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Novel Locus *FER* Is Associated With Serum HMW Adiponectin Levels

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OBJECTIVE—High molecular weight (HMW) adiponectin is a predominant isoform of circulating adiponectin and has been related to type 2 diabetes. Previous linkage studies suggest that different genetic components might be involved in determining HMW and total adiponectin levels.

RESEARCH DESIGN AND METHODS—We performed a genome-wide association study (GWAS) of serum HMW adiponectin levels in individuals of European ancestry drawn from the Nurses' Health Study (NHS) (N = 1,591). The single nucleotide polymorphisms (SNPs) identified in the GWAS analysis were replicated in an independent cohort of Europeans (N = 626). We examined the associations of the identified variations with diabetes risk and metabolic syndrome.

RESULTS—We identified a novel locus near the *FER* gene (5q21) at a genome-wide significance level, best represented by SNP rs10447248 ($P = 4.69 \times 10^{-8}$). We also confirmed that variations near the adiponectin-encoding *ADIPOQ* locus (3q27) were related to serum HMW adiponectin levels. In addition, we found that *FER* SNP rs10447248 was related to HDL cholesterol levels ($P = 0.009$); *ADIPOQ* variation was associated with fasting glucose ($P = 0.04$), HDL cholesterol ($P = 0.04$), and a metabolic syndrome score ($P = 0.002$).

CONCLUSIONS—Our results suggest that different loci may be involved in regulation of circulating HMW adiponectin levels and provide novel insight into the mechanisms that affect HMW adiponectin homeostasis. *Diabetes* 60:2197–2201, 2011

Adiponectin is the most abundant adipocyte-secreted hormone in blood (1), which regulates the inflammatory response, enhances insulin action, and affects metabolism of glucose and lipids (2,3). In the circulation, adiponectin is present in multimers of various molecular weights including high (HMW), medium (MMW), and low (LMW) molecular weight, with the predominant form being HMW adiponectin (4), which is thought to represent the biological active form that is better correlated with insulin sensitivity and risk of obesity and diabetes than total adiponectin (5,6).

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Family and twin studies indicate that 30–88% variance in adiponectin concentration is potentially accounted for by genetic influence (4,7,8). Candidate gene studies and genome-wide association studies (GWAS) of total adiponectin levels have identified loci near genes *ADIPOQ*, *ARL15*, and *CDH13* (8–14). Previous linkage studies indicate that different loci might be involved in determining HMW adiponectin (15). We therefore undertook a genome-wide analysis on the serum levels of HMW adiponectin. We describe the identification of a novel locus at genome-wide significance level.

RESEARCH DESIGN AND METHODS

The Nurses' Health Study (NHS) was established in 1976 when 121,700 female registered nurses aged 30–55 years and residing in 11 large U.S. states completed a mailed questionnaire on their medical history and lifestyle (16). The lifestyle factors have been updated by validated questionnaires every 2 years. The current study was approved by the institutional review board at Brigham and Women's Hospital. Participants for the current study were a subset of women (N = 1,591) included in a nested case-control study of type 2 diabetes (17,18). To test the association with diabetes risk, we also included a nested case-control study in the Health Professional Follow-up Study (HPFS) (17) (Supplementary Materials).

Replication study. A total of 626 nondiabetic individuals (240 men and 386 women) from 235 families were recruited in the Gargano area in center-east Italy and examined as previously described. The study and the informed consent procedures were approved by the local research committee. A metabolic syndrome score was calculated according to Adult Treatment Panel III criteria.

Assessment of serum adiponectin. In NHS, study samples were analyzed in randomly ordered case-control pairs to further reduce systematic bias and interassay variation. Serum HMW adiponectin concentrations were determined by ELISA (Millipore, St. Charles, MO) with a sensitivity of 0.5 µg/mL.

In the Italian sample, serum total adiponectin HMW and MMW plus HMW adiponectin concentrations were measured by ELISA (ALPCO) as previously described (4). MMW values were obtained by subtracting the concentrations of HMW from the combined concentrations of MMW plus HMW. LMW adiponectin fractions were obtained by subtracting the combined concentrations of MMW plus HMW from the total adiponectin concentrations. The intra-assay coefficients of variation were 5.4 and 5.0, 5.2 and 4.9, and 5.0 and 4.8% for total adiponectin, MMW plus HMW adiponectin, and HMW adiponectin, respectively.

