Genome-Wide Linkage Scan for Genes Influencing Plasma Triglyceride Levels in the Veterans Administration Genetic Epidemiology Study

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OBJECTIVE—Elevated plasma triglyceride concentration is a component of the insulin resistance syndrome and is commonly associated with type 2 diabetes, obesity, and coronary heart disease. The goal of our study was to perform a genome-wide linkage scan to identify genetic regions that influence variation in plasma triglyceride levels in families that are enriched with individuals with type 2 diabetes.

RESEARCH DESIGN AND METHODS—We used phenotypic and genotypic data from 1,026 individuals distributed across 294 Mexican-American families, who were ascertained for type 2 diabetes, from the Veterans Administration Genetic Epidemiology Study (VAGES). Plasma triglyceride values were transformed, and a variance-components technique was used to conduct multipoint linkage analysis.

RESULTS—After adjusting for the significant effects of sex and BMI, heritability for plasma triglycerides was estimated as $46 \pm 7\%$ ($P < 0.0001$). Multipoint linkage analysis yielded the strongest evidence for linkage of plasma triglycerides near marker D12S391 on chromosome 12p (logarithm of odds [LOD] = 2.4). Our linkage signal on chromosome 12p provides independent replication of a similar finding in another Mexican-American sample from the San Antonio Family Diabetes Study (SAFDS). Combined multipoint linkage analysis of the VAGES and SAFDS data yielded significant evidence for linkage of plasma triglycerides to a genetic location between markers GATA49D12 and D12S391 on 12p (LOD = 3.8, empirical P value = 2.0×10^{-5}). This region on 12p harbors the gene-encoding adiponectin receptor 2 (*AdipoR2*), where we previously have shown that multiple single nucleotide polymorphisms are associated with plasma triglyceride concentrations in the SAFDS. In the present study, we provided suggestive evidence in favor of association for rs929434 with triglyceride concentrations in the VAGES.

CONCLUSIONS—Collectively, these results provide strong evidence for a major locus on chromosome 12p that influences plasma triglyceride levels in Mexican Americans. *Diabetes* 58: 279–284, 2009

The insulin resistance (metabolic) syndrome is characterized by an increase in plasma triglyceride concentration, reduced HDL, visceral obesity, elevated blood pressure, and glucose intolerance (1,2). The prevalence of the insulin resistance syndrome and/or related diseases, including obesity and type 2 diabetes, is rapidly increasing. Although major risk factors for these disorders have been identified, the genetic factors remain largely unknown. Elevated plasma triglyceride concentration is a component of the insulin resistance syndrome and is commonly associated with type 2 diabetes, obesity, and coronary heart disease (1,2). Since knowledge of specific genetic determinants of the common forms of hypertriglyceridemia is limited, we conducted a genome-wide linkage scan to localize genes that influence variation in plasma triglyceride levels in Mexican-American families ascertained for type 2 diabetes from the Veterans Administration Genetic Epidemiology Study (VAGES).

Genome-wide linkage screens for plasma triglyceride levels have been performed in several populations, including our previous study in Mexican Americans from the San Antonio Family Diabetes Study (SAFDS) (3,4). In that study, we reported significant evidence for the linkage of triglycerides to a genetic location on chromosome 15q and found potential evidence for linkage on several chromosomal regions including 12p (3,4). Linkage to chromosomes 2p, 11p, and 11q also have been reported for plasma triglyceride levels in French-Canadian families from the Quebec Family Study (5). In addition, a study performed in families with northern European ancestry demonstrated evidence for linkage to plasma triglyceride levels on chromosome 19 (6). Additionally, a study in a Chinese population from the Hong Kong Family Diabetes Study identified several chromosomes, including 2q, 3q, 6q, 9q, 10q, and 17q, containing genes that influence variation in triglyceride levels (7). Other evidence for the linkage of plasma triglyceride levels has been reported on 8q in African Americans (8), 2q in Hutterites (9), 11q in Old Order Amish (10), and 10p in Finnish families (11). More recently, a meta-analysis of genome-wide linkage studies for plasma triglyceride levels provided suggestive evidence for linkage at chromosome 7p (12). The purpose of this study was to perform a genome-wide linkage scan to identify genetic regions that influence variation in plasma triglyceride levels in Mexican-American individuals from VAGES, in which the families were ascertained for type 2 diabetes.

