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Association of ADH and ALDH Genes With Alcohol Dependence in the Irish Affected Sib Pair Study of Alcohol Dependence (IASPSAD) Sample

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Abstract

Background: The genes coding for ethanol metabolism enzymes [alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH)] have been widely studied for their influence on the risk to develop alcohol dependence (AD). However, the relation between polymorphisms of these metabolism genes and AD in Caucasian subjects has not been clearly established. The present study examined evidence for the association of alcohol metabolism genes with AD in the Irish Affected Sib Pair Study of alcohol dependence.

Methods: We conducted a case–control association study with 575 independent subjects who met Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, AD diagnosis and 530 controls. A total of 77 single nucleotide polymorphisms (SNPs) in the seven *ADH* (*ADH1-7*) and two *ALDH* genes (*ALDH1A1* and *ALDH2*) were genotyped using the Illumina GoldenGate protocols. Several statistical procedures were implemented to control for false discoveries.

Results: All markers with minor allele frequency greater than 0.01 were in Hardy–Weinberg equilibrium. Numerous SNPs in *ADH* genes showed association with AD, including one marker in the coding region of *ADH1C* (rs1693482 in exon6, Ile271Gln). Haplotypic association was observed in the *ADH5* and *ADH1C* genes, and in a long haplotype block formed by the *ADH1A* and *ADH1B* loci. We detected two significant interactions between pairs of markers in intron 6 of *ADH6* and intron 12 of *ALDH2* ($p = 5 \times 10^{-5}$), and 5′ of both *ADH4* and *ADH1A* ($p = 2 \times 10^{-4}$).

Conclusion: We found evidence for the association of several *ADH* genes with AD in a sample of Western European origin. The significant interaction effects between markers in *ADH* and *ALDH* genes suggest possible epistatic roles between alcohol metabolic enzymes in the risk for AD.

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Keywords

Ethanol Metabolism Genes; Alcohol Dehydrogenase; Aldehyde Dehydrogenase; Gene Interaction; Epistasis

Alcohol dependence (AD) is a complex trait that has significant genetic influences but exhibits great clinical and etiological heterogeneity (Prescott et al., 2006a). Identification of specific genes that contribute to susceptibility to develop AD has been a focus of intense research but progress has been slow. Many candidate genes have been examined in association. The genes coding for alcohol metabolizing enzymes are clearly relevant and the most studied.

Two sets of genes code for enzymes in the oxidative pathway of ethanol metabolism: the alcohol dehydrogenases (ADH) convert ethanol into acetaldehyde and the aldehyde dehydrogenases (ALDH) transform acetaldehyde into acetic acid which can then be easily excreted. Several significant associations with AD have been reported for subsets of alcohol metabolism genes (e.g. *ADH1B*, *ADH1C*, *ALDH2*)[†], primarily in East Asian populations (Chen et al., 1999; Cheng et al., 2004; Higuchi et al., 2004; Osier et al., 1999). A meta-analysis including different ethnic groups reported a significantly higher risk of alcoholism[‡] was associated with subjects having *ADH1B*1* (odds ratio, OR = 2.23), *ADH1C*2* (OR = 1.91), and *ALDH2*1* (OR = 4.35) alleles, but only in East Asian populations (Zintzaras et al., 2006). The roles of these genes in non-Asian populations are less clear.

There are 7 human *ADH* genes cluster in a small region (approximately 365 kb) on chromosome 4 q21–q24. It has been suggested that 3 genes are class I (*ADH1A,1B, 1C*) and 1 genes is class II (*ADH4*), which code for primary enzymes for conversion of ethanol to acetaldehyde are involved in ethanol oxidation in vivo (Crabb et al., 2004). The class I enzymes are mainly expressed in liver and contribute about 70% of the total ethanol oxidizing capacity (Lee et al., 2004). Most prior studies have focused on class I *ADH* genes, especially *ADH1B* and *ADH1C*, which have known functional polymorphisms in their coding regions.

The functional variants of allele *ADH1B*2* (His47) and *ADH1B*3* (Cys369) have high enzyme activity and unusually rapid conversion of ethanol to acetaldehyde. This causes facial flushing and aversive effects after consuming alcohol and is protective against AD (Osier et al., 1999). These variants have markedly different frequencies in different ethnic groups. *ADH1B*2* allele is common in Asian populations (approximately 90%) (Eriksson et al., 2001) but is much less common in Caucasians. *ADH1B*3* has been found in African and native American populations (Osier et al., 2002; Wall et al., 1997). Results of meta-analysis for *ADH1B*2* comparing homozygous versus heterozygous genotypes of *ADH1B*2* with AD exhibited an OR of 5 in Han-Chinese and Japanese, but an OR of 2 in Europeans (Whitfield, 2002). Positive association of *ADH1B*3* with AD was also found in African and native American populations (Ehlers et al., 2001; Wall et al., 2003).

