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Genome-wide linkage scan for plasma high density lipoprotein cholesterol, apolipoprotein A-1 and triglyceride variation among American Indian populations: the Strong Heart Family Study

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Abstract

Background—Recent studies have identified chromosomal regions linked to variation in high density lipoprotein cholesterol (HDL-C), apolipoprotein A-1 (Apo A-1) and triglyceride (TG), although results have been inconsistent and previous studies of American Indian populations are limited

Objective—In an attempt to localize quantitative trait loci (QTLs) influencing HDL-C, Apo A-1 and TG, we conducted genome-wide linkage scans of subjects of the Strong Heart Family Study.

Methods—We implemented analyses in 3484 men and women aged 18 years or older, at three study centers.

Results—With adjustment for age, sex and center, we detected a QTL influencing both HDL-C (LOD = 4.4, genome-wide $P = 0.001$) and Apo A-1 (LOD = 3.2, genome-wide $P = 0.020$) nearest marker *D6S289* at 6p23 in the Arizona sample. Another QTL influencing Apo A-1 was found nearest marker *D9S287* at 9q22.2 (LOD = 3.0, genome-wide $P = 0.033$) in the North and South Dakotas. We detected a QTL influencing TG nearest marker *D15S153* at 15q22.31 (LOD = 4.5 in the overall sample and LOD = 3.8 in the Dakotas sample, genome-wide $P = 0.0044$) and when additionally adjusted for waist, current smoking, current alcohol, current estrogen, lipid treatment, impaired fasting glucose, and diabetes, nearest marker *D10S217* at 10q26.2 (LOD = 3.7, genome-wide $P = 0.0058$) in the Arizona population.

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Conclusions—The replication of QTLs in regions of the genome that harbor well-known candidate genes suggest that chromosomes 6p, 9q and 15q warrant further investigation with fine mapping for causative polymorphisms in American Indians.

Keywords

linkage; genome-wide scan; high density lipoprotein cholesterol; apolipoprotein A-1; triglyceride

INTRODUCTION

High density lipoprotein cholesterol (HDL-C) and triglyceride (TG) concentrations are both independent risk factors for cardiovascular disease (CVD).[1] Apolipoprotein A-1 (Apo A-1), the major apoplipoprotein in HDL-C, is important for biochemical pathways that make HDL-C antiatherogenic. It is estimated that each 1% increase in HDL-C concentration reduces the risk of coronary heart disease by approximately 2% [2], although its etiologic role has been debated, and that each 21% decrease in TG concentration reduces the risk of non-fatal myocardial infarction and CVD death by 40% [3].

According to the Atlas of Heart Disease and Stroke among American Indians and Alaska Natives [4], the age-adjusted prevalence of high blood cholesterol levels (>240 mg/dL) (NCEP [1]) among American Indians living in 35 U.S. states was 30%, notably higher than the approximately 17% prevalence [5] noted among the general United States (U.S.) population. These prevalence measures do not differ significantly between female (29%) and male (31%) American Indians. Similar, as for the prevalence of elevated HDL-C, data from the Strong Heart Study (SHS), prevalence of low HDL-C levels (<40 mg/dL) was 34.2 – 36.7% among American Indian populations, and increased with age [6]. Another study among an urban American Indian population demonstrated a strikingly high prevalence of low HDL-C levels of 54.9% among males and of 54.2% among females [7], higher than the general U.S. population (25.1% for males and 9.1% for females) [5]. This study also reported 32.3% of males and 28.6% of females had high levels of triglycerides (≥ 150 mg/dl) [7], similar to its prevalence in the general U.S. population (30%) [8].

Strong aggregate genetic effects on HDL-C, Apo A-1, and TG variability have been recognized across multiple populations, although findings from candidate gene studies have been conflicting. Nonetheless, recent genome-wide association studies (GWAS) have identified several variants influencing HDL-C, Apo- A-1, and TG. For example, evidence for association between HDL-C and several previously implicated lipid metabolism genes, such as *ABCA1* on chromosome (chr) 9 (rs4149268 [9], rs3890182 [10], and rs9282541 [11]) and *LIPC* on chr 15 (rs261332 and rs11858164 [11]) have been noted. In addition, evidence for association between *LIPC* rs4775041 and TG and *CETP* rs1800775 on chr 16 and Apo A-1 concentrations [12] have been reported. Despite these exciting findings, much of the heritable component of HDL-C, Apo-A1, and TG remains to be explained, suggesting that other susceptibility variants are segregating in populations.

Genome-wide linkage scans have been performed to identify novel regions of the genome harboring genes that regulate HDL-C, Apo A-1 and TG levels, but the identification of quantitative trait loci (QTL) has been challenging. As with most common complex phenotypes, several regions of the genome have been implicated, perhaps suggesting locus heterogeneity. A genome-wide scan of HDL-C in a kindred with familial hypercholesterolemia reported genome-wide significant linkage of HDL-C to chromosome 6 (LOD=3.1).[13]. Genome-wide significant linkage of Apo A-1 to chromosome 9q21.21-q33.1 (LOD=3.28), 8p21.1-q13.1 (LOD=3.71) and 10p15.1-p13 (LOD=5.51).[14] Genome wide significant evidence of linkage of TG to chromosome 4q28.3-32.1 (LOD=3.71) [14]

and to chromosome 15 (LOD=3.88 at approximately 20–30 cM) [15] has been noted. Few previous gene mapping studies of HDL-C, Apo A-1 and TG in American Indian populations have been conducted.

More studies are necessary to advance our knowledge of the genetic factors influencing HDL-C, Apo A-1 and TG, especially because genome-wide association studies have largely been implemented in Caucasian samples. American Indians are an important United States minority population that can inform knowledge in distinct ways due to their geographic and genetic isolation and high rates of cardiovascular disease risk factors. For instance, because of these qualities we may discover genetic variants that are unique to their genetic and environmental context which can further our understanding of pathways to cardiovascular disease. Further, replication of existing QTLs in independent samples is essential to prioritize regions of the genome for costly comprehensive fine-mapping and ultimately identification of functional variants. Thus, our objectives were to localize new QTLs influencing HDL-C, Apo A-1, and TG as well as to provide new evidence in support of previously identified QTLs by conducting a genome-wide scan in American Indian subjects of the Strong Heart Family Study (SHFS).