Genotyping and quality control. For the GWAS samples, genotyping was done using the Affymetrix Genome-Wide Human 6.0 array. Genotypic data were checked for quality as described elsewhere (17,18). We used MACH (http:// www.sph.umich.edu/csg/abecasis/MACH) to impute 2,543,887 single nucleotide polymorphisms (SNPs) on chromosomes 1–22 with NCBI build 36 of Phase II HapMap CEU data (release 22) as the reference panel. Imputed SNPs with minor allele frequency <0.02 and/or with poor imputation quality scores (MACH $r^2 \leq 0.30$) were filtered from analysis. Population structure was investigated by principal component analysis. Adjustment for the top four principal components did not appreciably change the association results.

Replication SNPs were genotyped by Taqman SNP allelic discrimination technique by means of an ABI 7000 (Applied Biosystems, Foster City, CA). Call rate and concordance rate were ≥96 and >99%, respectively. All the SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$).

Statistical analyses. GWAS analysis on HMW adiponectin levels in NHS was performed with the linear regression analysis using PLINK software. HMW adiponectin was normally distributed and analyzed without transformation. Associations between adiponectin isoforms and each SNP in the replication

sample were tested by a linear mixed-effects model implemented in SOLAR that accounts for within-family correlations. Each SNP was included in a model as a fixed effect with additive coding. We combined study-specific β-estimates from discovery and replication analyses using the inverse of the variance of the study-specific β-estimates to weight the contribution of each study with the fixed-effect model. The associations between SNPs and the risk of type 2 diabetes in NHS and HPFS were analyzed using a logistic regression model. To assess phenotypic correlations between HMW adiponectin and metabolic traits in the Italian samples, we used a mixed-effects model by SOLAR that includes fixed covariate effects. This method could account for the dependence of the family data and provide a more stringent P value. All analyses were adjusted for sex, age, and BMI.

RESULTS

We performed GWAS analyses with serum HMW adiponectin levels in 1,591 women (698 diabetic patients) from the NHS. The characteristics of the participants are presented in Supplementary Table 1. We fit a linear regression model for genotype trend effects (1 df), adjusting for age, BMI, and diabetes status. Quantile-quantile plots (Supplementary Fig. 1) suggest that there was no systemic bias for analyses (genomic inflation factor, $\lambda = 0.998$). Further adjustment for fasting status, smoking, alcohol consumption, and physical activity did not change the results.

Supplementary Fig. 2 shows the plots of the $-\log_{10}$ P values for the trend test for serum HMW adiponectin. No associations with HMW adiponectin levels reached genome-wide significance level ($P = 5 \times 10^{-8}$). We took SNPs representing independent loci with $P < 5 \times 10^{-6}$ for further replication (Table 2). In addition, we chose the two best associated but not correlated ($r^2 = 0.05$) SNPs at the ADIPOQ (3q27) loci, rs3774261 and rs822354, for replication.

The replication samples include 626 white Italian subjects (240 men and 386 women). Two ADIPOQ SNPs, rs822354 and rs3774261, showed directionally consistent and nominally significant associations with serum HMW adiponectin in the replication sample (Table 1), and the association of rs822354 reached genome-wide significance in meta-analysis of the two studies ($P = 3.67 \times 10^{-8}$). SNP rs822354 accounted for 1.1 and 2.5% of the variance in HMW adiponectin levels in the NHS and Italian samples, respectively. Another SNP, rs10447248 (5q21), also showed directionally consistent associations with HMW adiponectin levels ($P = 4.27 \times 10^{-3}$) in the replication samples. In the meta-analysis, the association of rs10447248 reached genome-wide significance level (4.69×10^{-8}) (Table 1; Fig. 1). Each A allele of SNP rs10447248 was related to 0.76 and 0.39 μg/mL lower HMW adiponectin and accounted for 1.3 and 2% of the variance in the NHS and Italian samples, respectively.

SNP rs2468677 (8q24) also showed directionally consistent and marginal association with HMW adiponectin levels in the replication set ($P = 0.0607$). In the meta-analysis, the association between rs2468677 and HMW adiponectin levels was near genome-wide significance ($P = 7.95 \times 10^{-7}$).