Three functional polymorphisms have been identified for *ADH1C*. The wild-type *ADH1C*1* has about twice the enzyme activity of *ADH1C*2* and is very common in Asian and African populations (approximately 90%), whereas the allele frequency is about 50% in Caucasians (Goedde et al., 1992; Osier et al., 2002). Some studies report protective effects of *ADH1C*1* against AD in Asian samples (Chen et al., 1996; Higuchi, 1994; Osier et al., 1999) and a native

[†]We use newer HUGO nomenclature for the *ADH* genes throughout this paper, including to refer to findings from previous studies (e.g. *ADH1B*, *ADH1C* refer to *ADH2*, *ADH3*, respectively, in the old nomenclature).

[‡]In this meta-analysis, strict criteria for alcoholism was defined by: individuals whose diagnoses using published criteria (DSM-III-R, DSM-IV, CAGE, SAPS, Feighner, ICD-10) and/or alcohol consumption greater than 80 g/d for at least 10 years, and individuals who unequivocally had any type of alcoholic end-organ damage.

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American sample (Mulligan et al., 2003). Other studies report no association of *ADH1C*1* with AD in European samples (Borras et al., 2000; Pares et al., 1994). Strong linkage disequilibrium (LD, i.e., the nonrandom association of alleles at two or more loci) has been observed between *ADH1B*2* and *ADH1C*1* (Chen et al., 1999; Osier et al., 1999), so whether there is an independent effect of *ADH1C* on AD is unclear.

Recently, functional polymorphisms were identified in the class II *ADH4* gene; one variant in the promoter region of *ADH4* (C-75A) alters expression level (Edenberg et al., 2004). Some studies reported association of *ADH4* with AD in European-Americans (Luo et al., 2006a), Brazilians (Guindalini et al., 2005) and another sample of European- and African-Americans (Edenberg et al., 2006). The class IV *ADH7* gene, which is mainly expressed in upper digestive tract (Farres et al., 1994; Moreno et al., 1994), codes for the enzyme with the highest activity for oxidizing retinol. Previous studies suggest that *ADH7* has an epistatic effect with *ADH1B* for protection against AD in Taiwanese Han and European populations (Han et al., 2005; Luo et al., 2006b; Osier et al., 2004).

Genes that code for ALDH enzymes are located on several different chromosomes. Nineteen putatively functional genes and 3 pseudogenes in the *ALDH* gene superfamily have been identified to be encoding *ALDH* isozymes (Vasiliou and Nebert, 2005), but only two of them, *ALDH1* (*ALDH1A1*, 9q21.13, cytosolic isozyme) and *ALDH2* (12q24.2, mitochondrial isozyme) are thought to be significantly involved in acetaldehyde oxidation (Ramchandani et al., 2001). The *ALDH2* gene is highly expressed in liver and stomach with a very high affinity for acetaldehyde and plays a central role in human acetaldehyde metabolism. The *ALDH1* gene also has high affinity for acetaldehyde. A functional polymorphism of the *ALDH2* gene, *ALDH2*2* (Lys487RAA) has lower enzyme activity than the wild-type allele. This mutant allele is mainly present in East Asians (approximately 30%). Although homozygous individuals have no ALDH2 activity; heterozygous individuals maintain 30 to 50% activity. The deficient *ALDH2*2* allele is associated with decreasing risk of AD (Chai et al., 2005; Higuchi et al., 2004) or alcoholism[‡] (Zintzaras et al., 2006) in Asian populations. In addition, individuals with the combination of a fast form of *ADH1B* and slow form of *ALDH2* had particularly reduced risk for AD in a Han-Chinese samples (Chen et al., 1999).

Two genetic variants are known in *ALDH1A1*, *ALDH1A1*2* (a 17 bp deletion in the promoter region present in many populations) and *ALDH1A1*3* (a 3 bp insertion in the promoter region present only in populations of African descent). Both have protective effects against AD in African-Americans (Spence et al., 2003), and *ALDH1A1*2* was further reported to be protective in a native American sample (Ehlers et al., 2004).

Overall, both ADH and ALDH enzymes exhibit genetic polymorphism and ethnic variation. The functional polymorphisms of *ADH* and *ALDH* with protective effects against AD are mainly reported in Asian and native American populations, or in African-Americans. Several of these functional polymorphisms have extremely low minor allele frequency (MAF) in European populations (Goedde et al., 1992; Oota et al., 2004; Osier et al., 1999), making it difficult to generalize findings across populations. Consequently, the roles of *ADH* and *ALDH* genes in the development of AD are less clear in Caucasians. We are aware of only 2 studies that examined all 7 *ADH* genes. Edenberg et al. (2006) genotyped 110 single nucleotide polymorphisms (SNPs) in *ADH* genes in the Collaborative Studies on Genetics of Alcoholism samples (comprised by Americans of European and African origin) and found significant ethnic differences in allele frequency for approximately 80% of these markers (Edenberg et al., 2006). They identified associations of *ADH4* SNPs with AD but only in European descended samples (results were not reported in African-American samples because of small numbers of African origin families in their study). In another study of European- and African-Americans, 23 SNPs in *ADH* genes plus 4 SNPs in *ALDH2* were genotyped (Luo et al., 2006b). Significant

associations reported with AD included *ADH5* genotypes and diplotypes of *ADH1A*, *ADH1B*, *ADH7*, and *ALDH2*, although the associated genotypes or diplotypes differed in the European- and African-American samples.