TABLE 1
Distribution of relative pairs by category in the VAGES and SAFDS families

Relative pair	Relationship coefficient*	Number of VAGES pairs†	Number of SAFDS pairs‡
Parent-offspring	0.5	416	463
Siblings	0.5	1,003	586
Grandparent-grandchild	0.25	19	123
Avuncular	0.25	184	810
Half-siblings	0.25	47	88
First and second cousins	0.15625	—	20
Double first cousins	0.25	—	10
Great grandparent-grandchild	0.125	—	12
Grand avuncular	0.125	6	204
Half-avuncular	0.125	15	106
First cousins	0.125	24	793
Double first cousins, once removed	0.125	—	35
First and second cousins, once removed	0.078125	—	22
Great grand avuncular	0.0625	—	2
Half grand avuncular	0.0625	—	5
First cousins, once removed	0.0625	1	759
Half first cousins	0.0625	—	77
Double second cousins	0.0625	—	25
Second and third cousins	0.0390625	—	6
First cousins, twice removed	0.03125	—	30
Half first cousins, once removed	0.03125	—	13
Second cousins	0.03125	—	287
Second cousins, once removed	0.015625	—	75
Third cousins	0.0078125	—	8
Total		1,715	4,559

*The relationship coefficient is $2 \times$ coefficient of kinship of two individuals. †The total data used for the present study were obtained from 1,026 individuals distributed across 294 families. Of 1,026, however, 41 were unrelated individuals (see text for details). ‡The total data used for the present study were obtained from 537 individuals distributed across 31 families.

RESEARCH DESIGN AND METHODS

VAGES. The study participants for whom both genetic and phenotypic data were available consisted of 1,026 Mexican Americans distributed across 294 mostly nuclear families from VAGES. Of 294 families, however, 41 were found to be represented by single individuals with triglyceride data. These 41 single, unrelated individuals were considered for the analysis because they contribute to the evaluation of covariate effects. The remaining 985 individuals from 253 families generated 1,715 relative pairs that were distributed across nine relative-pair categories (Table 1). The VAGES families were ascertained on at least two siblings affected with type 2 diabetes and only one parent with type 2 diabetes. Metabolic, anthropometric, demographic, and medical history information were obtained on all examined individuals. All procedures were approved by the institutional review board of the University of Texas Health Science Centre at San Antonio, and all subjects gave informed written consent before their participation.

Blood samples were obtained after a 12-h fast. Plasma triglyceride concentrations were determined by an enzymatic colorimetric quantification method (Wako Chemicals, Nuess, Germany). After excluding three extreme outliers, plasma triglyceride values were available for 1,023 individuals and were used to conduct the present study, and no values >1 SD from the next lowest value were included. To address the issue of continued nonnormality, plasma triglyceride values were transformed using an inverse-normal transformation.
Genotype data, genetic map, and estimation of identity-by-descent matrices. DNA was prepared from whole blood (Gentra Systems, Minneapolis, MN). The Center for Inherited Disease Research (CIDR) performed a 10-cM genome scan using the DNA samples from the VAGES participants. We used CIDR genotypic data on 385 highly polymorphic autosomal markers. The program PREST (13) was used to resolve pedigree discrepancies. The genotype data were cleaned for both Mendelian inconsistencies and spurious double recombinants using the program SimWalk2 (14,15). Overall, the blanking rate for errors was <0.22% of the total number of genotypes. The PEDSYS (16) program INFER was used to infer unknown genotypes from the genotypes of relatives, when possible without ambiguity. The genetic maps used for the present analyses were similar to the Marshfield maps. The multipoint identical-by-descent (IBD) matrices given a number of genetic markers (map distance in Haldane cM) were calculated using Markov chain Monte Carlo methods implemented in the program Loki (17,18).

