

Genome-Wide Linkage of Plasma Adiponectin Reveals a Major Locus on Chromosome 3q Distinct From the Adiponectin Structural Gene

The IRAS Family Study

Xiuqing Guo,^{1,2} Mohammed F. Saad,³ Carl D. Langefeld,⁴ Adrienne H. Williams,⁴ Jinrui Cui,¹ Kent D. Taylor,^{1,2} Jill M. Norris,⁵ Sujata Jinagouda,³ Christine H. Darwin,³ Braxton D. Mitchell,⁶ Richard N. Bergman,⁷ Beth Sutton,⁸ Y.-D. Ida Chen,^{1,2} Lynne E. Wagenknecht,⁴ Donald W. Bowden,^{4,8} and Jerome I. Rotter^{1,2}

Adiponectin (APM1) is an adipocyte-derived peptide that contributes to glucose, lipid, and energy homeostasis. We assessed the genetic basis of plasma adiponectin in Hispanic-American and African-American families enrolled through the Insulin Resistance Atherosclerosis Study Family Study. A 10-cM genome scan was performed in two batches: an original set (set 1) consisting of 66 families (45 Hispanic American and 21 African American) and a replication set (set 2) consisting of 66 families (45 Hispanic American and 21 African American). Adiponectin levels were measured by radioimmunoassay in 1,727 individuals from 131 of 132 families. Linkage analysis was carried out in Hispanic Americans and African Americans separately in set 1, set 2, and the pooled set (set 1 plus set 2), with and without diabetic subjects. A major gene was mapped to 3q27 with a logarithm of odds (LOD) score of 8.21 in the Hispanic-American sample. Ninety-six unrelated individuals were screened for polymorphisms in the APM1 gene, and 18 single nucleotide polymorphisms (SNPs) were genotyped in the Hispanic-American sample. Plasma adiponectin level was modestly associated with two SNPs and their accompanying haplotypes. Incorporating each or both SNPs in the linkage analysis, however, did not significantly re-

duce the LOD score. Therefore, a quantitative trait locus at 3q27, likely distinct from the APM1 gene, contributes to the variation of plasma adiponectin levels in the Hispanic-American population. *Diabetes* 55:1723–1730, 2006

Plasma adiponectin is an adipocytokine produced by fat cells; its concentration was found to be negatively correlated with BMI, insulin resistance, hyperinsulinemia, plasma triglyceride concentration, and fasting and postprandial plasma glucose concentrations (1–3). Recent data provide evidence for genetic modulation of adiponectin levels. Family studies (4–6) have shown adiponectin to be heritable, with heritabilities reported to be in the range of 40–70%. Genome-wide linkage scans found evidence, or suggestive evidence, of linkage on chromosomes 5p, 14p, 2p, 3q, 10p, and 17p in a predominantly northern European ancestry population (4); evidence for linkage on chromosome 9p and tentative evidence of linkage on chromosomes 2p, 3q, and 10p in the Pima Indian population (5); evidence of linkage on chromosome 15p in Chinese and on 18p in Japanese, as well as suggestive evidence of linkage on chromosomes 3, 18, and 20 in Japanese (6); and evidence of linkage on chromosome 3q in the Old Order Amish (7). In this study, we assessed the genetic basis of plasma adiponectin in Hispanic-American and African-American families. The genome scan study identified a major quantitative trait loci (QTLs) in Hispanic Americans but not in African Americans in the region where the APM1 (ACDC) gene is located. However, association analysis indicated that the linkage signal could not be explained by the assessed variation in the APM1 gene.

RESEARCH DESIGN AND METHODS

The Insulin Resistance and Atherosclerosis Study Family Study (IRASFS) was designed to identify the genetic basis of glucose homeostasis and adiposity traits. Details of the study design, recruitment, and phenotyping have been described in detail (8). Briefly, large multigenerational African-American and Hispanic-American families were recruited through probands of the parent IRAS (9). Two-thirds (88) of the total 132 families were ascertained based on a large family structure as reported in an IRAS family history interview, not on clinical characteristics. Because the parent IRAS overrecruited people with impaired glucose tolerance and type 2 diabetes, the prevalence of type 2 diabetes (28%) was higher in these probands than in the general population.

From the ¹Medical Genetics Institute, Steven Spielberg Pediatric Research Center, Cedars-Sinai Medical Center, Los Angeles, California; the ²Departments of Pediatrics and Medicine, David Geffen School of Medicine at UCLA, Los Angeles, California; the ³Division of Clinical Epidemiology, Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, California; the ⁴Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, North Carolina; the ⁵Section of Epidemiology and Community Health, Department of Preventive Medicine and Biometrics, University of Colorado Health Sciences Center, Denver, Colorado; the ⁶Division of Endocrinology, Diabetes, and Nutrition, University of Maryland School of Medicine, Baltimore, Maryland; the ⁷Department of Physiology and Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, California; and the ⁸Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, North Carolina.

Address correspondence and reprint requests to Xiuqing Guo, PhD, Medical Genetics Institutes, Cedars-Sinai Medical Center, 8700 Beverly Blvd., 665W, Los Angeles, CA 90048. E-mail: xiuqing.guo@cshs.org.