In the literature, only a few studies examined the whole *ADH* genes cluster in relation to AD, and examination of both *ADH* and *ALDH* genes is very limited. Furthermore, the roles of these genes in non-Asian populations are less clear. The primary goal of the present study was to test for association of AD with SNPs in all 7 *ADH* genes as well as *ALDH1A1* and *ALDH2* in a sample of Western European origin that is more culturally and genetically homogeneous than samples used in many previous studies. The *ADH* gene cluster is of particular interest in this sample because we found a significant linkage peak for AD symptoms on a region of 4q that includes the *ADH* gene cluster (Prescott et al., 2006b). In addition, because of the related biological roles of ADH and ALDH enzymes in ethanol metabolism, a second goal was to test for interactions between markers in the *ADH* and *ALDH* genes.

MATERIALS AND METHODS

Subjects and Phenotype Measurement

Participants in this study were recruited in Ireland and Northern Ireland between 1998 and 2002. Details of the study design, sample ascertainment, and clinical characteristics of this sample are described elsewhere (Prescott et al., 2005). In brief, ascertainment of probands was mainly conducted in community alcoholism treatment facilities and public and private hospitals. Probands were eligible for study inclusion if they met the current DSM-IV criteria for AD and if all four grandparents had been born in Ireland, Northern Ireland, Scotland, Wales, or England. After a prospective family was identified through probands, parents and potentially affected siblings whom the probands provided permission to contact were recruited.

Probands, siblings and parents were interviewed by clinically-trained research interviewers, most of whom had extensive clinical experience with alcoholism. The assessment included demographic characteristics, lifetime history of AD and other comorbid conditions, alcohol-related traits, personality features, and clinical records. The DSM-IV AD diagnosis was assessed in probands and siblings using the SSAGA (semi-structured assessment of the genetics of alcoholism) interview (version II, Bucholz et al., 1994), modified to reduce assessment time by omitting items that address onset age of each symptom.

All participants provided informed consent. There were 1238 individuals who completed the SSAGA interview and met DSM-IV AD diagnosis, including 591 probands, 620 affected siblings, and 27 other relatives from 10 complex families. Controls were recruited in the Northern Ireland from volunteers donating at the Northern Ireland Blood Transfusion Service (n = 554) and in Ireland from the Garda Siochana (the national police force, n = 38) and the Forsa Cosanta Aituil (the army reserve, n = 34). Controls were screened and their samples excluded if they reported a history of heavy drinking or problematic alcohol use. In the present case–control study design, we included 530 controls and 575 independent AD cases (399 probands and 176 sibs) from the Irish Affected Sib Pair Study of alcohol dependence families. Samples were selected based on high yield of high quality DNA for genotyping and only one case per family was included.

Genotyping

Genotypes for a total of 77 SNPs in the seven *ADH* (*ADH1-7*)and 2 *ALDH* genes (*ALDH1A1* and *ALDH2*) were obtained as part of a large candidate gene study using an Illumina (San Diego, CA) custom genotyping array designed in Dr. David Goldman's Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism. A total of 130 candidate

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genes were selected from multiple functional systems implicated in substance dependence phenotypes (primarily alcohol, cocaine and opiates).

A genomic region including 5 kb upstream and 1 kb downstream of each candidate gene were retrieved from NCBI Human Genome Build 35.1. Genotype data from the African population, which is the most diverse, were obtained from HapMap Project Public Release No. 18 to re-construct haplotypes for each gene using SNPHAP (http://www-gene.cimr.cam.ac.uk/clayton/software/snphap.txt). A double classification tree search algorithm (Zhang et al., 2004) was applied to select minimum index SNPs that represent maximum haplotype information for each gene. Probable functional SNPs (nonsynonymous, splice site and putative functional SNPs from the literature) were forced in during the selection process. Physically large genes were split into two or three regions, which were processed separately, if the complexity of their haplotype structures was high. Finally, the performance of the initially selected SNP set was validated by the manufacturer and replacements made where necessary.

All genotyping was conducted in Dr. David Goldman's Laboratory. Genotyping was performed using the Illumina GoldenGate genotyping protocols on 96-well format Sentrix® arrays and 500 ng of sample DNA were used per assay. All pre-PCR processing was performed using a Tecan liquid handling robot running Illumina protocols. Arrays were imaged using an Illumina Beadstation GX500 and the data analyzed using GENCALL Version 6.2.0.4 and GTS REPORTS software Version 5.1.2.0 (Illumina).

Statistical Methods

Case–control association analyses were conducted using PLINK (Purcell, http://pngu.mgh.harvard.edu/~purcell/plink/) at both single marker and haplotype levels, which provides significance tests and estimated ORs for risk alleles. We explored all possible pair-wise marker-marker interactions among all genotyped SNPs in PLINK. The SNP pair-wise interactions were reported if *p*-values less than or equal to 0.01. Among all the examined SNPs, five of the *ADH* cluster SNPs were in coding regions: rs1126671 (*ADH4*), rs698 (*ADH1C*), rs1693482 (*ADH1C*), rs971074 (*ADH7*), and rs1573496 (*ADH7*). None of the 22 *ALDH* SNPs were in coding regions.