MATERIALS AND METHODS

Study population

The SHFS is a multicenter, population-based, family study designed to localize genes that influence risk factors for clinical and subclinical cardiovascular disease, diabetes, and obesity and their progression over time. SHFS data were collected in two phases, the first completed in 1999 and the second initiated in 2001. More than 3600 resident American Indian tribal members aged 18 and older were recruited and examined in three study centers in Arizona (AZ), North and South Dakota (ND/SD), and Oklahoma (OK). The SHFS protocols were approved by the Indian Health Service Institutional Review Board, the institutional review boards of the participating institutions, all participating communities involved, and by all participants. All participants have given informed consent for the genetic study of CVD and associated risk factors, including the present study. Detailed descriptions of the SHFS protocols have been published.[16]

Phenotypic and genotypic data

Phenotypic data—Protocols for the collection of phenotypic data have been described previously [17], and, briefly, are as follows. Fasting blood samples were obtained during a physical examination and assayed at MedStar Research Institute using standard laboratory methods [17]. TG, total cholesterol, HDL-C, and apolipoproteins were measured using enzymatic reagents and the Hitachi 717. Glucose was measured by the hexokinase method (Glucose-HK; BMD, Inc) on the Hitachi 717. Type II diabetes, impaired fasting glucose, and normal glucose tolerance status were defined according to American Diabetes Association criteria [18]. Waist circumference was measured with participants wearing light clothing and without shoes. Information on demographic characteristics was obtained by questionnaire. Ever smoking was defined as having had at least 100 cigarettes during ones lifetime; current smoking was defined when participants answered “yes” to both of the questions: whether they were current smokers and whether they had smoked more than 100 cigarettes in their life. Current and ever alcohol consumption were defined as having had at least 12 alcoholic beverages in the last year (at least one day per month) and/or lifetime, respectively. Reproductive history was queried including parity, gravidity, menopausal status, and estrogen use.

Genotypic data—The SHFS genotyping procedures have been documented previously [19]. Specifically, DNA was isolated from fasting blood samples using organic solvents and then amplified in separate PCR reactions with primers specific for short tandem repeat markers using the ABI PRISM Linkage Mapping Set-MD10 version 2.5 (Applied Biosystems, Foster City, CA). Paticipants were genotyped for approximately 400 microsatellite markers spaced at average 10 cM intervals (range, 2.4 to 24.1 cM) across the autosomal chromosomes, with the average heterozygosity of these markers for 0.69 in the Arizon subsample, 0.76 for the Dakotas, and 0.74 for Oklahoma. Analyses and assignment of the marker alleles were done using electrophoresis and computerized algorithms (Applied Biosystems). Sex-averaged chromosomal maps obtained from deCODE Genetics (http://www.decodegenetics.com) were used. All genetic distances are reported in Haldane centimorgans (cM). Pedigree relationships were verified using the PREST (Pedigree Relationship Statistical Tests) package [20]. Mendelian inconsistencies and spurious double recombinants were detected using the SimWalk2 package [21]. The overall blanking rate for both types of errors was <1% of the total number of genotypes for Arizona, North and South Dakota, and Oklahoma. We used web resources of the University of California Santa Cruz (http://genome/ucsc.edu) and Online Mendelian Inheritance in Man (http://www3.ncbi.nlm.nih.gov/entrez/query.fcgi?db 5 OMIM) to determine the cytogenetic location of markers and to search for candidate genes.

Statistical analysis

Fasting plasma HDL-C and TG values were transformed by natural logarithm, and apo A-1 was transformed by dividing 1 by the apo A-1 value, because of their skewed distributions. Multipoint variance component linkage analysis of ln(HDL-C), 1/(apo A-1) and ln(TG) was performed among approximately 3,400 SHFS participants with complete covariate data using SOLAR, version 2.1.4. Details of this model have been described previously.[22] The use of the variance component approach requires an estimate of the identity-by-descent matrices, which were computed using the Loki package [23] with a Markov chain Monte Carlo stochastic procedure. Genome-wide p-values were obtained by the method of Feingold et al. [24]

Stepwise linear regression was used to screen covariates for statistical significance using SAS, version 9.1. Given the sensitivity of variance component analysis to kurtosis, all phenotypic outliers of the trait of interest and all covariate data points (here defined as any value ≥3 standard deviations from the mean) were removed before analysis (the number excluded varies by variable; in all cases $n < 30$ or approximately 5% of the study observations). To maximize our power to detect genetic effects, we considered two different models of covariate adjustment in each population separately. In model 1, adjustments were made for age, sex, and field center. In model 2, we additionally adjusted for waist circumference, current smoking, current alcohol consumption, current estrogen use, lipid-lowering treatment, diabetes status, and impaired fasting glucose. All analyses were also run, and results reported, stratified by field center. Residuals were generated for both models and used in all subsequent quantitative genetic analyses. Kurtosis values for ln(HDL-C), 1/(apo A-1) and ln(TG) were <0.50 for all analyses.

RESULTS

Descriptive characteristics of the SHFS participants are summarized in Supplementary table 1. The average age ± SD of participants by center was ~37 ± 16, 39 ± 17, and 44 ± 17 years in Arizona, Dakotas, and Oklahoma, respectively. Consistent with the results from our previous studies of a subsample of these family members,[25] a high prevalence of current cigarette smoking and alcohol consumption was observed. As expected, a high prevalence of

diabetes was noted, especially in the Arizona center (32%). HDL-C, apo A-1 and TG concentrations varied by center, where HDL-C was lowest in the Arizona population (48.52 ±14 mg/dL), and apo A-1 (143.92 ± 27 mg/dL) and TG (163.59 ± 92 mg/dl) were highest in Oklahoma. In contrast to many similarly-aged U.S. populations, we found a very low prevalence of lipid-lowering therapy (≤ 6%).

Table 1 presents the multipoint genome-wide adjusted LOD scores and their locations from the variance component linkage analyses of HDL-C, apo A-1 and TG for all linkage peaks with LOD scores ≥1.8 [suggestive evidence for linkage [26]]. A QTL for HDL-C (adjusted LOD=4.4, genome-wide P =0.001) was detected in the Arizona population on chromosome 6p23 at 36 cM, nearest marker *D6S289* (fig 1). The 1-cM LOD unit support interval spanned 12 cM, from 29 to 41 cM (10.033–20.578 Mb P terminus), between marker *D6S470* and *D6S422*. Upon adjustment for multiple confounders (model 2), the magnitude of LOD score decreased by 1.6 units, yet suggestive evidence for linkage was still noted (LOD =2.8, genome-wide P =0.054).