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IBD, identity by descent; IRASFS, Insulin Resistance Atherosclerosis Study Family Study; LD, linkage disequilibrium; LOD, logarithm of odds; QTL, quantitative trait locus; SNP, single nucleotide polymorphism; SOLAR, Sequential Oligogenic Analysis Routines; UTR, untranslated region.

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The cohort was supplemented with 44 additional families from the community and from ongoing research programs. The prevalence of type 2 diabetes was also high in these probands (32%). Hispanic Americans were enrolled in San Antonio, Texas, an urban Hispanic population, and San Luis Valley, Colorado, a rural Hispanic population, and African Americans were enrolled in Los Angeles, California. Institutional review board approval was obtained at each collaborating institute. Informed consent was obtained from all subjects after the procedures were explained.

Adiponectin assay. Plasma adiponectin levels were measured by radioimmunoassay (Linco Research, St. Charles, MO) in 1,727 individuals (1,153 Hispanic Americans and 574 African Americans) from 131 IRASFS families (89 Hispanic American and 42 African American) for whom a ~10-cM genome scan had been completed (see below).

Microsatellite marker genotyping. A 10-cM genome scan using microsatellite markers was conducted on 132 families of the IRASFS by the Mammalian Genotyping Center in Marshfield, Wisconsin (available at <http://research.marshfieldclinic.org/genetics>), in two separate batches. Set 1 consisted of 66 families (45 Hispanic American and 21 African American) with 988 individuals (674 Hispanic Americans and 314 African Americans), while set 2 comprised 66 families (45 Hispanic American and 21 African American) with 739 individuals (479 Hispanic Americans and 260 African Americans). Adiponectin data were not available in one family. Mendelian inconsistency was examined using the software PEDCHECK (10), and probable genotyping errors were converted to missing. Pedigree relationships were confirmed using the entire genome scan data (383 markers) and the software PREST (11). Ninety-nine likely misspecified familial relationships were modified from 43 families. To account for possible founder effect, center-specific maximum likelihood estimates of allele frequencies based on unrelated individuals were obtained using the software LOKI (12–13) in the following linkage analyses.

Search for adiponectin polymorphisms. The APM1 gene maps to chromosome 3q27 and consists of three exons and two introns spanning 17 kb (14). Several investigators have searched the APM1 gene for sequence variants and have identified a range of variants located throughout the gene (14–16). We have carried out an extensive search for additional variants in the proximal 5' promoter, coding sequence, and 3' untranslated region (UTR) sequence of the APM1 gene using denaturing high-performance liquid chromatography. A total of 96 DNA samples from unrelated African-American subjects were tested for polymorphism screening (sequencing in African Americans should reveal the most variation compared with that in Hispanics) (17). This search identified two novel coding single nucleotide polymorphisms (SNPs): G113A (G38E) in exon 2 and C1233T (Y111H) in exon 3 (previously observed in 16,18), in addition to the previously described T45G (G15G), 13 SNPs in the long 3' UTR sequence of the APM1 gene, and 3 promoter SNPs that had also been identified in other laboratories (15–16). However, none of these 3' UTR SNPs fall within known regulatory elements, e.g., AUUUA. The SNPs identified in the 3' region were fairly close together and some had relatively low minor allele frequency; thus, we chose only 2 polymorphic SNPs out of the 13 in the 3' UTR for further genotyping. All three promoter SNPs were genotyped. In total, 18 SNPs (6 were identified by denaturing high-performance liquid chromatography in our laboratory and 12 polymorphisms were identified through the National Center for Biotechnology Information dbSNP database [available at <http://www.ncbi.nlm.nih.gov/SNP/and/or literature searches>]) occurring in two linkage disequilibrium (LD) blocks covering the entire APM1 gene (17) were genotyped.

SNP genotyping. The 18 selected SNPs in the APM1 gene were genotyped in the Hispanic-American sample using the Sequenom MassArray Genotyping System (Sequenom, San Diego, CA). This genotyping system uses single base extension reactions to create allele-specific products of SNPs that are readily separated and automatically read by mass spectrometer in the MALDI-TOF MassARRAY system (Bruker Daltonics, Billerica, MA). Fifty quality control individuals were randomly placed throughout the plates and checked for accuracy. Each SNP was examined for Mendelian consistencies using PEDCHECK (10). Any genotypes inconsistent with Mendelian inheritance were converted to missing.

Statistical analysis. Descriptive statistics were calculated for the study sample. GEE1 (general estimating equations methods) (19) were used to test for differences in adiponectin levels between men and women and between Hispanic Americans and African Americans. To better approximate conditional normality and homogeneity of variance, we examined a family of power transformations, and natural log provided the best transformation for adiponectin. However, modest departures from conditional normality may still exist for log adiponectin, e.g., in the Hispanic-American sample, we observed a skewness of -0.45 and kurtosis of 1.25 for the residuals of log(adiponectin) after adjusting for age, sex, BMI, and clinic site. We therefore also obtained empirical logarithm of odds (LOD) scores in our linkage analyses.