Because of prior evidence in our sample for linkage to AD symptoms on chromosome 4q22 to 4q32 (Prescott et al., 2006b) (peak multipoint logarithm of the odds score = 4.59 at D4S1611), we adopted a weighted false discovery rate (FDR) approach (Roeder et al., 2006) for associations of single markers in *ADH* loci. This approach weighs the association *p*-values using prior linkage data while applying the FDR method to obtain weighted *p*-values. If the linkage findings are informative, applying this procedure can increase power. Therefore, for single marker tests, we reported weighted *p*-values for *ADH* loci and unweighted *p*-values for *ALDH* loci.

To address multiple testing issues and control the risk of false discoveries in these analyses, we calculated for each *p*-value, a so-called *q*-value (Storey, 2003; Storey and Tibshirani, 2003). A *q*-value is an estimate of the proportion of false discoveries among all significant markers (i.e., *q*-values are FDRs) when the corresponding *p*-value is used as the threshold for declaring significance. We use a *q*-value threshold of 0.2 for declaring significance, meaning that we allow 20% of the significant findings to be false discoveries. Reasons for this choice are that this threshold provides a reasonable balance between the competing goals of controlling false positives versus detecting true positive associations (van den Oord and Sullivan, 2003). This FDR approach and threshold was applied for both single marker and interaction tests; *q*-values were calculated assuming that the proportion of markers without effects (P_0) equals one. As this results in a conservative bias (i.e., *q*-values that are too high), we also estimated P_0

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from the data (for interaction tests only because we have too few data entry to get reliable P_0 estimate for single marker tests) and re-calculated the *q*-values using that estimate. For this purpose, we selected the maximum likelihood estimator that takes advantage of the knowledge that in genetic studies P_0 has to be close to one and produces the most precise estimates in this scenario (Kuo et al., in press; Meinshausen and Rice, 2006) for interaction tests.

RESULTS

Genotyping Completion

Genotyping was completed for 43 SNPs in the *ADH* genes cluster and 34 SNPs in the two *ALDH* genes. Among the 77 genotyped SNPs, we excluded 5 SNPs with low genotyping rate (<50%) and 10 with MAF less than 0.01. Sixty-two SNPs were retained in the analysis and all were in Hardy-Weinberg equilibrium (using a regular cut-off *p*-value of 0.001) in the overall sample and in controls alone. Among the 1105 genotyped individuals, 17 (10 cases, 7 controls) were removed from analysis as genotyping call rates were less than 80%. The sample included in the final analysis thus consisted of 565 AD cases and 523 controls. The average genotyping rate among these individuals is 92.46%.

Single Marker Association and Linkage Disequilibrium Structure

Marker information, allele frequency, and single marker association results are displayed in Table 1 for 40 SNPs in the seven *ADH* genes across a 364.7 kb region, 17 SNPs in *ALDH1A1* in a 136.6 kb region, and 5 SNPs in the *ALDH2* gene in a 35.2 kb region. Many *ADH* markers showed association with AD, but no markers in the *ALDH* genes showed association. ORs were in the range of 1.18 to 1.48 among associated markers. The SNP showing the greatest evidence of association, rs1154414 in the *ADH5* intron region, yielded a weighted *p*-value of 0.004 (OR = 1.48). The 3 class I *ADH* genes (*ADH1A, 2, 3*) consistently exhibited association with AD, with the strongest association to marker rs1353621 in *ADH1B* intron 1 with a weighted *p*-value of 0.005 (OR = 1.27). The two significant markers, rs1154414 and rs1353621 have low *q*-values and pass our prespecified threshold of 0.2. Among the 5 SNP coding region in the *ADH* gene cluster, only marker rs1693482 (*ADH1C* exon6, Ile271Gln) showed association with AD ($p = 0.047$, OR = 1.18) in our sample.

Figure 1 shows the LD of the *ADH* gene cluster along with significance levels of the 40 SNPs (The two *ALDH* genes are excluded because of lack of evidence for association). Overall, there was higher LD within each gene and lower LD between genes. Six of the 7 *ADH* genes formed a region of moderate LD (rs7684986-rs1692126, Fig. 1). All were in very low LD with *ADH7*, which itself had two separate LD blocks. Among these six *ADH* genes, *ADH5* and *ADH4* exhibited high LD between genes (D′ estimates for adjacent SNPs greater than 0.80 for 83% of the comparisons) as well as the region formed by *ADH6*, *ADH1A*, and *ADH1B* (D′ estimates for adjacent SNPs across these 3 genes greater than 0.80 for 73% of the comparisons).