Further suggestive evidence of linkage to HDL-C level was observed in the North and South Dakotas population on chromosome 9q31.1-q31.3 at 110 cM (LOD=1.9) between *D9S1690* and *D9S167*, and in the Oklahoma population on chromosome 9q22.2-q31.1 at 101 cM (LOD=2.1), close to marker *D9S287*. In addition, we found suggestive evidence of linkage to HDL-C in the Oklahoma population at 193 cM on chromosome 3q26.31-q27.3 (LOD=1.9), nearest marker *D3S1262*.

Genome-wide significant evidence of linkage to apo A-1 levels was detected in the Arizona population on chromosome 6p23 (LOD = 3.2, genome-wide P = 0.020) at 36 cM, nearest marker D6S289 (fig 2). The 1-cM LOD unit support interval spanned 10 cM, from 31 to 41 cM (10.033–20.578 Mb P terminus), between markers D6S470 and D6S422. Upon adjustment for multiple confounders (model 2), the magnitude of the LOD score decreased by 1.1 units, yet suggestive evidence for linkage was still noted (LOD =2.1). We also detected genome wide significant linkage to apo A-1 levels in the North and South Dakotas population on chromosome 9q22.2 at 99 cM (LOD = 3.0, genome-wide P = 0.033), nearest marker D9S287 (fig 3). The 1-cM LOD unit support interval spanned 22 cM from 90 to 112 cM (84.873–111.077 Mb q terminus), between D9S167 and D9S1677.

Further suggestive evidence of linkage to apo A-1 level was observed in the North and South Dakotas population on chromosome 2q33.3-q35 at 214 cM (LOD=2.2), nearest marker *D2S2382*, on chromosome 9q22.2-q31.1 at 103 cM (LOD=2.5) between *D9S287* and *D9S1690* and on chromosome 12q24.32 at 162 cM (LOD=2.1), close to marker *D12S1659*, and in the Oklahoma population on chromosome 7q21.11-q21.13 at 92 cM (LOD=1.9), nearest marker *D7S669* (table 1).

Genome-wide significant evidence of linkage to TG levels was detected in the North and South Dakotas population on chromosome 15q22.31 (LOD = 3.8, genome-wide P =0.0044) at 68 cM, nearest marker D15S153 (fig 4). The 1-cM LOD unit support interval spanned 15 cM from 61 to 76 cM (56.166–69.071 Mb q terminus), between D15S117 and D15S131. When excluding participants on lipid lowering therapy, this LOD score increased to 5.0 (data not shown). We also detected genome wide significant linkage to TG levels in the Arizona population on chromosome 10q26.2 at 164 cM (LOD = 3.7, genome-wide P =0.0058), nearest marker *D10S217* (fig 5). This LOD score was slightly lower when adjustments were made for age and sex only (model 1) (LOD = 2.9, genome-wide P =0.042). The 1-cM LOD unit support interval spanned 11 cM from 156 to 167 cM (125.078–129.530 Mb q terminus), between D10S587 and D10S217.

Suggestive evidence of linkage to TG levels was detected in the Arizona population on chromosome 4q32.3-q34.1 at 168 and 169 cM between *D4S1597* and *D4S1539*, on chromosome 6p12.1-q14.1 at 87 cM, between *D6S257* and *D6S460*, on chromosome 15q15.1-q21.1 at 44 cM, close to *D15S994*, and on chromosome 18p11.21-q12.1 at 40 cM, near *D18S53*. In the Oklahoma population, suggestive evidence of linkage to TG levels was noted at 67 cM on chromosome 11q12.1-q13.2 between *D11S4191* and *D11S987*, and at 10 cM on chromosome 21q21.1, near *D21S1899* (table 1).

DISCUSSION

The present study was conducted to identify regions of the genome harboring genes that influence HDL-C, Apo A-1 and TG levels in large extended American Indian families. Here, we present evidence for a QTL for HDL-C and apo A-1 levels in the Arizona population on chromosome 6p nearest marker *D6S289*, and for apo A-1 levels in the North and South Dakotas population on chromosome 9 nearest marker *D9S287* at 9q22.2. We also detected significant QTLs for TG levels in the North and South Dakotas population on chromosome 15 nearest marker *D15S153*, and in the Arizona population on chromosome 10, nearest marker *D10S217* at 10q26.2.

Chromosome 6p, where we observe our strongest linkage evidence for HDL-C and apo A-1, has previously been implicated in four genome scans of lipid-related traits.[13, 27–29] Genome-wide significant LOD scores were reported in this region for HDL-C in a kindred with familial hypercholesterolemia (LOD=3.1) [13]. Suggestive linkage has also been noted for total apoB in French-Canadian families (LOD=1.35) [29] and in French-Canadian families (LOD=1.32) [28]. The strongest positional candidate genes are located ~4Mb from our highest LOD score, the *ELOVL2* and *TFAP2A* genes. Cell over-expression of *ELOVL2* gene, encoding a polyunsaturated fatty acid elongase, enhances TG synthesis and accumulation of lipid droplets [30]. *TFAP2A* (transcription factor AP-2 alpha) encodes the activator protein (AP)2α and negatively regulates *ABCA*1 promoter activity [31]. However, to date no previous studies have examined whether *ELOVL2 or TFAP2A* polymorphisms influence variation in HDL-C or apo A-1. The same QTL detected for both HDL-C and apo A-1 is consistent with the biological structure for apo A-1, the major structural protein of HDL-C.

To further support our results on 6p, we evaluated the *P-value*s from the association between SNP genotypes and HDL-C, apo A-1 and TG concentrations for SNPs within or in LD with biological candidate genes underlying the 1 LOD support interval of our linkage peak on 6p among approximately 2,600 participants of the Diabetes Genetic Initiative (DGI) study (available at http://www.broad.mit.edu/diabetes/). Of the 1,870 SNPs within the 1-cM LOD unit support interval on chromosome 6p, evidence for association with HDL-C was observed for *ELOVL2* SNP rs3798712 (P =0.0147), and evidence for association with apo A-1 was observed for *ELOVL2* SNP rs3798712 (P =0.0032) and *TFAP2A* SNP rs12663250 (P =0.0214) (Supplementary table 2).