Linkage analysis. The variance component linkage analysis method, as implemented in the Sequential Oligogenic Linkage Analysis Routines (SOLAR)

program, was used to test for evidence of linkage (20). In this approach, the total variation in log(adiponectin) (σ^2_P) is partitioned into components of variance due to a major gene (σ^2_{MG}), additive polygenes (σ^2_G), and individual-specific environment (σ^2_E)

$$\sigma^2_P = \sigma^2_{MG} + \sigma^2_G + \sigma^2_E \quad (1)$$

Heritability (h^2) is estimated by the proportion of total variation in adiponectin levels due to genetic effects ($h^2 = \sigma^2_G/\sigma^2_P$). Similarly, the expected genetic covariances between arbitrary relatives i and j can be specified as a function of the identity by descent (IBD) and relationships at a given marker locus as the following:

$$\text{Cov}(X_i, X_j) = \sigma^2_{MG} \text{IBD}_{ij} + \sigma^2_G 2\phi_{ij} + \sigma^2_E \delta_{ij} \quad (2)$$

where IBD_{ij} is the probability of individuals i and j share both alleles identical by descent, ϕ_{ij} is the corresponding kinship coefficient, and $\delta_{ij} = 1$ if $i = j$ and 0 otherwise. All results reported are based on multipoint estimates of the IBD statistics. SOLAR tests for linkage by testing the null hypothesis that the additive genetic variance due to a QTL equals zero (no linkage). Age, sex, BMI, and center (in Hispanic-American sample) were all significant predictors for adiponectin ($P < 0.01$ each in the Hispanic-American sample), and the proportion of total variance due to these covariates is 0.17. Therefore, all of the four covariates were adjusted in the following analyses. Empirical P values were generated by simulating a fully informative marker and gene dropping to minimize any effect of departures from distributional assumptions using the LODADJ option. These data were simulated and subsequent linkage analysis computed 10,000 times to obtain the distribution of the test statistic under the null hypothesis of no linkage. Given that this approach estimates a linear correlation factor (21,22), 10,000 replications are a reasonable estimate. The empirical P values were obtained as the proportion of the 10,000 replicates that had an LOD score greater than or equal to the nominal LOD score that was observed for the original linked locus. P values were converted to LOD scores by $\text{LOD} = \chi^2/[2^* \ln(10)]$.

In set 1, we carried out a 10-cM genome scan linkage analysis for each ethnic group (674 Hispanic-American individuals from 45 families resulting in 4,015 relative pairs including 694 full sibpairs; 314 African-American individuals from 21 families resulting in 1,800 relative pairs including 243 full sibpairs). The Hispanic-American set 2 sample was used for the confirmation study, which comprised 479 individuals from 44 families (3,779 relative pairs including 657 full sibpairs). The partitioning of the data into two samples provides an opportunity to conduct a confirmatory analysis in regions identified in the set 1 linkage analysis. To exclude the possible effect of diabetes status on the linkage signal, the linkage analysis was repeated in the Hispanic-American sample after excluding individuals with diabetes.

Association analysis. Two methods were utilized to test for an association between individual SNPs in the APM1 gene and plasma adiponectin levels, including the generalized estimating equations methods as described above (19) and a variance components-based method as implemented in SOLAR. Two degrees of freedom overall test of genotypic association and the three a priori genetic models (i.e., additive, dominant, or recessive) when appropriate were computed using generalized estimating equations methods as implemented in the GENMOD procedure of SAS. In SOLAR association analysis, we used dummy variables for each of the genetic models. A consistent finding of association from both methods ensures to certain degree of reliability.

Haplotype analysis. As discussed in Sutton et al. (17), the level of LD among the SNPs was estimated using the following equation:

$$\hat{D}' = \begin{cases} \frac{\hat{p}_{11} - \hat{p}_1 \hat{p}_1}{\min(\hat{p}_1 \hat{p}_2, \hat{p}_1 \hat{p}_{2+})}, \hat{D} > 0 \\ \frac{\hat{p}_{11} - \hat{p}_1 \hat{p}_1}{\min(\hat{p}_1 \hat{p}_1, \hat{p}_2 \hat{p}_{2+})}, \hat{D} < 0 \end{cases} \quad (3)$$

where for alleles 1 and 2 at the respective loci, $\hat{D} = \hat{p}_{11} - \hat{p}_1 \hat{p}_1$ is the classic LD coefficient. Only the founders within the families were included in LD analysis to eliminate bias due to family structure. Haplotype association analysis was then carried out using the quantitative pedigree disequilibrium tests, using one, two, three, and four marker moving windows to assess haplotype association (23).

Linkage analysis conditional on the SNPs in the APM1 gene. To evaluate the contribution of the APM1 gene variations to the linkage signal, linkage analyses were repeated in the Hispanic-American sample on chromosome 3 adjusting for each of the informative APM1 SNPs and/or combination of SNPs. Specifically, SNP(s) was included as a covariate, assuming a dominant or an additive model depends on the minor allele frequency. The percentage of variance explained by SNP(s) was also estimated.