Variance-components linkage analysis. We used a pedigree-based multipoint variance-components approach to test for linkage between genetic locations and the transformed triglyceride levels using maximum-likelihood methods (19–21). The variance-components method uses information from all possible biological relationships simultaneously to disentangle the genetic architecture of the quantitative trait. This method specifies the expected genetic covariances between relatives as a function of their IBD relationships at a marker locus (which is hypothesized to be linked to a locus influencing the quantitative trait [QTL]). It allows for locus-specific effects (h^2_q is heritability attributed to the QTL), residual additive genetic effects (h^2 is heritability attributed to the residual genetic effects), and individual-specific random environmental factors [$e^2 = 1 - (h^2_q + h^2)$].

In addition to the variance components, mean $\pm$ SD of the phenotype and covariate effects (e.g., age and BMI) were simultaneously estimated by maximum-likelihood techniques. Hypothesis testing was performed by likelihood ratio tests. The hypothesis of no linkage (i.e., additive genetic variance due to the QTL = 0) was tested by comparing the likelihood of this restricted model with that of a model in which the additive genetic variance due to the QTL is estimated. The difference in the two $\log_{10}$ likelihoods produces a logarithm of odds (LOD) score. Twice the difference in the $\log_e$ likelihoods of these models yields a test statistic that is asymptotically distributed as the ½:½ mixture of χ^2_1 and a point mass at 0, denoted by χ^2_0, where the degree of freedom is equal to the difference in the number of parameters estimated between the two competing models (22). An LOD score of ≥ 3.0 was considered as strong evidence in support of linkage. For the purpose of discussion, other genetic regions across the genome with LOD scores ≥ 1.2 and ≥ 1.9 are considered evidence for potential linkage and suggestive linkage, respectively. Given the initial set of covariates of transformed triglyceride levels considered for an analysis, the program SOLAR was used to evaluate the statistical significance ($P < 0.05$) of a given covariate using likelihood ratio tests. Each of these tests involved comparison for a given parameter and had one degree of freedom. Given the VAGES family ascertainment criteria, all of our genetic analyses incorporated correction for ascertainment bias by computing the likelihood of a pedigree conditional on the transformed triglyceride value of the proband, using the program SOLAR. Since the VAGES families had more than one proband per family, we randomly chose one of the probands from each family to correct for ascertainment bias since correcting

TABLE 2
Characteristics of VAGES and SAFDS participants for whom both phenotypic and genotypic data were available

Variable	VAGES	SAFDS
n	1,026	537
Age at examination (years)	49.6 ± 13.1	42.1 ± 16.8
Female subjects (%)	65.9	58.3
Diabetic subjects (%)	68.6	24.6
BMI (kg/m^2)	33.2 ± 8.0	30.2 ± 6.6
ln BMI*	3.5 ± 0.2	3.4 ± 0.2
Plasma triglycerides (mg/dl)†	159 ± 136	180 ± 135

Data are means ± SD or percent. *ln = log transformed. †Plasma triglyceride values were transformed for the genetic analysis using inverse normal transformation.

for ascertainment bias in datasets ascertained on multiple affected probands can result in a loss of power to detect linkage (23,24). We performed simulation analysis to determine the empirical P value to verify our major linkage finding. A fully informative marker, not linked to triglyceride, was simulated, IBD information for this simulated marker was calculated, and linkage analysis was performed. The empirical P value was determined based on information obtained from 100,000 replicates. The statistical genetic analytical procedures were implemented in the SOLAR program.

Single nucleotide polymorphism genotyping and association analysis. We typed rs929434 in *AdipoR2* in the VAGES data to perform association analysis with triglyceride concentrations using methods described previously (4). DNA samples for genotyping were available for 907 individuals only.

SAFDS. The goals and related genetic investigations of SAFDS have been extensively described previously (25–27). Briefly, probands for SAFDS were individuals with type 2 diabetes, and all of the probands' first-, second-, and third-degree relatives aged ≥18 years were invited to participate in the study. Type 2 diabetes–related phenotypes were collected, and a 10-cM genome scan

was performed by CIDR. The SAFDS baseline data used for this study were reported in Table 2, and 537 individuals from 31 complex, multigenerational families generated 4,559 relative pairs that were distributed across 24 relative-pair categories (Table 1).