We also attempted to support our results on 6p using GWAS data from 1087 participants of the offspring cohort of the Framingham Heart Study (FHS) SHARe 100K online resource. All results for autosomal SNPs are available through NCBI dbGaP (http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?id=phs000007). We examined if any SNP with a *P-value* ≤ 0.05 was located within a candidate gene for HDL-C, apo A-1 or TG or tagged a candidate gene under our genome wide significant linkage peaks. No evidence for association was observed among the FHS offspring cohort.

Chromosome 9q, where we observe our strongest linkage evidence for Apo A-1 levels, has previously been implicated in six genome scans of lipid-related traits. [9, 14, 32–35] Genome-wide significant LOD scores were reported in this region for Apo A-1 (LOD=3.28) in female sib-pairs [14]. The strongest positional candidate gene is located ~9 Mb from our highest LOD score, the *ABCA1* gene. The ATP-binding cassette (ABC) protein encoded by this gene functions as a cholesterol efflux pump in the cellular lipid removal pathway, with cholesterol as its substrate. One study showed an association between apo A-1 levels and *ABCA1* 14C>T polymorphisms in a Malay population ($OR_{TT/CC}$=2.92, 95%CI=1.42–6.00; $OR_{CT/CC}$=1.29, 95%CI=0.81–2.04).[36]

It is notable that *ABCA1* is a rate-limiting factor for HDL-C biogenesis. Indeed, GWAS study findings also support an association between *ABCA1* variants and HDL-C, for example, rs9282541 (−3.2 mmol/L decrease per T allele, $P = 3.2 \times 10^{-5}$) [11] and rs4149274 (1.51 mg/dL increase per G allele, $P \sim 7.4 \times 10^{-8}$) [9]. On-line GWAS resources further support the association with *ABCA1* gene variants. Eight SNPs of the *ABCA1* gene within the 1-cM LOD unit support interval of chromosome 9q, were associated with apo A-1 ($P \leq 0.05$) in white participants of the DGI study (Supplementary table 2). In contrast, no evidence for this association was observed among the FHS offspring cohort (all $P > 0.05$).

Chromosome 15q, where we observe our strongest linkage evidence for TG levels, has previously been implicated in four genome scans of lipid-related traits.[37–40] Genome-wide significant LOD scores were reported in this region for TG and HDL-C levels among participants from NHLBI Family Heart study project (LOD=3.0), for HDL-C (LOD=3.3) among Mexican American individuals from the San Antonio Family Heart Study project [40], and for HDL-C among participants from the Genetic Epidemiology of Metabolic Syndrome project (LOD=3.1) [38]. Suggestive linkage has also been noted for TG in family members with familial hypertriglyceridemia (LOD=1.35).[37] Approximately 587 genes underlie the 1 LOD unit support interval (46 cM, 45.2 Mb) of the 15q signal. The strongest positional candidate gene is located ~8Mb from our highest LOD score, the *LIPC* gene. LIPC (hepatic triglyceride lipase) is a lipolytic enzyme that has dual functions of triglyceride hydrolase and ligand/bridging factor for receptor-mediated lipoprotein uptake [41]. Recent data show inconsistent results in the association between *LIPC* polymorphisms and TG variation [42, 43] and between LIPC and HDL and LDL concentrations [27, 44]. A recent GWAS study [9] identified a significant association between TG and *LIPC* SNP rs4775041 (3.62 mg/dL increase per C allele combined $P = 1.6 \times 10^{-8}$). With respect to the on-line DGI GWAS resource, of the 5,844 SNPs on chromosome 15q within the 1-cM LOD unit support interval, nine SNPs within the *LIPC* gene were associated with TG ($P \leq 0.05$) (Supplementary table 2). With respect to the Framingham 100K GWAS resource, *LIPC* SNPs, rs2899632 ($P = 0.0032$) and rs8028759 ($P = 0.02$) were associated with TG measured at FHS Examinations 7 and 4, respectively.

Significant linkage of TG levels to chromosome 10q has also been reported in several other studies. Pajukanta et al [45] studied Finnish families with familial combined hyperlipidemia and found a LOD of 2.6 for cholesterol at the same location on chromosome 10. Approximately 33 known genes underlie the 1 LOD unit support interval (12 cM, 4.5 Mb) of the 10q signal. However, this chromosome 10q locus is in a region lacking any obvious positional candidate genes with known functions related to lipid metabolism, suggesting that this locus may harbor a novel lipid gene.

We also observed several loci with suggestive linkage to HDL-C, apo A-1 and TG, some novel and others supporting positive findings from other studies. Supplementary table 3 summarizes the observed linkage signals from this study that are supported by evidence of linkage to lipid-related traits in other populations on 2q, 3q, 4q, 7q, 11q, 12q, 18, and 21q.

Although these signals do not meet the genome-wide significance threshold, these results still offer valuable information in that they may identify regions worthy of further study.

We found that when excluding American Indian participants on lipid lowering therapy (n = 66 participants in the Dakotas center), the magnitude of the LOD score for TG on chromosome 15q increased by 1.2 units (adjusted LOD =5.0). We can offer several possible explanations for these findings. First, as lipid-lowering medications may lower the concentration of TG [46], the TG values of these medicated subjects may not accurately reflect their true values. Secondly, it is possible that by excluding medicated individuals from the analysis, the genetic locus heterogeneity was reduced, thereby strengthening the evidence for linkage at this particular QTL. When additionally adjusting TG for waist circumference, current smoking status, current consumption of alcohol, current estrogen use, lipid-lowering treatment status, and diabetes status, the magnitude of the LOD score on chromosome 10q increased by 1.6 units (LOD =3.7). As the sample of examined individuals remained the same, we infer that by reducing the residual covariate effects (model 1 with only adjustment for sex and age), we had greater power to detect a QTL specific effect at this locus on chromosome 10q. As no other modifications (amplifications or attenuations) of LOD scores were noted upon adjustment for lipid lowering therapies and the percentage of participants taking lipid lowering drugs was low, we report both findings that have been adjusted for lipid lowering therapy and those that did not.

