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ASTN1 and Alcohol Dependence: Family-Based Association Analysis in Multiplex Alcohol Dependence Families

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Abstract

A previous genome-wide linkage study of alcohol dependence (AD) in multiplex families found a suggestive linkage result for a region on Chromosome 1 near microsatellite markers D1S196 and D1S2878. The ASTN1 gene is in this region, a gene previously reported to be associated with substance abuse, bipolar disorder and schizophrenia. Using the same family data consisting of 330 individuals with phenotypic data and DNA, finer mapping of a 26 cM region centered on D1S196 was undertaken using SNPs with minor allele frequency (MAF) ≥ 0.15 and pair-wise linkage disequilibrium (LD) of r^2 <0.8 using the HapMap CEU population. Significant FBAT *P*-values for SNPs within the ASTN1 gene were observed for four SNPs (rs465066, rs228008, rs6668092, and rs172917), the most significant, rs228008, within intron 8 had a *P*-value of 0.001. Using MQLS, which allows for inclusion of all families, we find three of these SNPs with MQLS *P*-values <0.003. In addition, two additional neighboring SNPs (rs10798496 and rs6667588) showed significance at $P = 0.002$ and 0.03, respectively. Haplotype analysis was performed using the haplotype-based test function of FBAT for a block that included rs228008, rs6668092, and rs172917. This analysis found one block (GCG) over-transmitted and another (ATA) under-transmitted to affected offspring. Linkage analysis identified a region consistent with the association results. Family-based association analysis shows the ASTN1 gene significantly associated with alcohol dependence. The potential importance of the ASTN1 gene for AD risk may be related its role in glial-guided neuronal migration.

Keywords

ASTN1; alcohol dependence; multiplex families

INTRODUCTION

Excessive use of alcohol is the third leading cause of preventable death [Mokdad et al., 2004] in the US. The economic and social costs have been estimated to be $184 billion due to alcohol-related accidents, lost productivity, incarceration and other alcohol related morbidity [Harwood, 2000]. In spite of the fact that use of alcohol is quite common, a smaller proportion of the population drink in sufficient quantity and with associated health, family, and work-related problems to be considered alcohol dependent (AD). Data from the National Comorbidity Survey, a survey of respondents ages 15–54 found 20.1% of men and

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8.2% of women meeting criteria for alcohol dependence (AD) [Kessler et al., 1997]. There is now evidence that those individuals with the greatest propensity for AD may carry an increased genetic risk for developing alcohol dependence.

Although there is considerable heritability for alcohol dependence (0.49–0.64) in males [Caldwell and Gottesman, 1991; Heath et al., 1997] and females (0.56–0.59) [Kendler et al., 1992; Prescott et al., 1999], few genes have been identified that reliably confer susceptibility. However, studies employing well-designed sampling strategies that over sample families with a high density of cases have revealed important clues for gene finding as seen in the Collaborative Study on the Genetics of Alcoholism (COGA) studies [Reich et al., 1998; Edenberg et al., 2004]. Genome-wide association (GWAS) studies have also revealed potentially important loci but require large samples to detect loci having genome-wide significance. A meta-analysis of two GWAS studies of alcohol dependence totaling 4,979 cases and controls has identified three loci with statistical significance of $\alpha' < 5 \times 10^{-7}$ [Wang et al., 2011].

In a genome wide scan of multiplex families ascertained through a pair of affected probands [Hill et al., 2004], we found evidence for linkage in multiple chromosomal regions. The present report is based on efforts to follow up on linkage findings for a region on Chromosome 1q23.3–1q25.1 that included a maximal LOD score of 3.46 ($P = 0.002$) at marker D1S196 and at an adjacent marker D1S2878 with a LOD value of 3.45 ($P = 0.002$).

MATERIALS AND METHODS

Study Sample

All members of the multiplex families who participated in the study gave their written consent to do so after the nature and purpose of the study was fully explained to them. (Consent forms were approved by the University of Pittsburgh Institutional Review Board.)

Multiplex Families

Multiplex families were selected on the basis of the presence of a pair of alcohol dependent brothers or sisters. The probands were selected from among individuals in treatment for alcohol dependence in the Pittsburgh area. Probands were eligible if they met DSM-III criteria for AD and had a same sex sibling who similarly met criteria for AD. All proband pairs and their cooperative relatives (siblings and parents) were personally interviewed using a structured psychiatric interview (Diagnostic Interview Schedule [DIS]). The DIS provides good reliability and validity [Helzer et al., 1985] for alcohol dependence and alcohol abuse by DSM-III and IIIR criteria [American Psychiatric Association, 1980, 1987] and alcoholism by Feighner Criteria [Feighner et al., 1972], an early diagnostic set of criteria used in the Collaborative Studies on the Genetics of Alcoholism (COGA) family [Reich et al., 1998].