RESULTS

The characteristics of the VAGES participants are reported in Table 2. After accounting for the significant covariate effects of sex and log-transformed BMI (ln BMI), the overall polygenic heritability (h^2) for transformed plasma triglyceride values was estimated to be 46 ± 7%, with high statistical significance ($P < 0.0001$). It should be noted that normality was maintained approximately, after adjustment for covariate influences (i.e., the skewness and kurtosis coefficients of the residuals is −0.01 and −0.11, respectively). The initial set of covariates considered for evaluation of statistical significance included sex, age, age^2, age × sex, age^2 × sex, and ln BMI. Following the determination of the heritability for triglycerides, the multipoint linkage analysis was conducted, and the results of our genome-wide linkage screen (i.e., peak LOD score by chromosome) are summarized in Fig. 1.

The strongest evidence for linkage (LOD = 2.4) of triglycerides occurred on chromosome 12 at ~26 cM near marker D12S391. The multipoint LOD scores obtained for transformed plasma triglyceride were plotted against map positions on chromosome 12 (Fig. 2). In addition to the evidence for suggestive triglyceride linkage on chromosome 12 near marker D12S391, four other regions on four
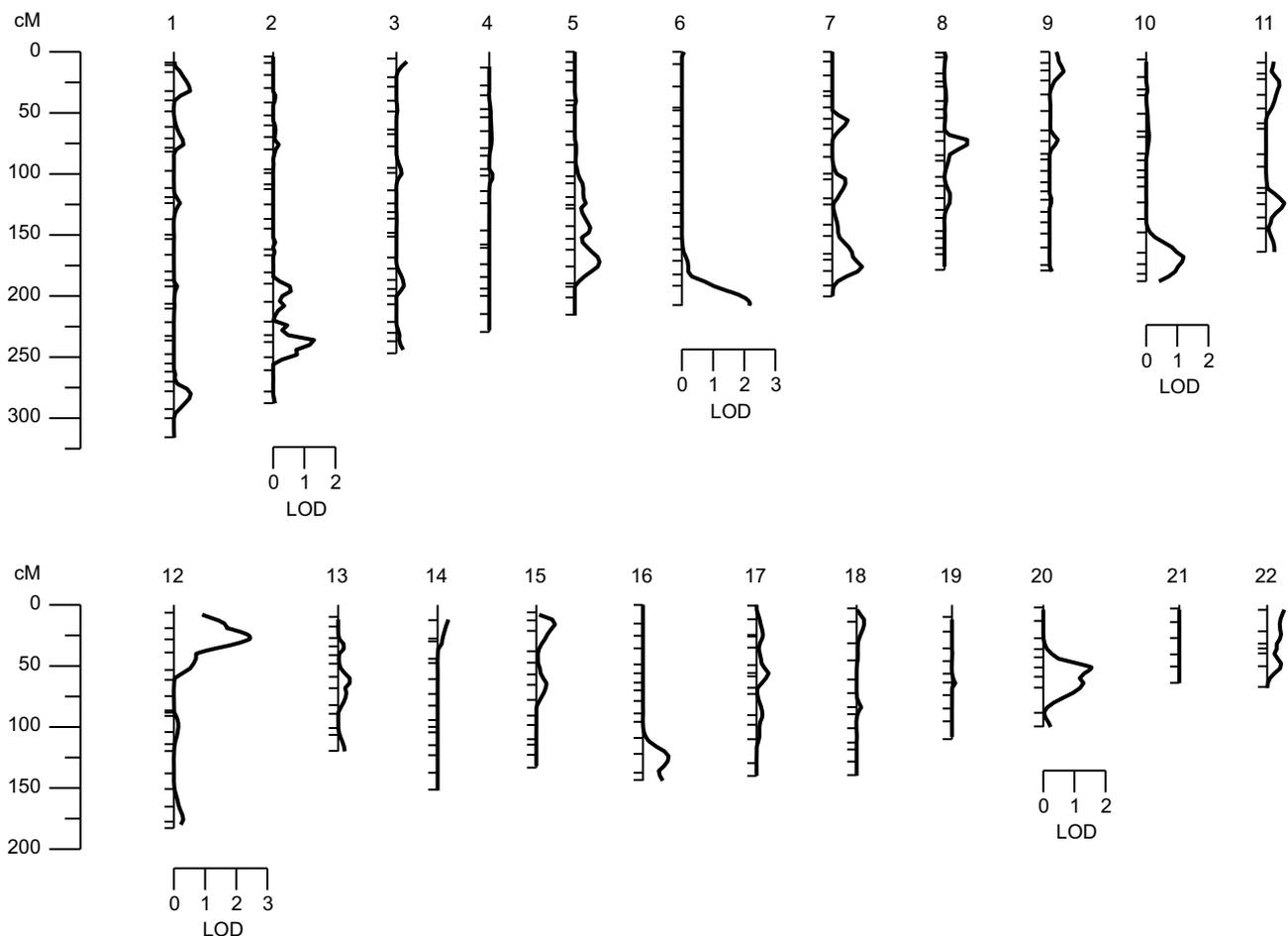