In conclusion, our findings suggest that one or more genes on chromosomes 6 and 9 regulate HDL cholesterol and apopliprotein A-1 concentrations, and on chromosomes 10 and 15 regulate triglyceride concentrations. Excellent candidate genes are implicated in each of these genomic regions and future research should pursue these positional candidate genes, particularly for the *ELOV2,* the *ABCA1* and the *LIPC* genes, to determine whether polymorphisms in these genes are the source of these highly replicated linkage signals. Our study also provides corroboration of genomic regions that possibly influence interindividual variation in HDL-C, apo A-1 and TG concentrations on chromosome 2, 3, 4, 7, 9, 12, and 18, and new information about genomic regions on 11 and 21. The identification and confirmation of QTLs for HDL-C, apo A-1 and TG concentrations may bring us closer to the identification of the functional genes that influence these phenotypes and aid in the targeting of preventative therapies for individuals with low circulating levels of HDL-C and apo A-1, and high fasting triglyceride concentrations, established risk factors for CVD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key points

- More studies are necessary to advance our knowledge of the genetic factors influencing HDL-C, Apo A-1 and TG among US minority populations, particularly American Indians which can inform knowledge in distinct ways due to their geographic and genetic isolation and high rates of cardiovascular disease risk factors.
- In this study, four QTLs influencing HDL-C, apo A-1 , and TG levels were identified in the American Indian participants of the Strong Heart Family Study, on chromosomes 6p, 9q, 15q, and 10q.
- These signals implicate excellent candidate genes, particularly for the *ELOV2,* the *ABCA1* and the *LIPC* genes, and are supported by several linkage and association studies across multiple ethnic groups, suggesting a generalization of lipid related genetic effects across multiple populations.

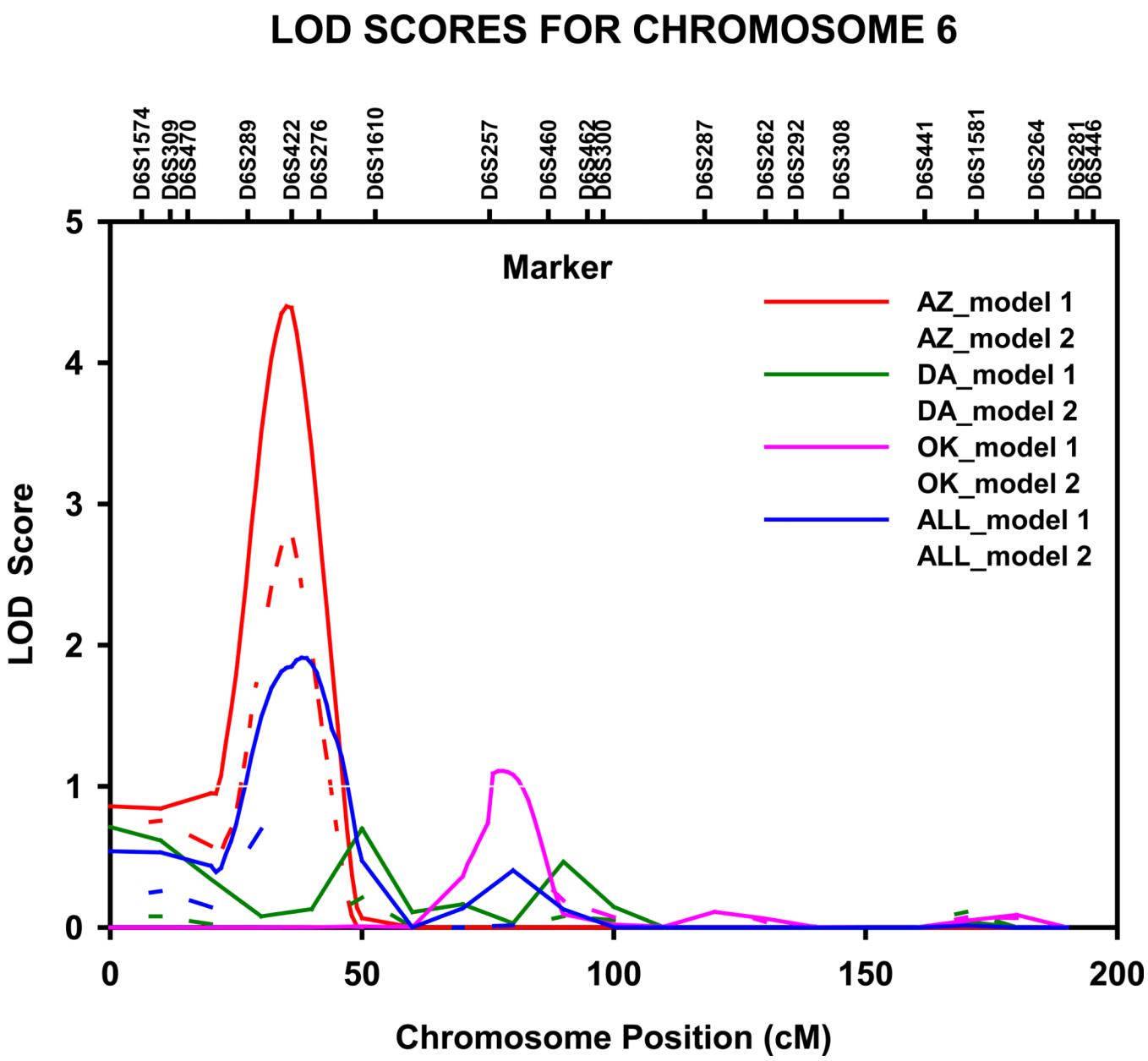

Figure 1.

Multipoint LOD scores on chromosome 6 for HDL cholesterol. Results for combined and stratified analyses shown. Model 1 was adjusted for age, sex, and field center (combined analysis). Model 2 was additionally adjusted for waist circumference, current smoking status, current alcohol consumption, current estrogen use, lipid treatment status, impaired fasting glucose and diabetes status. AZ, Arizona field center; DA, Dakotas field centers; OK, Oklahoma field center; cM, Haldane centimorgans.