Haplotype Association

Because of the high LD of SNPs within each *ADH* gene (with the exception of *ADH7*), we examined association of haplotypes based on genes instead of algorithm-defined blocks. LD between *ADH1A* and *ADH1B* is strong and yields a highly correlated haplotype distribution for the common haplotypes in both genes. The most common haplotype in *ADH1A* (frequency 42.4%) occurs with the most common haplotype in *ADH1B* (42.6%); the second and third most common haplotypes at *ADH1A* are similarly related to the corresponding haplotypes at *ADH1B*. One to one correspondence of the three common haplotypes results in a high correlation of 0.93 between *ADH1A* and *ADH1B*. Therefore, we examined the haplotypic association using all markers in the two genes to form a long haplotype block. For *ADH1C*, D

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′ was extremely high among 5 genotyped markers (mean = 0.995). Consequently, two tagging SNPs (rs1614972 and rs1693482) were used for haplotype analysis.

Table 2 displays the results of haplotype analyses. Compatible with the single marker results, haplotypes formed by SNPs in *ADH5* and 3 class I *ADH* genes (*ADH1A*, *ADH1B*, *ADH1C*) showed haplotypic evidence of association with AD. No other *ADH* and none of the *ALDH* genes exhibited haplotypic association with AD (data not shown). For *ADH5*, the strongest effect was seen with a protective haplotype of 7 markers that is overrepresented in controls (8.4% in cases vs. 11.7% in controls, $p = 0.01$). For *ADH1A-ADH1B*, two haplotypes of 8 markers are associated with AD, one overrepresented in cases (42.6% vs. 38.0%, $p = 0.036$), the other more common in controls (0.9% in cases vs. 2.1% in controls, $p = 0.02$). These results and the strong LD between the two loci suggest that we are observing only a single effect; which one has the functional effect is not clear. The results for *ADH1C* showed some evidence for a common risk haplotype (cases: 49.4% vs. controls: 45.6%, $p = 0.07$) and a common protective haplotype (cases: 25.8% vs. controls: 29.5%, $p = 0.05$).

Pairwise Interaction

The closely related biological roles of *ADH* and *ALDH* gene products in alcohol metabolism suggest strong potential for interaction effects on risk for AD. We explored these interaction effects by analyzing marker-marker interactions. There were 1891 possible pair-wise comparisons of 62 SNPs. Again, the aforementioned FDR approach and threshold were applied to assess the significance of the interaction analysis. We evaluated the probability of a false positive result for each interaction test based on the observed significance level. Because of the moderate sample size and the exploratory nature of this analysis, results of the interaction tests with *p*-values less than 0.01 are displayed in Table 3; *q*-values were also provided as an estimate of the confidence of the findings to be true.

There were 22 interaction effects with *p*-values less than 0.01, but only two of them demonstrated a *q*-value less than 0.2 (i.e., a 20% chance of the positive finding being false). With this more stringent criterion we get only two significant findings. However, as *q*-values are function of power (lower power means higher *q*-values) we can also look at these results in a more exploratory fashion by increasing the threshold to avoid missing potential interactions. The remaining interaction tests all had high *q*-values, indicating that a *p*-value of approximately 0.0002 is required to give reasonable support for this putative interaction being a true effect in our sample of this size ($n = 1088$). For comparison, an interaction *p*-value of 0.001 (the third highest) in this sample and data structure has an 80% chance of being false. Because of the smoothing procedure in FDR calculation, smaller *p*-values require smaller *q*-values; the "smoothing" procedure explains why many of the tests all have the same *q*-values of 0.805.

Markers rs3857224-rs7296651 (intron 6 in *ADH6* and intron 12 in *ALDH2*, respectively) showed significant evidence for interaction with an OR of 2.3 ($p = 5 \times 10^{-5}$). Marker rs3857224 also had a borderline main effect mentioned previously. Marker pair rs3762894/rs904092 (5′ of *ADH4* and *ADH1A*) yielded an OR of 3.8 for the interaction term, but neither showed significant association at the single marker level. One *ADH7* coding SNP, rs971074, which did not show significant association with AD in prior analysis was in this list of potential interaction effects with two SNPs in the *ALDH1A1* gene. However, the biological function of these possible interactions with rs971074 is unknown.

DISCUSSION

The development of AD is a function of the interplay of genetic and environmental factors. Some proportion of variation in risk for AD may be because of individual differences in

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metabolism. Interindividual variation in alcohol absorption and metabolism is in part because of allelic variants in the genes coding for alcohol metabolizing enzymes. Previous studies have predominantly reported evidence from a few functional polymorphisms in these genes, but *ADH1B*2*, *ADH1C*1*, and *ALDH2*2* are rare outside the Asian or native American samples. As a result, these studies gave only limited understanding of the involvement of these genes in AD in Caucasian populations. The present study examined the association of AD with all 7 *ADH* genes and two genes in the *ALDH* gene family, as well as possible interactions between these genes in an Irish sample. This comprehensive examination of genes involved in alcohol metabolism is important and may provide insight into AD risk in Caucasians.