Families were excluded if the probands or any first-degree relative were considered to be primary for drug dependence (preceded alcohol dependence onset by at least 1 year), or the proband or first-degree relative met criteria for schizophrenia, or a recurrent major depressive disorder. Probands and relatives with mental retardation or physical illness precluding participation were excluded. Complete details regarding participant selection may be seen in Hill et al. [2004]. The majority of probands (80%) had three or more siblings who contributed DNA, consented to a clinical interview, and provided family history. These large sibships resulted in a total of 418 sib pairs of all types (201 affected–affected, 172 unaffected–affected, and 45 unaffected–unaffected. One or both parents have been genotyped in 86% of the families. An average of 5.1 individuals per family were genotyped.

SNP Selection

Previously, we carried out a genome-wide linkage analysis finding potentially important linkage results for multiple regions including Chromosome 1 [Hill et al., 2004]. Our study included genotyping in a 26.6 cM region on Chromosome 1 that centered on the microsatellite marker D1S196. Using a binary alcohol dependence phenotype and including relevant covariates (age, gender, and Constraint) a LOD score of 3.46 was obtained. To study this region further, SNPs were chosen with minor allele frequency (MAF) ≥ 0.15 and pair-wise linkage disequilibrium (LD) of r^2 <0.8 using the HapMap CEU population at approximately 1 cM intervals in this region. The gentoyping and analysis was completed in three stages. First, we focused on a 19 cM region (Fig. 1) extending from rs7522166 to rs2816187. This region, bounded by these SNPs was chosen because rs7522166 is 7 cM proximal to D1S196 and rs2816187 is 13 cM distal to D1S196. We genotyped 18 SNPs at approximately 1 cM intervals in this region. Preliminary analysis revealed that the most significant SNP was rs228008 located in ASTN1 gene. In step 2, 31 SNPs were chosen to cover a region from rs1229355 near D1S196 distally to rs7542180, covering a 19 cM region at approximately 500 kb, and including three SNPs proximal and three distal to the ASTN1 gene at 125 kb intervals. Twelve additional SNPs were then chosen within the ASTN1 gene at an average distance of 28.9 kb.

DNA Isolation and Genotyping

Genomic DNA was extracted from whole blood with a second aliquot prepared for EBV transformation and cryopreservation. PCR conditions were as described in Hill et al. [2004]. Genotyping was completed on a Biotage PSQ 96MA Pyrosequencer (Biotage AB, Uppsala, Sweden). Each polymorphism was analyzed by PCR amplification incorporating a biotinylated primer. Thermal cycling included 45 cycles at an annealing temperature of 60°C. The Biotage workstation was used to isolate the biotinylated single strand from the double strand PCR products. The isolated product was then sequenced using the complementary sequencing primer.

Quality Control

SNP genotyping quality control involved ongoing monitoring of SNP signals provided by Qiagen software. Output is provided using three categories for each SNP: pass, fail and check. Data analysis was performed for only those signals meeting the “pass” criterion. Signals that failed or were returned as needing further checking were rerun. If after three attempts the SNP did not meet the “pass” criterion, it was eliminated from the analysis and another SNP chosen as a replacement.

Statistical Methods

Mendelian inconsistency—The PedCheck program [O’Connell and Weeks, 1998] was used to evaluate individual SNPs for Mendelian inconsistencies based on the pedigree structures. As a result of the evaluation, 43 marker genotypes from among 19,470 were coded as missing to resolve the reported inconsistencies.

Hardy–Weinberg equilibrium (HWE)—Estimates of population allele frequencies were calculated using MENDEL version 11 [Lange et al., 2001]. Files required by the MENDEL program were generated via the program Mega2 [Mukhopadhyay et al., 2005]. Marker allele frequencies were tested for departures from Hardy–Weinberg equilibrium using the allele frequency option in MENDEL. None of the 59 SNPs analyzed were found to have *P*-values below the Bonferroni adjusted threshold (<0.00085) that would indicate significant HWE departures.

Genetic maps—Our Genetic Map Interpolator (GMI) software [Mukhopadhyay et al., 2010] was used to retrieve current physical map positions from Ensembl (Ensembl 63); these physical positions were then used to linearly interpolate genetic map positions based on the Rutgers Combined Linkage-Physical Map [Kong et al., 2004; Matise et al., 2007].