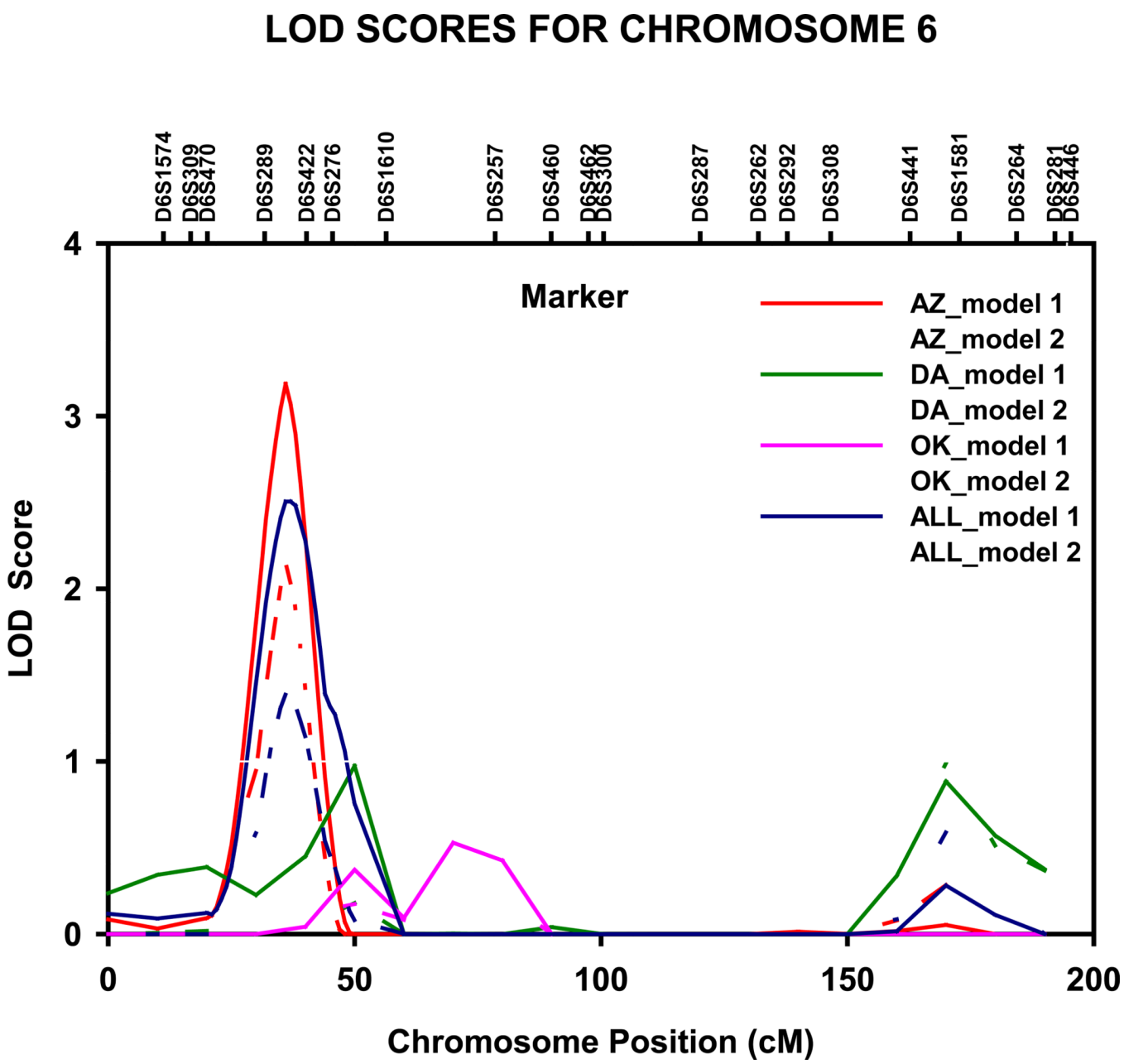

Figure 2.

Multipoint LOD scores on chromosome 6 for apolipoprotein A-I. Results for combined and stratified analyses shown. Model 1 was adjusted for age, sex, and field center (combined analysis). Model 2 was additionally adjusted for waist circumference, current smoking status, current alcohol consumption, current estrogen use, lipid treatment status, impaired fasting glucose and diabetes status. AZ, Arizona field center; DA, Dakotas field centers; OK, Oklahoma field center; cM, Haldane centimorgans.