We further examined the impact of the newly identified SNP rs10447248 in the FER locus on the serum levels of other isoforms, MMW and LMW adiponectin, that were measured in the Italian samples. The A allele of SNP rs10447248 was nominally associated with lower MMW ($P = 0.016$) but was not associated with LMW ($P > 0.05$) (Fig. 2) adiponectin levels.

To get deeper insights about the associations between the newly identify FER locus and HMW adiponectin levels, we performed genotyping imputation in the NHS samples

TABLE 1
Associations of SNPs with serum concentration of HMW adiponectin

SNPs	Chromosome	BP	Gene	Effect/reference alleles*	NHS				Italian				Combined P
					EAF	n	β	P	EAF	n	β	P	
Novel loci													
rs13358260	5	78881467	PAPD4	C/T	0.93	1591	1.45	8.85E-07	0.94	625	0.04	4.51E-01	4.96E-06
rs10517133	4	45059671	AC108043.3–1	C/G	0.90	1583	1.28	1.28E-06	0.90	622	0.09	3.67E-01	4.55E-06
rs10447248	5	107943635	FER	A/G	0.34	1583	-0.76	3.23E-06	0.38	620	-0.39	4.27E-03	4.69E-08
rs2120576	12	91754320	EEA1	C/T	0.89	1591	1.14	3.32E-06	0.90	623	-0.19	2.34E-01	9.30E-04
rs2468677	8	140588699	KCNK9	G/T	0.52	1590	0.72	3.33E-06	0.51	622	0.24	6.07E-02	7.95E-07
Known loci													
rs822354	3	187962900	ADIPOQ	A/G	0.68	1590	0.71	3.17E-05	0.64	622	0.58	1.96E-04	3.67E-08
rs3774261	3	188054253	ADIPOQ	A/G	0.62	1590	0.67	3.09E-05	0.52	616	0.36	9.97E-03	9.63E-07

BP, base pair physical position; EAF, effect allele frequency. *Effect alleles represent the alleles associated with serum HMW adiponectin levels.
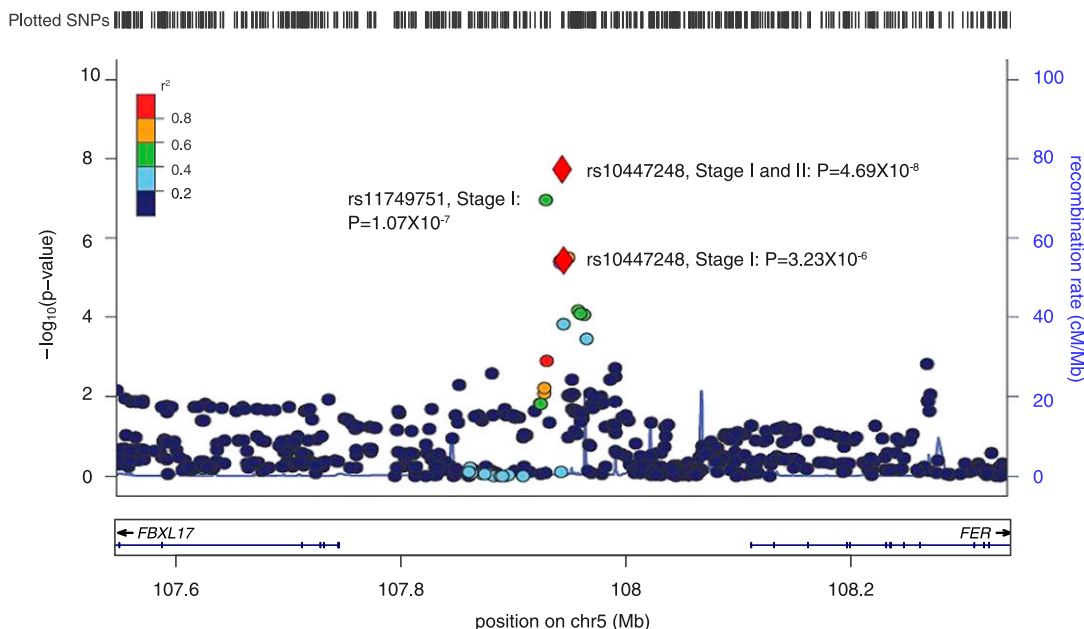
FIG. 1. Region plot of *FER* locus. Association signals at chromosome 5q21, across a region centering on *SNP* rs10447248, with HMW adiponectin levels in NHS. The vertical axis represents the –log$_{10}$ *P* values from the linear regression analysis for the genotyped and imputed SNPs.