TABLE 1
Characteristics of the IRAS family cohort

	HA		AA	
	All	Excluding diabetes	All	Excluding diabetes
<i>n</i> families	89	88	42	42
<i>n</i> individuals	1,153	1,027	574	512
Age (years)	41.1 ± 13.9 (39.9)	39.6 ± 13.2 (38.5)	42.6 ± 13.9 (41.2)	41.0 ± 13.4 (40.0)
Sex	Men 482 Women 671	Men 422 Women 605	Men 237 Women 337	Men 219 Women 293
BMI (kg/m ²)	28.4 ± 5.4 (28.0)	29.2 ± 6.7 (28.1)	28.7 ± 5.3 (28.2)	28.4 ± 5.2 (28.0)
Adiponectin (μg/ml)*	11.5 ± 6.5 (10.2)†‡	15.1 ± 7.6 (13.9)§	7.1 ± 3.9 (6.1)	10.5 ± 5.0 (9.6)

Data are means ± SD (median), unless otherwise indicated. *Adiponectin was log transformed for the comparison. *P* < 0.0001 for †men vs. women in Hispanic Americans (HA); ‡HA vs. African American (AA) for men; §HA vs. AA for women; ||men vs. women in AA; and ¶*P* = 0.0003 for HA vs. AA for nondiabetic women.

RESULTS

Characteristics of the family cohort. Consistent with the literature, mean plasma adiponectin levels were higher in female than male subjects in both Hispanic Americans ([means ± SD] 15.1 ± 7.6 vs. 11.5 ± 6.5 μg/ml in the entire Hispanic-American sample, 15.0 ± 6.6 vs. 11.4 ± 5.9 μg/ml in nondiabetic subjects, *P* < 0.0001 for each) and in African Americans (10.4 ± 5.6 vs. 7.1 ± 3.9 μg/ml in the entire African-American group, 10.5 ± 5.0 vs. 7.0 ± 3.8 μg/ml in nondiabetic subjects, *P* < 0.0001 for each) (Table 1). Interestingly, African Americans had significantly lower mean adiponectin levels than Hispanic Americans in both male (all individuals: 7.1 ± 3.9 vs. 11.5 ± 6.5 μg/ml; nondiabetic subjects: 7.0 ± 3.8 vs. 11.4 ± 5.9 μg/ml, *P* < 0.0001 for each) and female (all individuals: 10.4 ± 5.6 vs. 15.1 ± 7.6 μg/ml, *P* < 0.0001; nondiabetic subjects: 10.5 ± 5.0 vs. 15.0 ± 6.6 μg/ml, *P* = 0.0003) subjects. Plasma adiponectin levels were demonstrated to be highly heritable with an estimated heritability of 0.71 ± 0.06 in Hispanic Americans after adjusting for age, sex, BMI, and center and 0.64 ± 0.08 in African Americans after adjusting for age, sex, and BMI. When diabetic subjects were excluded, the heritability estimates increased to 0.84 ± 0.07 in Hispanic Americans and 0.82 ± 0.09 in African Americans.

Genome scan linkage analysis in set 1. Strong evidence for linkage on chromosome 3q27 between markers D3S2418 and MFD427 was observed in the Hispanic-American sample (LOD = 6.35 at 218 cM) and suggestive evidence of linkage on chromosome 12 (LOD = 2.18 at 111 cM between markers PAH and D12S2070) (Table 2). When we further examined the chromosome 3 region by each clinic site (San Antonio and San Luis Valley), we found consistent evidences for linkage. In San Antonio alone, the maximum LOD score was 3.22 observed at 217 cM, while the maximum LOD score in San Luis Valley was 3.40, occurring at 218 cM. However, no significant evidence of linkage was observed in the African-American families (LOD = 0.0) in this region.

Confirmation linkage study in the Hispanic-American sample. In the confirmation studies of the Hispanic-American set 2 sample, which comprised 479 individuals from 44 families (3,779 relative pairs including 657 full sibpairs), an LOD score of 1.62 was observed in the same region on chromosome 3, and the LOD score increased to 8.21 at 217 cM in the combined Hispanic-American sample (set 1 plus set 2: 8,274 relative pairs including 1,432 full sibpairs). The LOD-1 support interval surrounding the Hispanic-American peak was between 213 and 222 cM (9 cM in length) between markers D3S2418 and MFD427. A consistent linkage signal was again observed in San Antonio (LOD = 3.61 at 217 cM) and San Luis Valley (LOD = 4.64 at 218 cM) in the combined Hispanic-American sample. A new peak was identified on chromosome 7 (LOD = 2.68 at 132 cM between the markers D7S3061 and D7S1804) in the Hispanic-American set 2 sample. In the combined Hispanic-American sample, two additional regions, chromosome 2 (LOD = 2.48 at 207 cM, between D2S1384 and D2S2944) and chromosome 12 (LOD = 2.12 at 112 cM, between PAH and D12S395), showed suggestive evidence of linkage. When excluding diabetic subjects, the LOD score remained 5.98 on chromosome 3 at 218 cM and 2.09 on chromosome 2 at 199 cM. Interestingly, the maximum LOD score on chromosome 16 increased from 0.57