Family based association test (FBAT)—Transmission rates of marker alleles were examined using the family-based association test program, FBAT [Laird et al., 2000; Rabinowitz and Laird, 2000], assuming an additive genetic model with robust variance estimation (-e option) to account for the relatedness. This family-based method is a generalization of the transmission disequilibrium test (TDT) [Spielman et al., 1993] which provides a valid test of association even if admixture present. FBAT converts each pedigree into nuclear families, which are then treated as independent families for the test statistic calculation. Informative families consisting of parent-child trios are utilized in the FBAT analysis.

More powerful quasi-likelihood score (MQLS)—We also computed the "more powerful quasi-likelihood score" (MQLS) test [Thornton and McPeek, 2007], which is designed to test for case–control association on data sets containing related individuals. This method uses kinship coefficients to account for relatedness in the sample, using these coefficients to assign weights to individuals within pedigrees. The MQLS test, which uses the intact pedigree structures was performed assuming 10% population prevalence for alcohol dependence. Results did not differ in a meaningful way when we varied the assumed prevalence from 5% to 20%.

LODPAL linkage analysis—Nonparametric linkage analysis of 106 affected sibpairs was performed using each subject's gender and age, and their scores on the Constraint scale of the Multidimensional Personality Questionnaire [Tellegen, 1982; Tellegen and Waller, 1982] as covariates and implemented using the LODPAL program [S.A.G.E 6.1.0, 2010]. The Constraint scale measures behavioral constraint, a construct that overlaps with risk-taking behavior, a tendency that has been linked to adverse health consequences including alcohol and drug dependence [DiClemente et al., 1995]. Using data from twins reared apart and together, Tellegen et al. [1988] reported that among the personality traits tested Constraint was among those with the greatest genetic variance (0.58). Adding covariates to the linkage analysis can increase the power to capture linkage by accounting for potential disease heterogeneity. Use of the personality trait Constraint appears to reduce heterogeneity among substance users. McGue et al. [1999] reported that within a sample of alcohol dependent individuals a subset with drug use disorder showed elevated Constraint scale scores, concluding that behavioral disinhibition among alcohol dependent individuals may be attributable to those who abuse drugs other than alcohol. LODPAL uses a general conditional logistic model to test for identity by descent (IBD) allele sharing [Olson, 1999]. The covariates chosen were those utilized in a previous genome-wide linkage analysis [Hill et al., 2004]. Estimated multipoint marker IBD allele sharing for the affected sibpairs was obtained from GENIBD (S.A.G.E. 6.1.0) and utilized as input for LODPAL analysis.

Haplotype analysis—Linkage disequilibrium (LD) analysis was performed using the HAPLOVIEW program version 4.2 [Barrett et al., 2005]. The LD block structure was defined by calculating D′ values pairwise between SNPs. SNP haplotype blocks were created using the HAPLOVIEW default block determination method [Gabriel et al., 2002]. Only one haplotype block was identified. This haplotype block contained three SNPs (rs228008, rs6668092, and rs172917), spanning 0.03 cM within *ASTN1.* Pairwise linkage disequilibrium between the SNPs within *ASTN1* and the LD block are shown in Figure 2.

RESULTS

Stage 1 Association Results

The initial 19 cM region (Fig. 1) extending from rs7522166 to rs2816187 included 18 SNPs at approximately 1 cM intervals. FBAT results revealed significant results for rs465066 $P = 0.013$ and for rs228008 $P = 0.012$.

Stage 2 Association Results

An additional 31 SNPs were chosen to cover a 19 cM region from rs1229355 near D1S196 distally to rs7542180 at approximately 500 kb intervals, and including three SNPs proximal and three distal to the ASTN1 gene at 125 kb intervals. FBAT results for the 49 SNPs revealed significant results for only two SNPs, rs465066 and rs228008. The only SNP showing significance in an MQLS analysis was rs228008.

Stage 3 Association Results

Ten additional SNPs were included at this stage to allow for finer mapping of the region immediately proximal and distal to rs228008 at 25 kb intervals to include rs6413830 proximally and rs1241039 distally. Analysis of the 59 SNPs revealed that the most strongly associated SNP for alcohol dependence affected status was rs228008 (MQLS $P = 0.0009$, FBAT $P = 0.012$) located within an intron of *ASTN1.* Four additional SNPs spanning a 0.03 cM region within the same intron were identified by MQLS as statistically significant (Table I). Results of the FBAT and MQLS analyses are summarized in Table I. LocusZoom was used to generate the association plot (http://csg.sph.umich.edu/locuszoom/; Fig. 3).