Using weighted *p*-values for *ADH* gene cluster, we found that our previous reported linkage evidence in this chromosome 4 region tended to increase significance over nonweighted *p*-values as expected. Marker rs1693482 is the only coding SNP (*ADH1C* exon6, Ile271Gln) which showed association with AD in our study. Other coding SNPs have previously shown association with alcoholism[‡] in different populations (Zintzaras et al., 2006). The SNP that distinguishes *ADH1B*1* from *ADH1B*2* (rs1229984, Arg47His in exon3) had very low MAF (0.006) in this Irish sample and was excluded from our standard analysis. However, this SNP consistently showed association with AD and several other endopheno-types in previous studies of Caucasians despite its low frequency. When we included rs1229984 in single marker analysis, it exhibited association with AD (weighted *p*-value = 0.05) in our sample. Another SNP which distinguishes *ADH1C*1* from *ADH1C*2*, (rs698; Val349Phe in exon8) had reasonable MAF and was retained in the standard analysis, and showed weak association with AD (weighted *p*-value = 0.07). In *ALDH2*, marker rs671 (Lys504Glu in exon12) had very low MAF (0.001) and was excluded from our analysis.

Edenberg et al. (2006) found significant association of *ADH4* with AD in the European-American sample, and a protective effect of ADH1B*3 in African-American sample, which is in prediction as the ADH1B*3 allele has been shown to be restricted to populations of African descent (Han et al., 2005). The significant association of *ADH4* with AD was also reported in another European-American sample using a Hardy–Weinberg disequilibrium test (Luo et al., 2005, 2006a). However, the association of *ADH4* with AD was not replicated in our Irish sample. Although the reason for this nonreplication is unclear, sample fluctuation and differences in allele frequency estimates and LD structure may all contribute. In our case, the lack of replication is less likely to be because of allele frequency difference: 3 markers (rs3762894, rs1126671, and rs1042364) were genotyped in studies of both Edenberg et al. (2006) and our group, with identical MAF for the latter two markers and a difference of 3% in MAF for rs3762894.

Our study is the first to examine both the *ADH* and *ALDH* genes in a European sample. In our data, the *ADH* genes are in moderate to high LD with each other except for *ADH7* (see Fig. 1). The LD structure is compatible with Hapmap CEPH (Centre d'Etude du Polymorphism Humain, the DNA samples provided from U.S. residents with northern and western European ancestry in the International HapMap Project) and Edenberg et al. (2006) data. Noncoding SNPs may have unknown effects on the function of *ADH* enzymes or may be in LD with other functional or regulatory SNPs. Our main significant findings are in the 3 class I genes (*ADH1A*, *ADH1B*, and *ADH1C*) and the *ADH5* gene. The class I enzyme is known to be liver expressed and contributes approximately 70% of the total ethanol oxidizing capacity. Polymorphisms in the class I genes *ADH1B* and *ADH1C* previously showed protective effects against AD by causing aversive reactions (Osier et al., 1999;Shen et al., 1997). The protective effects of *ADH1B*2, ADH1B*3*, and *ADH1C*1* are reported across several ethnic groups (African-American, native American, Japanese, Chinese, Taiwanese, Jewish, and Mexican), especially in East Asian populations where the *ADH1B*2* and *ADH1C*1* predominate and are in strong LD with each other (Goedde et al., 1992;Shen et al., 1997). These allele variants have

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very low MAF (less than 1%) in other Caucasian and our samples, so their impact on AD should be extremely low in European populations. However, other polymorphisms in these loci may contribute to the risk of AD. Nearly half of our genotyped markers in class I genes are significantly associated with AD singly or in haplotypes. Interestingly, associated markers in *ADH1A* and *ADH1B* loci are either upstream of the gene or close to promoter regions, consistent with a possible regulatory effect. Luo et al. (2006b) also reported significant association for *ADH1A* and *ADH1B* using diplotype tests in their European-American sample.

The most significant single marker (rs1154414) is in intron 4 of *ADH5* (OR = 1.48, $p = 0.004$). In our data, this marker has high LD with most of the markers in *ADH* genes except for *ADH7*. Luo et al. (2006b) also reported significant association for *ADH5* for marker rs1154400 using genotypic tests in both European- and African-Americans but the associated genotypes were opposite to those found in our study (they found C/C in European-Americans and T/T in African-Americans) and this may be because of a multilocus effect in *ADH5*. When only single loci are assessed, the interlocus correlation can contribute to the “flip-flop” association (Lin et al., 2007). Although we did not find an association for marker rs1154400, one of our significant associated marker (rs1154414) is in LD with rs1154400 (D′ = 0.834), and rs1154414 also tags the associated haplotype (1121111 in Table 2) for *ADH5*. The *ADH5* gene codes for a class III ADH and encodes glutathione-dependent formaldehyde dehydrogenase, which has very low affinity for ethanol. This enzyme is an important component of cellular metabolism for the elimination of formaldehyde but has virtually no activity for ethanol oxidation. Unlike most of the *ADH* genes, which are expressed in specific tissues, *ADH5* is expressed ubiquitously. The biological explanation for an *ADH5* association with AD is unclear, but may be caused by the extensive LD we observe between rs1154414 and SNPs in other genes in the cluster.