TABLE 2
Maximum LOD scores (position in cM) for plasma adiponectin levels*

Chromosome		HA			Combined HA (nondiabetic subjects)	Combined AA
		Combined	Set 1	Set 2		
2	Nominal	2.48 (207)	1.02 (202)	1.49 (207)	2.09 (199)	0.26 (266)
	Empirical	1.76 (207)	0.74 (202)	1.47 (207)	1.61 (199)	0.28 (266)
3	Nominal	8.21 (217)	6.35 (218)	1.62 (228)	5.98 (218)	0.63 (131)
	Empirical	5.82 (217)	4.64 (218)	1.60 (228)	4.60 (218)	0.68 (131)
7	Nominal	1.11 (81)	0.73 (80)	2.68 (132)	1.28 (82)	1.11 (107)
	Empirical	0.78 (81)	0.53 (80)	2.63 (132)	0.99 (82)	1.20 (107)
10†	Nominal	1.21 (152)	1.05 (151)	0.57 (164)	0.17 (154)	1.39 (75)
	Empirical	0.86 (152)	0.77 (151)	0.56 (164)	0.13 (154)	1.49 (75)
12	Nominal	2.12 (112)	2.18 (111)	0.49 (166)	0.69 (135)	0.15 (70)
	Empirical	1.50 (112)	1.59 (111)	0.48 (166)	0.53 (134)	0.16 (70)
16	Nominal	0.57 (96)	0.52 (114)	1.52 (66)	2.21 (70)	0.0 (0)
	Empirical	0.40 (96)	0.38 (114)	1.49 (66)	1.70 (70)	0.0 (0)

*Those chromosomes that had a maximum LOD score >2.0 in Hispanic Americans (HA) are reported here. †The highest LOD score in African Americans (AA).

(96 cM) to 2.21 (70 cM between D16S3396 and D16S3253). **Linkage analysis in the African-American sample.** The maximum LOD score on chromosome 3 was 0.63 at 131 cM in the entire African-American sample, while the LOD score at 218 cM, where the linkage peak was observed in the Hispanic-American sample, was 0. The best evidence of linkage in African Americans was on chromosome 10 (LOD = 1.39 at 75 cM) between markers D10S1208 and D10S1221, while no evidence of linkage was observed in the Hispanic-American sample at this location.

The genome scan results are shown in Fig. 1 for

Hispanic-American set 1, Hispanic-American set 2, the entire Hispanic-American sample, and the African-American sample. Figure 2 illustrates the detailed linkage results for chromosome 3.

Empirical LOD scores. The empirical LOD scores are also provided in Table 2. For the chromosome 3 peak, the nominal LOD score remained as high as 5.82 at 217 cM in the entire Hispanic-American sample and 4.60 at 218 cM in the nondiabetic Hispanic-American sample. These analyses further supported the existence of a QTLs on chromosome 3q27.