Stage 3 Haplotype Results

A within-family association analysis between alcohol dependence and the revealed haplotype was tested using haplotype FBAT [Laird et al., 2000] assuming an additive genetic model and using a robust estimate of variance (Fig. 2). The GCG haplotype block within *ASTN1* which included rs228008, rs6668092, and rs172917, with a frequency of 0.56, was found to be over-transmitted ($P = 0.041$) to affected offspring while the ATA haplotype block with a frequency of 0.40 was under-transmitted to affected offspring ($P = 0.014$).

Stage 3 Linkage results

With 59 SNPs available for linkage analysis, we performed a LODPAL analysis using the same covariates used in the initial linkage analysis of this region that had also utilized LODPAL analyses [Hill et al., 2004]. Results of the LODPAL linkage analysis revealed LOD values greater than 1.95 in a 12 cM region extending from rs7517175 to rs1073299 that included the ASTN1 gene (Fig. 4).

DISCUSSION

Linkage analysis (LODPAL) and within family association (FBAT and MQLS) analyses were performed for 59 SNPs in this region of Chromosome 1. FBAT analysis requires heterozygosity in parents for families to be informative and included in the analysis. Accordingly, only a subset of families could be included in our FBAT analyses potentially reducing the power to detect within-family variation. Therefore, MQLS was included in our data analytic plan to allow for use of all of our data.

The first goal of our Chromosome 1 search was determine if genes might be uncovered in a 26 cM region that included D1S196 and D1S2878, microsatellite markers that had provided

the strongest evidence for linkage on Chromosome 1. Linkage analysis of the 59 SNPs in this region found LOD scores between 1.83 and 3.47 in a region extending from rs7517175 to rs10798496, an approximately 11 cM region. Following the first stage analysis of Chromosome 1, our next goal was determine if association would be seen within this region using our family data. Our results point to SNPs within the ASTN1 gene being significantly associated with alcohol dependence.

Based on both linkage and association analyses, our results suggest that variation in the ASTN1 gene is associated with risk for alcohol dependence within multiplex for alcohol dependence families. In a previous study investigating 306 genes involved in neurotransmission and development, Gratacos and colleagues found rs2281180 within exon 19 associated with substance abuse. The present results confirm the Gratacos et al. [2008] case/control findings by identifying within family variation in alcohol dependence to be related to ASTN1 variation though an association with exonic rs2281180 was not seen.

Because our results point to the importance of the astrotactin neuronal protein (ASTN1) gene in the development of alcohol dependence, it may be useful to speculate on the origin of this relationship. ASTN1 has been extensively documented to be a receptor for glial-guided neuronal migration [Edmondson et al., 1988; Fishell and Hatten, 1991; Zheng et al., 1996; Adams et al., 2002]. ASTN1 along with a recently identified member of the astrotactin gene family ASTN2 [Wilson et al., 2010] has been shown to directly alter neuronal migration along glial fibers in the developing cerebellum. The early development of the mammalian brain is crucially dependent on the migration of neuronal precursors from germinal zones into the formation of neuronal laminae where synaptic connections are formed [Rakic, 1978; Hatten, 1999]. Molecular control of neuronal migration associated with the ASTN1 neuronal protein appears to hold promise for understanding a variety of human brain disorders. ASTN1 has been implicated in autism [Glessner et al., 2009], schizophrenia [Vrijenhoek et al., 2008; Kahler et al., 2008], bipolar disorder [Gratacos et al., 2008] and attention deficit hyperactivity disorder (ADHD) [Lesch et al., 2008]. One previous report has found a significant association between ASTN1 and substance use disorder contrasting 165 cases and 937 controls [Gratacos et al., 2008].

The potential importance of variation in a neuronal migration protein on alcohol dependence is apparent when one considers the multitude of studies now suggesting that volumetric differences in key brain areas are associated with risk for psychiatric disorders. An emerging literature has identified familial loading for alcohol dependence as a factor influencing brain structure and function [Hill et al., 2001, 2007, 2009, 2010a; Benegal et al., 2007; Herting et al., 2011]. Structural variation has been identified for the amygdala, orbitofrontal cortex and other components of the limbic network involved in emotion regulation as well as for the cerebellum [see Tessner and Hill, 2010 for review]. These anatomical alterations may provide the neurological substrate for excessive use of alcohol and development of alcohol dependence (AD) as a result of altered personality variation and cognitive functioning [see Hill, 2010b for review]. Therefore, it appears plausible that variation in ASTN1 gene may be related to brain morphological changes that could influence risk for AD. Genetic variation in other genes responsible for neuronal growth and differentiation has been found for the orbitofrontal cortex and cerebellum and risk for alcohol dependence [Hill et al., 2009, 2010a].