There is one marker each in intron 6 of *ADH7* and intron 6 of *ADH6* which showed single marker, but not haplotypic, association with AD. The associated marker (rs1154458) in *ADH7* is in the second block of this gene, and is in high LD (D′, 0.83 to 0.94) with markers in the associated haplotype of Luo et al. (2006b). *ADH7* has been suggested to have a protective effect against AD in Taiwanese Han-Chinese (Osier et al., 2004) despite controversial evidence about whether its effect is independent from the functional polymorphism in *ADH1B*. The coding SNP (rs971074) does not show association with AD in our sample, but the associated intronic marker (rs1154458) may tag other variants not assessed in the current dataset. In addition, we found that rs971074 has potential interaction effects with two intronic SNPs in the *ALDH1A1* gene with OR = 1.7 to 1.8. We observed a weak association for marker rs3857224 (intron 6 in *ADH6*) with AD, but there is no previous association evidence for *ADH6* in the literature. Nevertheless, this marker exhibited the most significant interaction effect ($p = 5 \times 10^{-5}$) with one marker in intron 12 of *ALDH2* (rs7296651). This may merit further study to clarify whether it is merely a statistical artifact, or represents a true molecular interaction. In terms of physiologic function, the *ADH7* gene is mainly expressed in the upper digestive tract (such as stomach and esophagus) (Farres et al., 1994; Moreno et al., 1994) and the *ADH6* gene is expressed mainly in liver (Yasunami et al., 1991; Zhi et al., 2000). *ADH7* is most active as a retinol dehydrogenase, and may participate in the synthesis of retinoic acid, a hormone important for cellular differentiation. *ADH6* metabolizes a wide variety of substrates, including ethanol, retinol, other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products. Both enzymes are inefficient in ethanol oxidation and are much less important in ethanol metabolism compared with class I and II ADH enzymes. Of potential relevance is that the cases in our sample are mainly severe alcoholics with average AD symptoms of 6 (of 7), who were ascertained in treatment settings. It is possible that the *ADH6* and *ADH7* genes are related to aspects of the AD phenotype other than clinical diagnosis, such as health effects that result in treatment-seeking or personality traits like extraversion that are associated with substance use (Luo et al., 2007).

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To account for multiple testing, we applied several FDR approaches and reported *q*-values for both single marker and interaction tests. According to the FDR estimate, there is likely to be at least one true positive interaction effect in our data. Although our results showed significant interaction effects in two marker pairs with *q*-values less than 0.2, it is very hard to say whether the statistical evidence of epistasis can be used to infer biological epistasis at the individual level. If the true genetic model is epistatic without strong main effects of any loci, association tests at the population level have virtually no power to detect these loci (Culverhouse et al., 2002). However, because several of our genotyped *ADH* genes showed significant to marginal effects in AD, we should have moderate power to detect possible interaction effects among these alcohol metabolism genes, though the underlying biological function of these potential gene-gene or protein-protein interactions will need to be addressed using other sorts of data and study designs.

Other than possible interaction effects, *ALDH1A1* and *ALDH2* show neither single marker nor haplotypic association with AD in our data. A previously associated variant, *ALDH2*2* (rs671) is almost monomorphic in Caucasians (MAF = 0.001 in our sample). In a linkage study we conducted for alcohol-related phenotypes, there was evidence for linkage to age-at-onset of AD in the vicinity of *ALDH1A1* gene (9q13-21) and another linkage peak for maximum drinking within 24 hours near the vicinity of *ALDH2* (Kuo et al., 2006). Although no significant association was found for AD in the present study for these two *ALDH* family genes, it would be worthwhile to test for association with those pheno-types producing linkage.

Overall speaking, the current study has several strengths, including unbiased assessment of *ADH* and two key *ALDH* genes in a ethnically and culturally homogeneous European sample. Because of the multiple genes and markers involved in the analyses, we adopted methods to control for false discoveries and weighted *p*-values of single marker associations by prior linkage evidence from the same Irish sample. Nevertheless, this study has some limitations. First, whereas ADH metabolizes the bulk of ethanol within the liver, other enzymes, such as cytochrome *P4502E1* and catalase also metabolize ethanol but were not included in this study. Second, there are gender-related differences in hepatic activity of ADH isoenzymes and ALDH as well as differences in body weight and body fluid volume on alcohol metabolism, but these were not controlled for in our analyses. A recent study using the alcohol clamp method to measure alcohol absorption and metabolism found that given the same blood levels of ethanol, there is no gender difference in the rate of alcohol elimination (Ramchandani et al., 2007). Therefore, an alternative way to check this influence maybe to see if the average consumption by genders differs after adjusting for age and body weight. Third, the interaction analyses were exploratory and we applied marker-based tests while it is more ideal to conduct gene sets analysis to study gene-gene interactions. However, the existent statistical method for gene-based interaction tests is not yet well-established. Finally, we genotyped 43 markers in seven *ADH* genes and 34 markers in *ALDH* genes. Although our LD patterns are compatible with HapMap data, it remains possible that we do not have a full coverage set of SNPs to detect potential association in these alcohol metabolism genes.