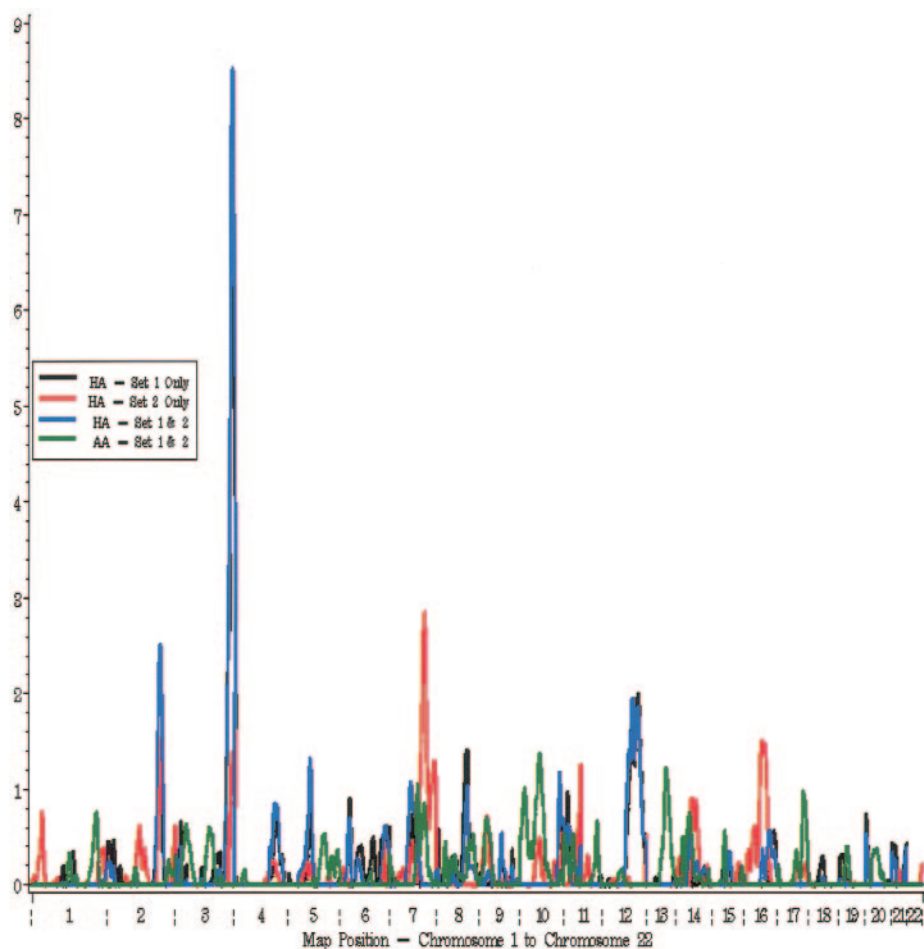


FIG. 1. Genome scan analysis of adiponectin adjusted for age, sex, BMI, and center. Maximum LOD scores for chromosomes 1–22 are presented as follows: Hispanic-American set 1, black lines; Hispanic-American set 2, red lines; Hispanic-American sets 1 and 2, blue lines; African Americans, green lines.

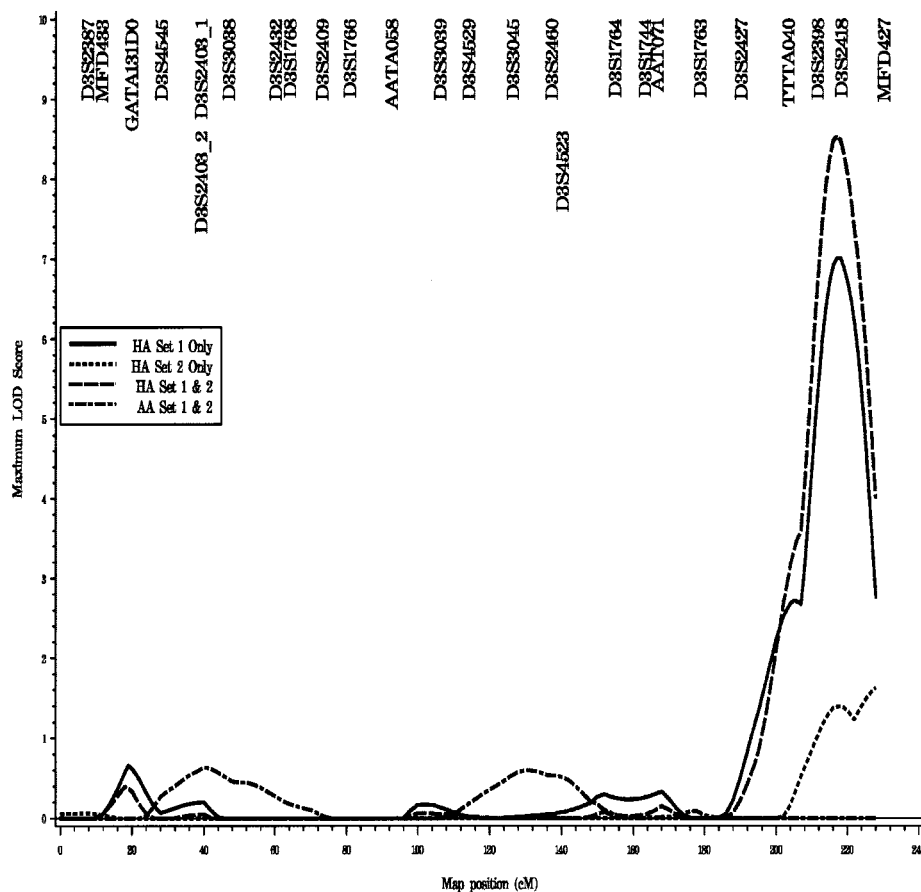


FIG. 2. Linkage analysis of adiponectin on chromosome 3 adjusted for age, sex, BMI, and center. Maximum LOD scores on chromosome 3 are presented as follows: Hispanic-American set 1, solid line; Hispanic-American set 2, dotted line; Hispanic-American sets 1 and 2, dashed line; African-American sets 1 and 2, dotted line.

Association analysis for each SNP. Among the 18 SNPs that were genotyped, 5 were found to be not polymorphic (i.e., minor allele frequency was <0.05) and were therefore eliminated from further association analyses. The association analysis of the 13 polymorphic SNPs in the APM1 structure gene revealed that only 2 SNPs, all located in the 5' promoter region, were associated with plasma adiponectin levels: rs1656943 ($P = 0.003$ from GENMOD and 0.03 from SOLAR) and G-11391A ($P < 0.0001$ from GENMOD and 0.01 from SOLAR) (Table 3). The minor allele in each of the two SNPs was associated with increased plasma adiponectin levels, as shown in Table 4.