The present results should be interpreted in the context of some limitations, however. First, the linkage peak originally reported [Hill et al., 2004] for this region of Chromosome 1 is relatively large though large peaks are typical for complex traits. Because our peak was large, it can be presumed that it contains many genes. For example, one potentially important gene, KIAA0040, that was not included in our planned analysis, has recently been

reported with genome-wide significance for alcohol dependence [Zuo et al., 2011]. This gene is within our originally identified linkage peak [Hill et al., 2004].

Although the originally identified linkage peak was confirmed in the present analysis, the LOD score obtained was modest and required inclusion of covariates to reveal the obtained results. However, selection of covariates for the current linkage analysis which included personality variation was based on phenotypic variation in AD that had proved important in our earlier analysis which had suggested that this region might harbor genes for alcohol dependence susceptibility [Hill et al., 2004]. Personality variation in traits such as Constraint, a determinant of behavioral tendencies to engage in risk-taking behavior [see Hill, 2010b for review], may have provided useful information for reducing heterogeneity among those with addictive behavior. Previous studies suggest that Constraint may be an endophenotype for addictive behavior [Hill et al., 1990; McGue et al., 1999].

Second, the families upon which the present report is based were ascertained through affected sib pairs, possibly rendering the results atypical for the general population of alcohol dependent families. Multiplex families appear to differ from alcohol dependent families in the general population by having greater transmission of alcohol dependence across generations. Follow up of offspring from these multiplex families indicates an exceptionally high rate of AD and associated substance use by young adulthood [Hill et al., 2008]. Although these families may not be representative of AD families in the general population, the study of multiplex families appears to provide an efficient means for identifying genes because of the greater likelihood that salient genes may be segregating within these families [Hill, 2010b]. Third, alcohol dependence was defined by DSM-III criteria a diagnostic system that requires the presence of tolerance and physical dependence. Use of the DSM IV diagnostic scheme may have provided differing results. Because subjects were selected at a time when DSM-III was the current diagnostic scheme, it was not possible to include the newer DSM systems.

Finally, the ascertainment of multiplex families along with assessment of multiple family members presents logistical challenges that limit the number of sibling pairs that can realistically be included. Our sample size was modest with 418 sibling pairs. Over the past decade there has been a shift toward large-scale genome-wide association studies (GWAS) instead of family-based methods where sample sizes are typically more modest. This trend was predicated on the notion that association studies are sometimes more powerful than linkage studies [Risch and Merikangas, 1996]. However, some have questioned whether GWAS methods that are designed to detect common rather than rare variants will explain a substantial portion of heritability in psychiatric disorders [Maher, 2008]. Others have argued that the GWAS approach may find common variants that provide statistically significant results, but only modest population attributable risk, comparing less favorably with focused investigations of families where genes can be identified with high predictive value [Mitchell and Porteous, 2009].

No one statistical genetic method can be expected to completely characterize the genetic underpinnings of complex phenotypes such as alcohol dependence. As Suarez et al. [2007] illustrated in simulations carried out using linkage, case–control association and family-based tests, each approach has limitations that are best addressed by using multiple methods. The potential value of family-based methods for detection of multiple rare variants within one locus or several more weakly associated loci within the same chromosomal region has been demonstrated for other complex psychiatric disorders using family data [Ng et al., 2009]. Recently, Ott et al. [2011] proposed that evaluating linkage and association simultaneously while taking combinations of data from pedigrees with different relationship structures (extended pedigrees and sibships) and case–control samples may provide maximal

power to identify new genetic variants for trait loci beyond those that can be identified in genome-wide association case–control designs. Although we did not simultaneously evaluate linkage and association, we did use both approaches with the same data set to uncover evidence for both. Also, because the power to detect association increases with available data from related individuals [Sahana et al., 2010], it appears that family-based data collection will remain an important source for detecting both linkage and association. Availability of parental genotypes is especially important for accurate linkage analyses [Suarez et al., 2007] though such data are often unavailable where parents of the proband generation are >50 years of age. Although the parents of our probands were older, our analyses were performed with the benefit of DNA for 86% of the parents. All of the parents including those without DNA had phenotypic information available through direct interview, or if deceased through multiple family history reports. However, parental DNA was not available for 9 of the 65 families.

Although the present study has limitations, the approach taken was hypothesis-driven and included genotyping successively smaller chromosomal regions in order to confirm or refute the direction of previous analyses. Each step of our investigation pointed to the ASTN1 gene as being associated with alcohol dependence within these multiplex families. Haplotype analysis utilizing SNP variation within the ASTN1 gene confirmed the influence of variation in this gene on risk for alcohol dependence. Finally, linkage analysis using the same methodology utilized in the initial genome-wide linkage report [Hill et al., 2004] (LODPAL with covariates) finds a region with suggestive LOD scores that overlaps the region in which family-based association results showed maximal significance.