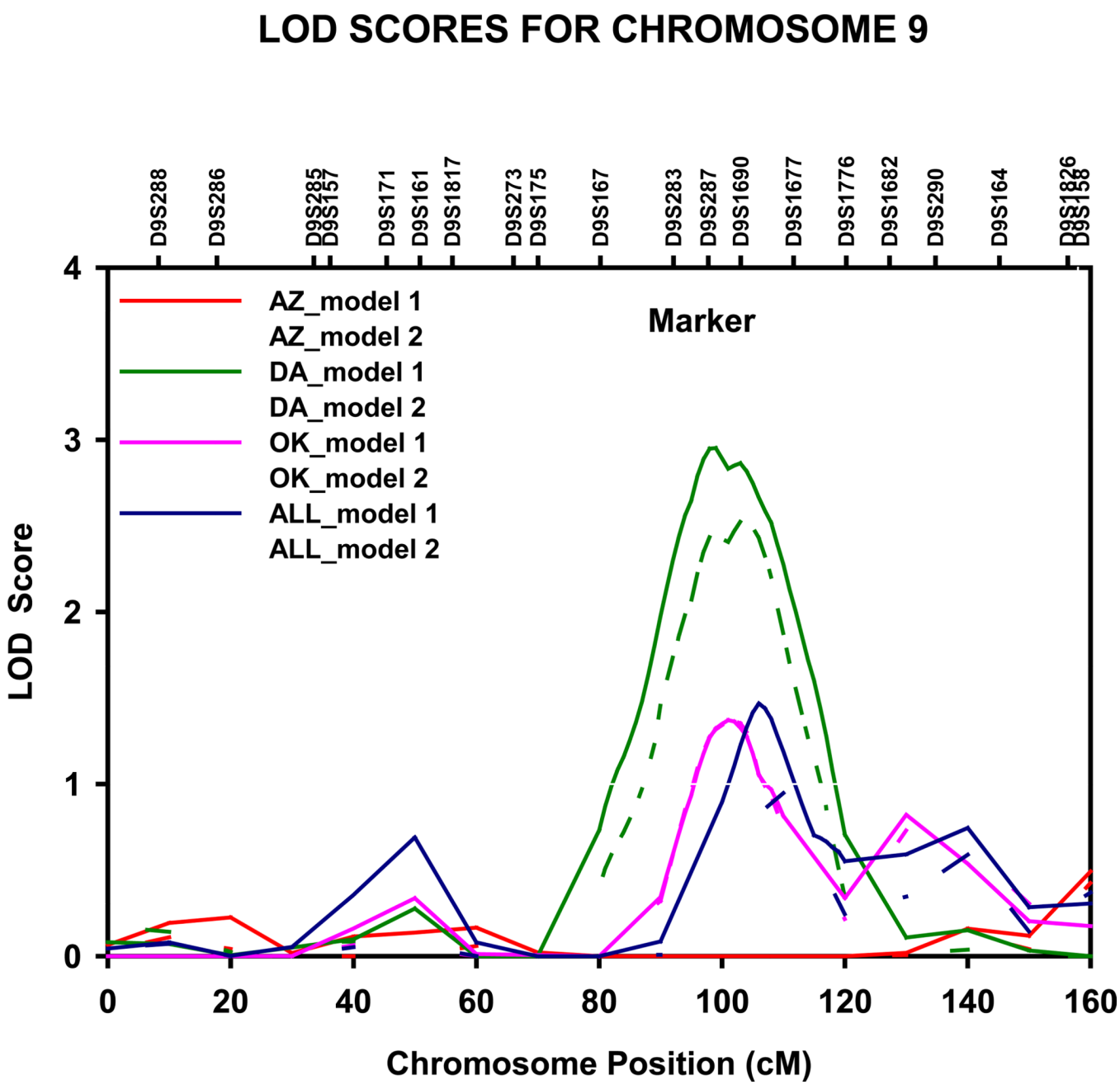

Figure 3.

Multipoint LOD scores on chromosome 9 for apolipoprotein A-I. Results for combined and stratified analyses shown. Model 1 was adjusted for age, sex, and field center (combined analysis). Model 2 was additionally adjusted for waist circumference, current smoking status, current alcohol consumption, current estrogen use, lipid treatment status, impaired fasting glucose and diabetes status. AZ, Arizona field center; DA, Dakotas field centers; OK, Oklahoma field center; cM, Haldane centimorgans.

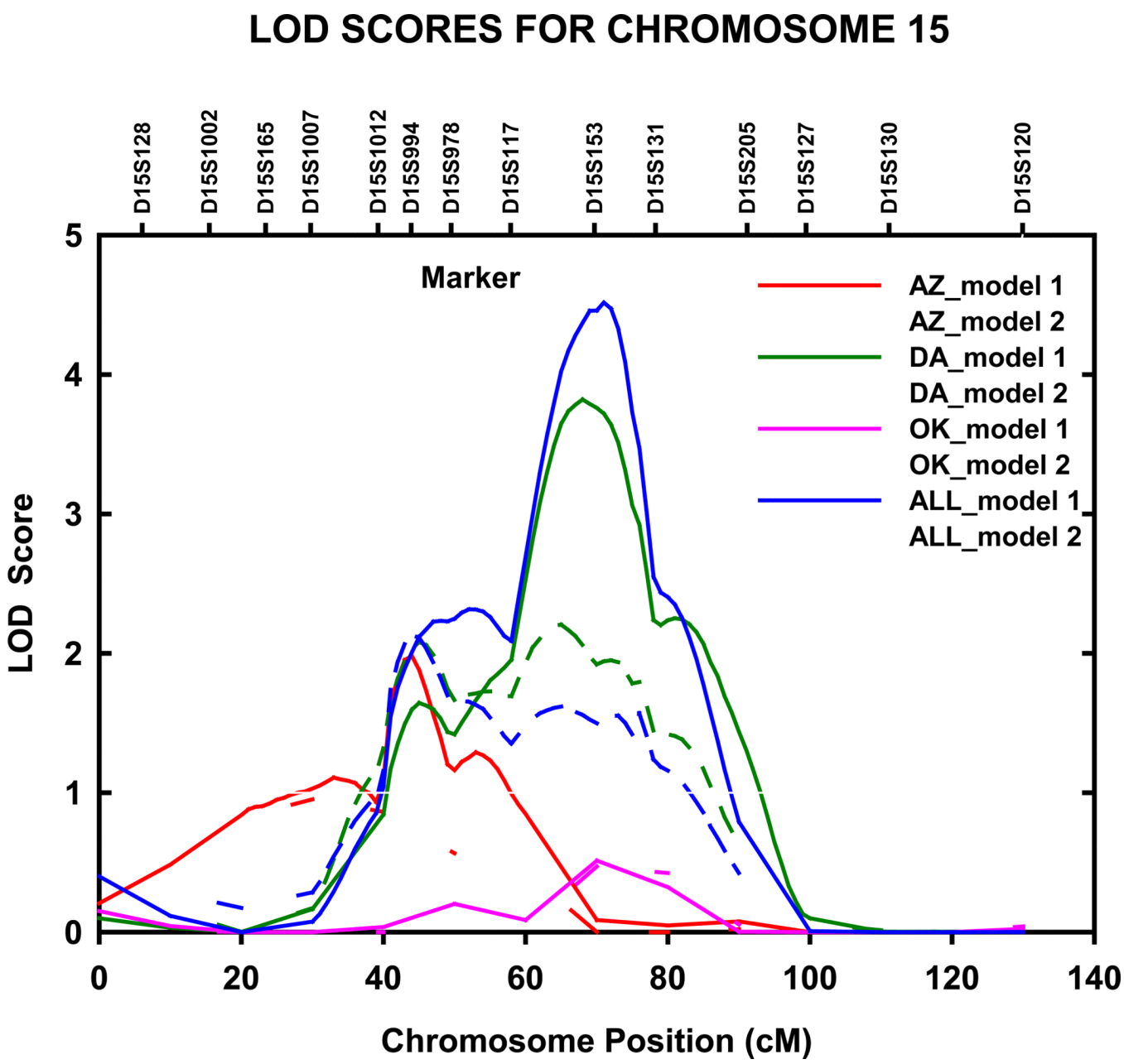

Figure 4.

Multipoint adjusted LOD scores on chromosome 15 for triglycerides. Results for combined and stratified analyses shown. Model 1 was adjusted for age, sex, and field center (combined analysis). Model 2 was additionally adjusted for waist circumference, current smoking status, current alcohol consumption, current estrogen use, lipid treatment status, impaired fasting glucose and diabetes status. AZ, Arizona field center; DA, Dakotas field centers; OK, Oklahoma field center; cM, Haldane centimorgans.