(19). Among the imputed SNPs, rs11749751 in the region near *FER* showed stronger association with serum HMW adiponectin ($P = 1.07 \times 10^{-7}$) than SNP rs10447248 (Fig. 1). The two SNPs are in moderate linkage disequilibrium ($r^2 = 0.5$). When included in the same regression model, both SNPs rs10447248 and rs11749751 showed independently and nominally significant associations with HMW levels ($P = 0.0018$ and 0.0044, respectively).

We further examined the associations between the HMW adiponectin–associated SNPs with the risk of type 2 diabetes in NHS and HPFS and with the metabolic traits in the Italian samples. After adjustment for age, BMI, and fasting status, the allele A of *ADIPOQ* SNP rs822354 was related to a decreased risk of diabetes in the HPFS (odds ratio [OR] 0.87 [95% CI 0.78–0.98]) but not in the NHS (1.00 [0.98–1.03]). *FER* SNPs were not associated with diabetes risk. In Italian samples, allele A of *ADIPOQ* SNP rs822354 was associated with a lower metabolic syndrome score ($P = 0.002$) (Table 2), lower fasting glucose ($P = 0.04$), and higher HDL cholesterol levels ($P = 0.04$). In our sample, HMW adiponectin and metabolic syndrome score share a common genetic background as indicated by a significant genetic correlation. Of note, *ADIPOQ* SNP rs822354 could explain 3% of the genetic correlations between the two variables ($\rho g = -0.32 \pm 0.14$) ($P = 0.04$). *FER* SNP rs10447248 was not significantly related to metabolic syndrome score but was associated with lower levels of HDL cholesterol ($P = 0.009$).

DISCUSSION

In this study, we found novel genome-wide significant associations of SNPs on chromosome 5q21 with serum HMW adiponectin levels in Caucasians with European ancestry. SNP rs10447248 is ~170 kb upstream the *FER* gene (Fig. 1). Fer protein is a member of the FPS/FES family of nontransmembrane receptor tyrosine kinases. Animal studies suggest that *FER* has a role in regulating inflammation and innate immunity (20,21). However, there

are no available data for the roles of this gene product in regulating adiponectin production.

We confirmed that common SNPs near/in *ADIPOQ* locus were associated with serum HMW adiponectin levels. The results are consistent with the previous studies (4,11,14). Interestingly, we found that multiple genetic variations in low linkage disequilibrium were related with HMW adiponectin levels, suggesting that allelic heterogeneity may exist at this locus in affecting the homeostasis of the marker. Another locus near the *KCNK9* gene on chromosome 8q24 also showed suggestive association with HMW adiponectin levels. Of note, in a previous genome-wide scan this locus was related to the levels of triglycerides (22). SNPs in genes *ARL15* (rs4311394) and *CDH13* (rs3865188 and rs7195409) were recently associated with total or HMW adiponectin levels (11,12,14). However, neither locus was
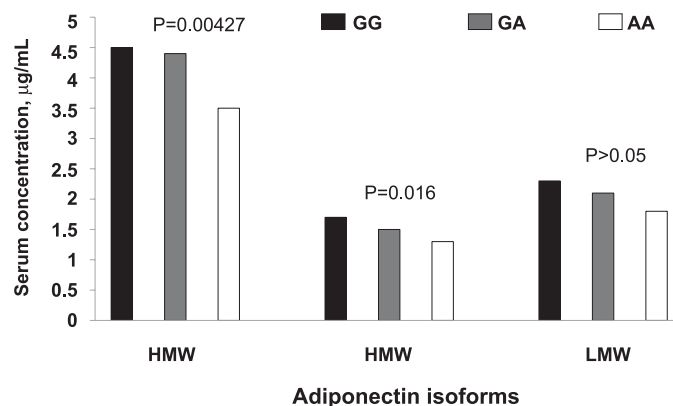

FIG. 2. Associations of SNP rs10447248 with adiponectin isoforms. Levels of circulating HMW, MMW, and LMW adiponectin by the genotypes of SNP rs10447248 in Italian samples. The *P* values are from the trend test in linear regression model. The analyses were adjusted for age, sex, and BMI.