Association analysis for haplotypes. Haplotype analysis revealed that haplotypes in the genomic region between SNPs rs1656943 and G-11391A were associated with adiponectin levels in the Hispanic-American sample. The P value was 0.026 for haplotypes reconstructed using SNPs rs1656943 and rs860291, while that for haplotypes reconstructed using SNPs rs860291 and G-11391A was 0.002. The three-SNP (rs1656943, rs860291, and G-11391A) haplotype analysis yielded the most significant association ($P = 0.001$). The third most frequent haplotype carries the rare variants at both rs1656943 and G-11391A and is associated with elevated adiponectin levels ($P = 0.016$),

TABLE 3
The SNPs in the APM1 gene and association analysis in the HA sample

SNP	Gene region	dbSNP	Minor allele frequency	P value GEE1	P value SOLAR
C-19148T	Promoter	rs4632532	0.45	0.91*	0.74
C-17760T	Promoter	rs6444169	0.18	0.45†	0.45
T-14816C	Promoter	rs1656943	0.05	0.003†	0.03
C-12896T	Promoter	rs860291	0.10	0.24†	0.18
G-11391A	Promoter	dHPLC	0.05	<0.0001 †	0.011
C-11377G	Promoter	rs266729	0.31	0.70*	0.56
InsCA_11156	Promoter	dHPLC	0.15	0.43†	0.64
A-10066G	Intron1	rs182052	0.45	0.98*	0.84
G-7950T	Intron1	rs822390	0.23	0.26†	0.13
A-4120C	Intron1	rs822394	0.22	0.18†	0.06
T45G	Exon2	rs2241766	0.18	0.78†	0.98
G276T	Intron2	rs1501299	0.24	0.17†	0.17
A712G	Intron2	rs3774261	0.44	0.82*	0.76

*General association model. †Dominant model. HA, Hispanic American.

TABLE 4

The mean adiponectin levels in the Hispanic-American sample classified by genotype of the two associated SNPs and by haplotypes, *P* value for association, and SNP(s)/haplotype contributions to the linkage signal

SNP	Mean adiponectin ($\mu\text{g/ml}$)		<i>P</i> value	LOD score reduction	Percent variance*
	1/1	1/2 + 2/2			
rs1656943	13.31 \pm 6.90 (995)	14.99 \pm 7.20 (118)	0.003	8.76 \rightarrow 8.40	0.16
G-11391A	13.29 \pm 6.88 (1,025)	15.80 \pm 7.43 (108)	<0.0001	8.59 \rightarrow 8.23	0.17
Both SNPs	—	—	—	9.11 \rightarrow 8.70	0.17
Haplotypes (rs1656943, rs860291, and G-11391A)			0.001	8.19 \rightarrow 7.17	0.18
	Noncarriers	Carriers			
Haplotype 1	16.29 \pm 7.04 (58)	13.39 \pm 6.33 (1,094)	0.005	—	—
Haplotype 3	13.32 \pm 6.32 (1,050)	15.82 \pm 6.72 (102)	0.016	—	—

Data are means \pm SD (*n*), unless otherwise indicated. *0.17% variance was explained by age, sex, and BMI for samples being typed on one of the three SNPs.

while the most frequent haplotype (111) is associated with decreased adiponectin levels (*P* = 0.005, Table 4).

Linkage analysis conditional on the SNPs and haplotypes in the APM1 gene. To have a consistent sample and to be able to compare the change in the LOD scores in the models with and without the SNP adjustment, we restricted the linkage analyses to those individuals with genotypes available for the SNPs of interest. Adjusting individually for each SNP did not change the maximum LOD significantly: estimated scores reduced from 8.76 to 8.40 after adjusting for rs1656943 and from 8.59 to 8.23 after adjusting for G-11391A. Adjusting for these two SNPs simultaneously reduced the LOD score from 9.11 to 8.70. When adjusting for haplotypes for SNPs rs1656943, rs860291, and G-11391A, the LOD score reduced from 8.19 to 7.17 (Table 4). This indicates that these SNPs are not major determinants of the large linkage peak observed in this study. Supporting this conclusion, the estimated percentage of adiponectin variation explained by the SNP(s) and/or haplotypes was also quite low (Table 4).

DISCUSSION

The goal of this study was to elucidate the genetic basis of plasma adiponectin in Hispanic-American and African-American families. We performed a genome-wide scan of plasma adiponectin levels in 89 Mexican-American and 42 African-American families including 1,727 individuals (1,153 Hispanic Americans and 574 African Americans). The initial linkage analysis carried out in the first half of the sample revealed strong evidence for linkage on chromosome 3q27 in the Hispanic-American sample. The confirmation study of the Hispanic-American set 2 sample found an LOD score of 1.62 in the same region. The LOD score increased to 8.21 in the combined Hispanic-American sample. Excluding diabetic subjects reduced the LOD score to 5.98 but still established the existence of a QTL on chromosome 3 near 218 cM, which excludes the possibility of a false-positive due to possible influence of diabetes status. The consistency of the linkage results, from set 1, set 2, the combined Hispanic-American sample, and combined Hispanic-American sample after excluding diabetic subjects, is reassuring of the existence of a QTL on chromosome 3q27.