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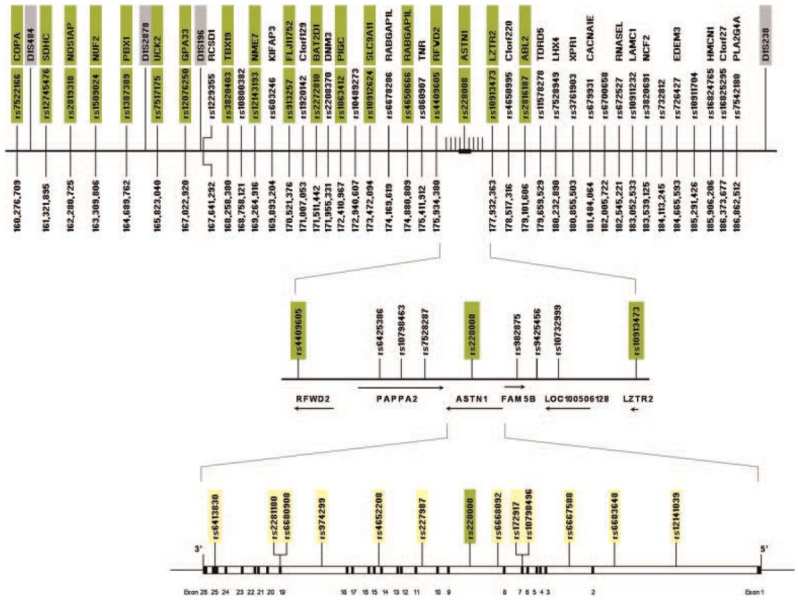

FIG. 1.

The figure depicts a 26.6 cM region of Chromosome 1q 25.2 that includes the human ASTN1 gene and single nucleotide polymorphism (SNP) sites included in the present analyses. Distances are based on physical maps from Ensembl 63.

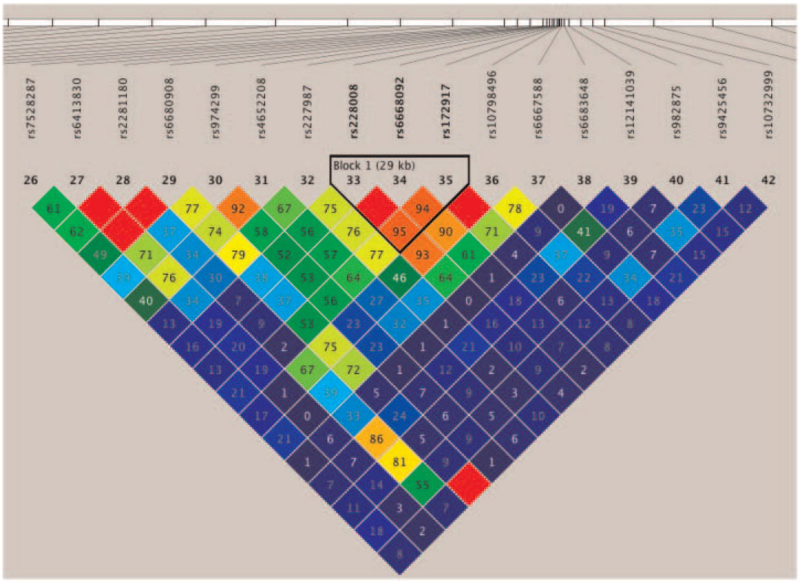

FIG. 2.

Linkage disequilibrium analysis was performed using HAPLOVIEW (version 4.2). The block structure was defined by calculating D′ values pairwise between SNPs. One block was identified containing three SNPs within the ASTN1 gene.

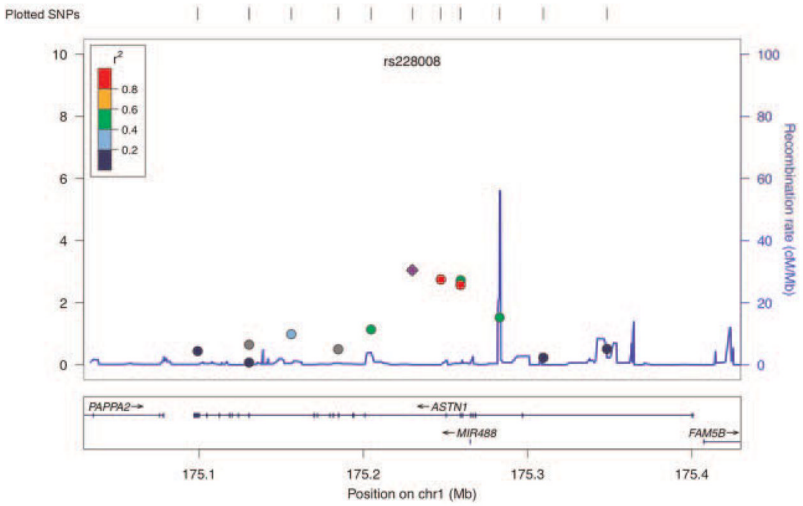

FIG. 3.