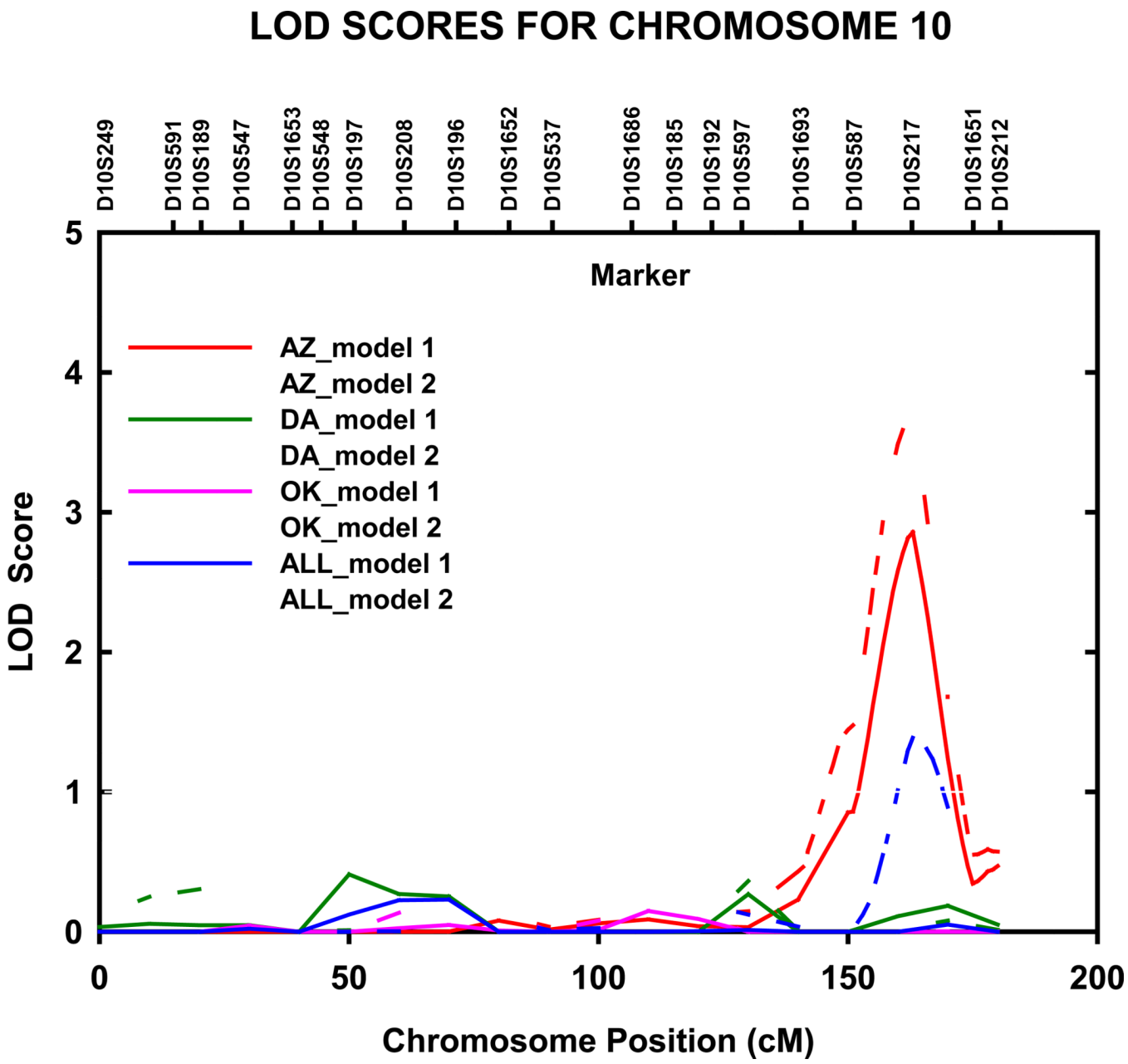

Figure 5.

Multipoint adjusted LOD scores on chromosome 10 for triglycerides. Results for combined and stratified analyses shown. Model 1 was adjusted for age, sex, and field center (combined analysis). Model 2 was additionally adjusted for waist circumference, current smoking status, current alcohol consumption, current estrogen use, lipid treatment status, impaired fasting glucose and diabetes status. AZ, Arizona field center; DA, Dakotas field centers; OK, Oklahoma field center; cM, Haldane centimorgans.

Table 1

Maximum LOD scores suggestive of linkage (LOD ≥ 1.8) for multipoint quantitative trait linkage analyses of HDL-C, apolipoprotein A-1 and triglyceride levels in American Indian Participants of the Strong Heart Family Study

Phenotype	Center and Model [a]	Chromosome	Centimorgan	Chromosomal Region	Maximum LOD Score
HDL-C					
	Oklahoma, model 2	3	193	3q26.31 – q27.3	1.9
	Arizona, model 1, model 2	6	35–36	6p22.3–p24.3	4.4, 2.8
	Dakotas, model 1	9	110	9q31.1–q31.3	1.9
	Oklahoma, model 2	9	101	9q22.32–q31.1	2.0
apolipoprotein A-1					
	Dakotas, model 1	2	214	2q33.3–q35	2.2
	Arizona, model 1, model 2	6	36	6p22.3–p24.3	3.2, 2.1
	Oklahoma, model 2	7	92	7q21.11–q21.13	1.9
	Dakotas, model 1	9	99	9q22.2	3.0
	Dakotas, model 2	9	103	9q22.2–q31.1	2.5
	Dakotas, model 2	12	162	12q24.32	2.1
Triglycerides					
	Arizona, model 1, model 2	4	168–169	4q32.3–q34.1	1.9, 3.0
	Arizona, model 1	6	87	6p12.1–q14.1	2.9
	Arizona, model 2	6	75	6p12.1–p21.2	2.6
	Arizona, model 1, model 2	10	163–164	10q26.2–q26.3	2.9, 3.7
	Oklahoma, model 1	11	67	11q12.1–q13.2	1.8
	Arizona, model 1	15	44	15q15.1–q21.1	2.0
	Dakotas, model 1, model 2	15	65–68	15q22.1–q22.31	3.8, 2.2
	Dakotas, model 1	18	40	18p11.21–q12.1	1.9
	Oklahoma, model 2	21	10	21q21.1	2.0

[a] Model 1: adjustment for sex, age, and stratified by field center.

Model 2: additional adjustments for waist circumference, current smoking status, current consumption of alcohol, current estrogen use, lipid treatment status, impaired fasting glucose and diabetes status.