TABLE 2
Associations with metabolic syndrome components in Italian samples

| | Genotype | | | |
	GG	GA	AA	P*
rs822354				
Fasting glucose (mg/dL)	91.4	90.2	89	0.04
HDL cholesterol (mg/dL)	52	53.5	53.9	0.04
Triglycerides (mg/dL)	99.2	100.7	95.2	0.66
Waist (cm)	85	84.6	83.8	0.11
SBP (mmHg)	117	117	115	0.22
DBP (mmHg)	77.2	76.7	77.3	0.73
MS score	0.97	0.9	0.73	0.002
rs10447248				
Fasting glucose (mg/dL)	89.8	91.3	90.3	0.35
HDL cholesterol (mg/dL)	54.3	52.4	51.2	0.009
Triglycerides (mg/dL)	94.9	101.9	102.9	0.21
Waist (cm)	84.5	84.8	84.5	0.86
SBP (mmHg)	116.8	117.1	116.3	0.86
DBP (mmHg)	76.9	77.3	76.3	0.48
MS score	0.87	0.94	0.90	0.57

DBP, diastolic blood pressure; MS, metabolic syndrome; SBP, systolic blood pressure. *Significance for testing linear trend.

related to HMW adiponectin in our sample (NHS, $P > 0.05$) or another GWAS in Europeans (13). It appears that the genetic effects of the *CDH13* locus were more significant in Asian populations (11,12).

Previous linkage analyses suggest that different loci may be involved in regulating total and HMW adiponectin levels (15,23). It is noteworthy that circulating adiponectin levels may be regulated at different levels including transcription, translation, and posttranslational levels such as protein modification, secretion, oligomerization, degradation, and excretion, as well as clearance (24). It is likely that the genetic variants affecting these various steps may modulate HMW adiponectin levels and that *ADIPOQ* locus may affect transcription of the gene. The mechanisms underlying the associations for *FER* locus remain to be clarified in future function studies.

HMW adiponectin levels have been previously related to metabolic traits such as plasma insulin, insulin resistance, and risk of type 2 diabetes (5,6,25). SNP rs822354 was related to a reduced risk of diabetes in HPFS and lower metabolic syndrome score in Italian samples; however, the SNP was not significantly associated with diabetes risk in NHS. No SNP by sex interaction in modulating metabolic syndrome score was observed in the Italian sample ($P = 0.48$). SNPs at the *FER* locus were not related to diabetes risk or metabolic syndrome score. Of note, these SNPs only account for a small proportion of the variance in HMW adiponectin levels. This may partly explain the weak relation between the genetic variations and disease risk.

The major strengths of our study included a well-defined cohort, high-quality genotype data, and minimal population stratification (17). We acknowledge several study limitations, including potential errors in biomarker measurements. Nevertheless, these errors more likely bias the association toward null because the measurement errors are uncorrelated with genotyping. The discovery samples include only women while the replication samples include both sexes. However, the previous GWAS did not report sex-specific genetic effects on adiponectin. In addition, the Italian cohort consists only of nondiabetic subjects and

may not represent the general population, and all study populations exclusively consist of Caucasians with European ancestry. Therefore, the findings may not be generalizable to other ethnicities.

In conclusion, we found a novel locus at the *FER* gene (5q21) associated with serum HMW adiponectin levels and provided confirmatory evidence for the *ADIPOQ* locus with HMW adiponectin levels. Future studies may investigate the potentially additive nature of multiple pathways in regulation of HMW adiponectin concentrations and whether these genetic variants may affect disease risk associated with adiponectin deficiency.

L.Q. conceived and designed the experiments; performed the experiments; analyzed data; contributed reagents, materials, and analysis tools; and wrote the manuscript. C.M. performed the experiments; analyzed data; contributed reagents, materials, and analysis tools; and wrote the manuscript. L.S. and C.D.B. critically reviewed the manuscript. V.T. contributed reagents, materials, and analysis tools and critically reviewed the manuscript. F.B.H. conceived and designed the experiments; contributed reagents, materials, and analysis tools; and critically reviewed the manuscript.

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