Association plot (−log 10 of the p values from MQLS) for SNPs within 300 kb of rs228008, the SNP with the maximum association observed.

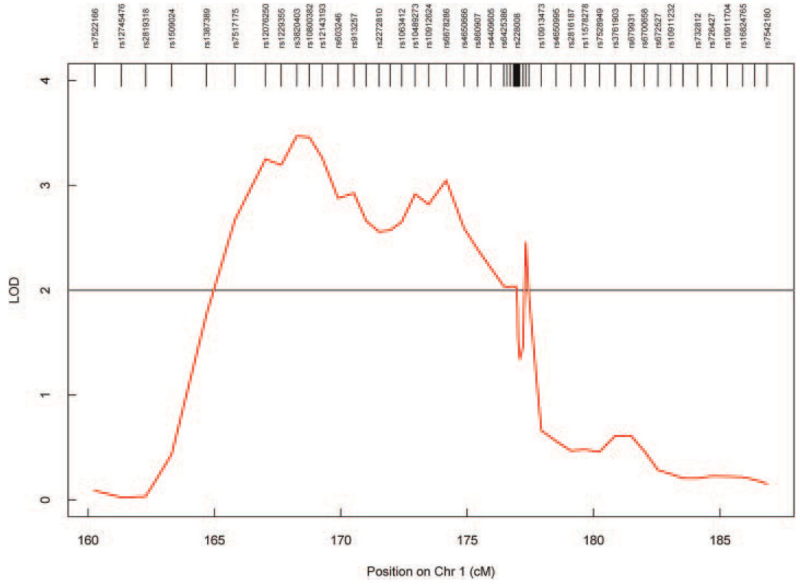

FIG. 4.
Nonparametric linkage results using LODPAL with covariates (age, sex, and constraint).

TABLE I

Results of FBAT, MQLS, and LODPAL Analyses

Marker	cM	LOD	Informative families—FBAT	FBAT *P*-value	MQLS *P*-value	Major/minor Allele[a]	Nucleotide	Allele freq.		Gene
								Affected	Unaffected	
rs7522166	164.75	0.089	23	0.629	0.233	2	C	0.478	0.523	COPA
rs12745476	167.01	0.024	18	0.709	0.529	1	C	0.774	0.785	SDHC
rs2819318	168.15	0.033	21	0.779	0.554	1	C	0.552	0.560	NOS1AP
rs1509024	170.13	0.435	27	0.148	0.250	1	G	0.724	0.705	NUF2
rs1387389	173.39	1.771	25	0.455	0.344	1	C	0.687	0.723	PBX1
rs7517175	174.66	2.676	27	0.366	0.129	1	T	0.537	0.504	UCK2
rs12076250	176.07	3.250	24	0.135	0.152	1	C	0.600	0.547	GPA33
rs1229355	177.46	3.193	26	0.915	0.979	1	T	0.542	0.563	RCSD1
rs3820403	178.71	3.471	20	0.220	0.834	1	A	0.629	0.593	TBX19
rs10800382	179.46	3.459	30	0.238	0.376	1	A	0.582	0.516	
rs12143193	180.03	3.262	19	0.205	0.367	1	T	0.684	0.642	NME7
rs603246	180.76	2.876	26	0.609	0.400	1	G	0.612	0.591	KIFAP3
rs913257	181.51	2.926	25	0.851	0.477	1	A	0.498	0.523	FLJ11752
rs1920142	182.01	2.660	25	0.359	0.409	2	C	0.343	0.356	C1orf129
rs2272810	182.46	2.557	26	0.864	0.458	2	A	0.490	0.485	BAT2D1
rs2208370	182.79	2.572	17	0.726	0.842	1	G	0.612	0.623	DNM3
rs1063412	183.12	2.650	29	0.970	0.302	1	T	0.525	0.609	PIGC
rs10489273	183.53	2.916	24	0.676	0.584	1	G	0.570	0.557	
rs10912624	183.86	2.817	20	0.501	0.599	1	T	0.780	0.797	SLC9A11
rs6678286	184.23	3.048	19	0.248	0.227	1	A	0.721	0.764	RABGAP1L
rs4650666	184.62	2.589	21	**0.014**	0.802	1	A	0.610	0.617	RABGAP1L
rs860907	185.00	2.390	26	0.992	0.199	1	G	0.622	0.650	TNR
rs4409605	185.58	2.212	29	0.382	0.836	1	A	0.580	0.590	RFWD2
rs6425386	186.10	2.039	22	0.480	0.316	2	T	0.403	0.377	PAPPA2
rs10798463	186.22	2.032	27	0.559	0.620	1	A	0.704	0.728	PAPPA2
rs7528287	186.33	2.034	30	0.587	0.323	1	G	0.620	0.663	PAPPA2
rs6413830	186.44	2.035	22	0.828	0.365	1	G	0.597	0.547	ASTN1
rs2281180	186.47	2.035	17	0.677	0.852	1	C	0.830	0.862	ASTN1