FIG. 1. Summary of the genome-wide linkage scan for genes influencing (transformed) plasma triglyceride levels in VAGES.
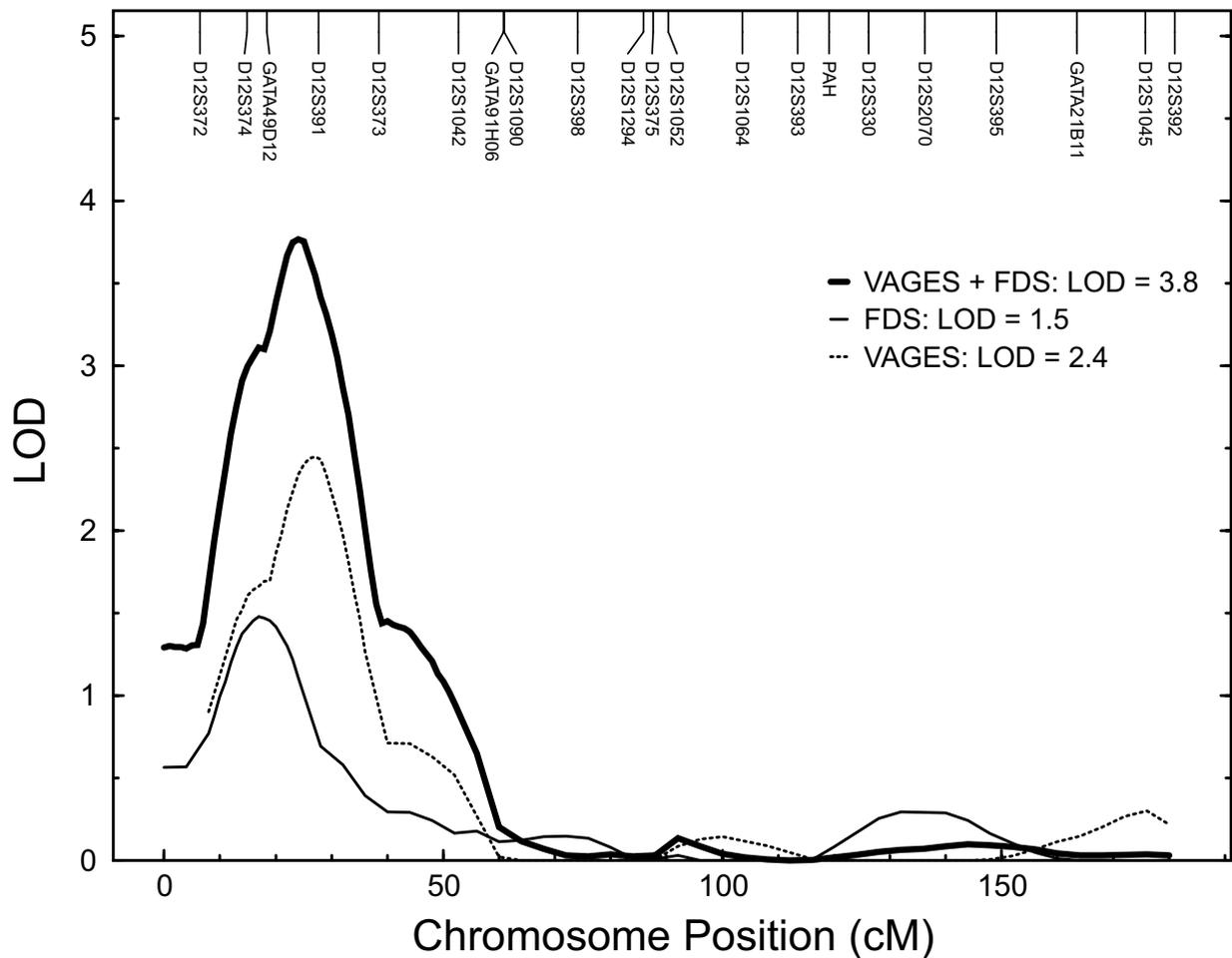
FIG. 2. Multipoint linkage results for (transformed) plasma triglyceride levels at the chromosome 12 region in the VAGES, SAFDS, and combined VAGES and SAFDS datasets.