Experimental data suggest that plasma adiponectin levels might be one of the key molecules in the development of the metabolic syndrome (24). Available genome-wide scans have mapped a susceptibility locus for type 2 diabetes and aspects of the metabolic syndrome to the same location, chromosome 3q27 (25–26). We therefore

investigated polymorphisms in the APM1 gene located at 3q27, using association and linkage analysis conditional on SNPs and/or haplotypes as an explanation for the linkage signal. Two SNPs (rs1656943 and G-11391A) in the 5' promoter region were found to be statistically associated with plasma adiponectin levels, but they explained only a very small amount of adiponectin variation, and adjusting for each of the two SNPs or adjusting for both simultaneously in the linkage analysis did not change the LOD score appreciably. These results indicate that the evidence of linkage in our sample cannot be explained by the assessed variation in the APM1 gene.

SNP +2019 delA, which was found to contribute to the linkage of adiponectin levels in Amish individuals (7), was not genotyped in this population. However, based on LD data from Vasseur et al. (16), +2019 delA is in complete LD with SNP A712G (*D'* = 0.99). Given the lack of association with SNP A712G as well as haplotypes generated from the region containing SNP A712G in the IRASFS participants and the high LD between SNPs, it is unlikely association would be identified with SNP +2019 delA. We also examined regions of high conservation among species (the highest conservation was the coding regions and the proximal 5' promoter), which was extensively screened. There may be additional regulatory regions that we did not identify and/or genotype. However, to the best of our knowledge, little work has been functionally done to determine the regulatory regions further up or downstream from APM1 that could be responsible for controlling APM1 levels. It should be noted that the next gene 5' to adiponectin (RFC4) is only 3.6 kb upstream from exon 1 of adiponectin and the next gene 3' (ST6GAL1) is only 7.2 kb away. There does not seem to be much room for additional regulatory elements. The discrepant findings in association with APM1 gene could be due to ethnic differences or differences in SNPs genotyped. Also, the functional SNP probably has yet to be identified in any population; thus, different studies are detecting association due to LD with the functional SNP, leading to different SNP associations. Further efforts to investigate other genes in the regions are underway.

BMI, body weight, and fasting insulin levels in Caucasians (25) and early-onset diabetes in French Caucasians (26) have been mapped to 3q27. Polymorphisms in the APM1 gene have been reported to be associated with BMI, insulin sensitivity, type 2 diabetes, coronary artery disease, and metabolic syndrome (15,27–28). To explore the relationship among the APM1 gene, adiposity, and plasma adiponectin levels, we examined the genetic correlation

between BMI and adiponectin. A significant genetic correlation was observed in the Hispanic-American sample (-0.22 ± 0.09 , $P = 0.016$), while a modest genetic correlation was observed in the African-American sample (-0.17 ± 0.13 , $P = 0.21$). The evidence of the existence of common gene(s) underlying adiponectin and BMI in the Hispanic-American sample leads to the question of whether the gene(s) affects adiponectin levels through BMI or through a different mechanism. We therefore also ran the multipoint linkage analysis in the combined Hispanic-American sample (set 1 plus set 2) for chromosome 3 without adjusting for BMI. A maximum LOD score of 8.18 was obtained at 218 cM compared with a maximum LOD score of 8.21 at 217 after adjusting for BMI. The gene(s) harbored in the 3q region appear to be affecting plasma adiponectin level independently of BMI.

Differences were observed between Hispanic Americans and African Americans within the IRAS families. First, the levels of adiponectin were very different in these groups, and this was not explained by BMI. Second, even though the heritabilities were equivalent, the linkage results were very different. In the 3q27 region, where we identified a QTL in the Hispanic-American sample, no evidence of linkage was observed in the African-American sample. We also identified several regions that showed suggestive evidence of linkage in the Hispanic-American sample, including chromosomes 2, 7, and 12, but no evidence of linkage was observed in the African-American sample in any of these regions. The best evidence of linkage in African Americans was on chromosome 10, while no evidence of linkage was observed in the Hispanic-American samples at this location. The newly identified QTLs location (3q) in the Hispanic-American samples of our study is the same as in the Old Order Amish (7) but is different from northern European (5p, 14p) (4), Pima Indian (9p) (5), Chinese (15p) (6), and Japanese (6), suggesting genetic heterogeneity among different populations. We also observed a higher heritability in both Hispanic Americans (0.71) and African Americans (0.64) compared with Caucasians and Pima Indians (40–45%). Since adiponectin is present in plasma in multimeric forms (29), the variation in laboratory approaches could account for some of the large variation of heritability values seen in various studies. However, there is no particular association between observing a higher or lower heritability and use of a specific method.

In conclusion, we have observed strong linkage evidence for a QTL on chromosome 3q27, apparently distinct from the APM1 gene, that contributes to the variation of plasma adiponectin levels in the Hispanic-American population. Furthermore, ethnic-based genetic heterogeneity that determines plasma adiponectin levels was evident. Identifying genes in these regions should provide insight into the regulation of adiponectin and its relation to insulin sensitivity, obesity, and diabetes. This may facilitate the development of more effective therapies for prevention and treatment of diabetes and associated cardiovascular diseases.

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