Marker	cM	LOD	Informative families—FBAT	FBAT *P*-value	MQLS *P*-value	Major/minor Allele[a]	Nucleotide	Allele freq. Affected	Allele freq. Unaffected	Gene
rs6680908	186.47	2.035	7	NA	0.227	1	C	0.910	0.937	ASTN1
rs974299	186.49	2.035	28	0.248	0.105	1	C	0.525	0.469	ASTN1
rs4652208	186.52	2.035	27	0.426	0.321	2	C	0.498	0.457	ASTN1
rs227987	186.53	2.035	27	0.185	0.074	1	T	0.530	0.472	ASTN1
rs228008	186.56	1.943	26	**0.012**	**0.001**	1	G	0.652	0.547	ASTN1
rs6668092	186.57	1.874	25	**0.021**	**0.002**	1	C	0.637	0.532	ASTN1
rs172917	186.58	1.826	29	**0.027**	**0.003**	1	G	0.652	0.547	ASTN1
rs10798496	186.58	1.826	28	0.128	**0.002**	1	C	0.500	0.421	ASTN1
rs6667588	186.60	1.544	33	0.134	**0.030**	1	G	0.545	0.508	ASTN1
rs6683648	186.63	1.450	23	0.778	0.592	1	A	0.512	0.535	ASTN1
rs12141039	186.66	1.343	30	0.147	0.312	1	T	0.635	0.661	ASTN1
rs982875	186.76	1.458	27	0.059	0.429	1	C	0.528	0.528	FAM5B
rs9425456	186.85	2.453	26	0.243	0.388	1	C	0.503	0.548	
rs10732999	186.94	1.953	21	0.007	0.184	1	G	0.719	0.693	
rs10913473	187.31	0.662	22	0.194	0.087	1	T	0.547	0.563	LZTR2
rs4650995	187.66	0.560	23	0.986	0.459	1	T	0.555	0.560	C1orf220
rs2816187	187.97	0.469	22	0.408	0.962	1	C	0.614	0.646	ABL2
rs11578278	188.18	0.480	28	0.984	0.632	1	C	0.639	0.673	TDRD5
rs7528949	188.47	0.458	24	0.611	0.635	1	G	0.557	0.540	LHX4
rs3761903	189.08	0.611	30	0.967	0.699	1	C	0.527	0.548	XPR1
rs679931	189.91	0.612	20	0.684	0.711	1	A	0.653	0.650	CACNA1E
rs6700658	190.66	0.465	19	0.791	0.367	1	C	0.557	0.595	
rs672527	191.50	0.286	21	0.227	0.910	1	C	0.702	0.701	RNASEL
rs10911232	192.21	0.248	26	0.332	0.098	1	C	0.557	0.599	LAMC1
rs3820691	192.70	0.207	22	0.787	0.410	1	T	0.525	0.524	NCF1
rs732812	193.15	0.206	29	0.557	0.796	1	T	0.532	0.532	
rs726427	193.58	0.227	22	0.994	0.453	1	C	0.542	0.598	EDEM3
rs10911704	194.04	0.225	26	0.322	0.386	1	T	0.575	0.598	
rs16824765	194.39	0.219	19	0.171	0.433	1	C	0.731	0.701	HMCN1
rs16825295	194.61	0.194	23	0.895	0.867	1	C	0.592	0.602	C1ord27

Marker	cM	LOD	Informative families—FBAT	FBAT *P*-value	MQLS *P*-value	Major/minor Allele[a]	Nucleotide	Allele freq.		Gene
								Affected	Unaffected	
rs7542180	194.94	0.154	17	0.880	0.866	2	G	0.500	0.480	PLA2G4A

[a]1, major allele and 2, minor allele (designation based on NCBI allele frequencies European Caucasians).

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