separate chromosomes exhibited potential evidence for linkage, with LOD scores ≥1.2 (Table 3).

Our linkage on chromosome 12p provides an independent replication of a similar finding in other Mexican-American data obtained from the SAFDS (3,4). Given that SAFDS has a new genome scan performed by CIDR (26), we reanalyzed the SAFDS data (3) but included all of the available triglyceride information ($n = 537$ compared with the initial linkage data based on a sample of 418 individuals) (Table 2) and the new (CIDR) chromosome 12 marker data. As in the case of VAGES, the plasma triglyceride values in SAFDS were transformed using the inverse-normal transformation and the analysis incorporated correction for the ascertainment bias given the SAFDS ascertainment scheme (3). After adjusting for the significant covariate effects of age, sex, age × sex, age², and ln

TABLE 3
Chromosomal regions potentially linked (LOD ≥1.2) to (transformed) plasma triglyceride levels

Nearest marker	Distance (cM)*	Chromosome location	LOD
D2S434	216	2q	1.3
D6S1027	187	6q	2.2
D10S1656	149	10q	1.2
D20S477	47	20p	1.5

*Marshfield data.

BMI on triglycerides, the reanalysis (the skewness and kurtosis values for residuals were 0.08 and 0.04, respectively) yielded potential evidence for linkage (LOD = 1.5) at ~17 cM near marker GATA49D12. Following this, we performed a combined VAGES and SAFDS plasma triglyceride linkage analysis using a combined chromosome 12 map and study-specific multipoint IBD matrices. After accounting for the above-said covariates and study as an additional covariate (the skewness and kurtosis coefficients for residuals were 0.07 and 0.02, respectively), the combined multipoint linkage analysis yielded significant evidence for linkage of transformed plasma triglycerides (LOD = 3.8, empirical P value = 2.0×10^{-5}) to a genetic location at ~ 24 cM between markers GATA49D12 (17 cM) and D12S391 (26 cM) on chromosome 12p (Fig. 2). The empirical P value that corresponds to our observed LOD score is similar to our nominal P value, which suggests that our nominal P value has not overstated the evidence of linkage.

As part of the present study, we genotyped ($n = 907$) the most strongly associated single nucleotide polymorphisms (SNPs) (rs929434) with triglycerides observed in the SAFDS ($P = 0.00016$) (4), in the VAGES data and found suggestive evidence in favor of association ($P = 0.06505$). The rare allele was associated with decreased triglyceride concentrations in both the SAFDS and VAGES datasets. We obtained the combined P value (0.00013) using Fisher's combined P value approach, in turn finding a slight im-

provement in overall evidence for association. In the VAGES data, however, the observed suggestive association explains only a small portion (~5%) of the initial linkage signal.

