (MAF = 0.10) increases adiponectin levels by 1.63 µg/ml, and assuming that each 1 µg/ml increase in adiponectin levels decreases diabetes risk incidence by 8.1% (24), we had <10% power to detect the effect of rs17300539 on diabetes incidence. Some previous associations could not be replicated; whether this is due to a false-positive original result or insufficient power to detect it in our samples is not clear. Also, novel associations need independent replication before we can confidently claim they represent true findings. Our sample is of European origin, and our findings may not be generalizable to populations with different linkage disequilibrium patterns. Finally, genetic associations do not prove that the SNP is the direct cause of the defect; fine-mapping and functional studies are needed to identify the true causal variants.

Conclusions. We confirmed significant associations between adiponectin levels and *ADIPOQ* SNPs in the 5′- promoter region (rs17300539 and rs822387) while unmasking a new association in the downstream 3′UTR region (rs6773957 and rs6444175). On the basis of linkage disequilibrium measures, we believe those two regions represent distinct association signals, and thus, adiponectin levels seem to be influenced by both regulatory segments. In addition, diabetes survival and mean glucose were associated with the nonsynonymous SNP rs17366743 (Y111H), which might influence alternative splicing. According to the hypothesis of Mendelian randomization, a causal variation in the genome should influence both the levels of a biomarker and the risk of disease. Our findings do not preclude the existence of the concept of Mendelian randomization but underline that it might not be always straightforward to demonstrate it, particularly when both effect sizes (that of genotype on the intermediate phenotype and that of the intermediate phenotype on the clinical endpoint) are modest. Our results suggest that the alteration in adiponectin function induced by SNP rs17366743 (Y111H) may have stronger effects than the change in levels induced by SNPs in the regulatory regions; thus, different variants in the same gene might be related to biomarker levels but not sufficiently to bring about associated disease states, and vice versa.

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