DISCUSSION

In this study, we report the results from a genome-wide linkage scan undertaken in VAGES to identify genes that influence variation in plasma triglyceride levels. We found that plasma triglyceride levels were suggestively linked to a genetic location near marker D12S391 on chromosome 12. Potential evidence for linkage also was observed on chromosomes 2q, 6q, 10q, and 20p. The linkage on chromosome 12 was an independent replication of our previously (or presently) reported triglyceride linkage finding in Mexican Americans from SAFDS, where we found modest evidence for linkage of plasma triglyceride levels at 12p (3,4). Our combined linkage analysis of VAGES and SAFDS increased our power to detect statistically significant evidence for linkage at 12p (LOD = 3.8, empirical P value = 2.0×10^{-5}). Taken together, these results provide strong evidence for a major susceptibility locus on 12p that influences plasma triglyceride levels in Mexican Americans.

Other genome-wide linkage screens in different populations reported evidence of linkage for plasma triglyceride levels on chromosomes 2p, 2q, 3q, 6q, 8q, 9q, 10p, 10q, 11p, 11q, 17q, and 19q (5–11). With the exception of 2q, 6q, and 10q, the majority of these loci appear to have no strong effects in the Mexican Americans. Consistent with our findings, a genome-wide linkage scan of plasma triglyceride concentrations in the Framingham Heart Study identified modest evidence for linkage on 12p (28). In contrast, the linkage of 12p to plasma triglyceride levels has not been replicated in the studies mentioned above (5–11), including a meta-analysis of genome-wide linkage studies for plasma triglyceride levels (12). This lack of replication is not surprising and may arise due to factors such as different study designs, sample size, choice or power of the statistical methods used, and genuine genetic heterogeneity. In addition, replication of a true linkage in a phenotype with complex inheritance patterns (e.g., oligogenic inheritance) appears to be difficult since it is likely to require a larger number of families than is required for initial detection (29). As can be seen from our present study, however, the combined VAGES and SAFDS linkage analysis provided stronger evidence for linkage of triglyceride levels on chromosome 12p compared with the independent linkage information provided by VAGES and SAFDS separately. In addition to these genome-wide linkage findings, more recently, genome-wide association studies have also identified additional loci/SNPs associated with triglyceride levels including *GCKR*, *LPL*, *APOA5*, and *TRIB1* (30–33). These reported loci/SNPs do not overlap with the chromosomal regions that we have identified in the present study.

Many genes that could plausibly play a role in the regulation of plasma triglyceride levels reside within the critical linkage region at 12p. One of these positional candidate genes is adiponectin receptor 2 (*AdipoR2*, 12p13.33). AdipoR2 is one of two receptors that mediate the effects of adiponectin, an important regulator of energy homeostasis and glucose and lipid metabolism (34). As we reported previously (4), multiple SNPs in *AdipoR2* were significantly associated with decreased plasma tri-

glyceride levels (4). In this same study, we demonstrated that one of these SNPs (rs929434) accounted for 47% of the linkage in the previously reported genome-wide linkage scan performed in a subset of SAFDS (3). In the present study, we found suggestive evidence in favor of association with rs929434 ($P = 0.06505$) in VAGES, and the observed suggestive association explains only a small portion (~5%) of the initial linkage signal. Following these findings, we have started typing a large battery of SNPs in *AdipoR2* to further confirm the association between genetic variation in *AdipoR2* and triglyceride levels.

In addition to our own findings, several other groups have reported that polymorphisms in *AdipoR2* are associated with type 2 diabetes, obesity, and other components of the insulin resistance syndrome (35–37). Interestingly, one of these association studies reported that *AdipoR2* polymorphisms were associated with decreased plasma triglyceride levels (35). Another gene of interest in this region of 12p is the wingless-type MMTV integration site family, member 5B (*WNT5B*). An association study in a Japanese population identified *WNT5B* as a new candidate for conferring susceptibility to type 2 diabetes (38). Their results suggest that the *WNT5B* gene plays an important role in the regulation of adipocyte function and might contribute to the pathogenesis of type 2 diabetes (38).

In conclusion, we found evidence for a major susceptibility locus on chromosome 12p influencing plasma triglyceride levels in Mexican Americans. The findings of the present study, combined with the SNP association studies performed in *AdipoR2* (4,35), indicate that this locus appears to have substantial influence on the phenotypic variation in plasma triglyceride levels in Mexican Americans. Further fine-structure mapping will be required to identify the remaining potential functional variants in this region contributing to the linkage signal.

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