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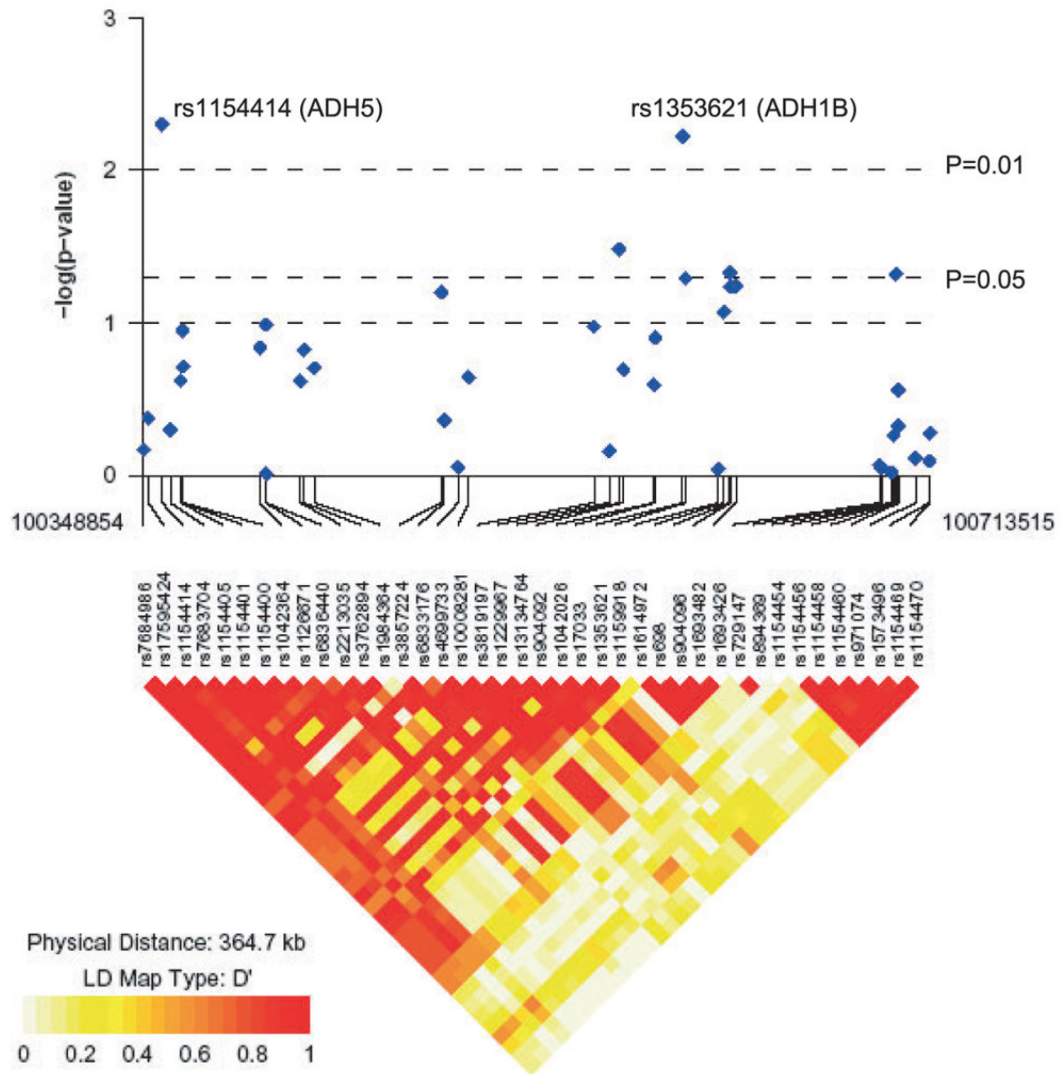

Fig. 1.

The linkage disequilibrium plot and nonadjusted *p*-values for markers in the *ADH* genes cluster. The single nucleotide polymorphisms are listed by chromosome position from pter toward qter.

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Table 1

Allele Frequency and Association Tests for Single Markers in the *ADH* and *ALDH* Genes

Gene	Ch	SNP	Position (bp)	Allele frequency		χ^2	*p*-value[a]	Odds ratio (95%CI)	*q*-value[b]
				Case	Control				
ADH5	4	rs7684986	100348854	0.119	0.113	0.17	0.56	1.06(0.81, 1.38)	0.859
	4	rs17595424	100351047	0.121	0.110	0.65	0.34	1.11(0.86, 1.45)	0.669
	4	rs1154414	100357314	0.913	0.876	7.88	0.004	1.48(1.13, 1.96)	0.186
	4	rs7683704	100361404	0.121	0.112	0.45	0.41	1.09(0.84, 1.42)	0.722
	4	rs1154405	100365928	0.300	0.277	1.39	0.20	1.12(0.93, 1.35)	0.496
	4	rs1154401	100366916	0.369	0.336	2.51	0.09	1.15(0.97, 1.38)	0.490
	4	rs1154400	100367188	0.347	0.321	1.69	0.16	1.13(0.94, 1.35)	0.496
ADH4	4	rs1042364	100402752	0.314	0.285	2.11	0.12	1.15(0.95, 1.38)	0.490
	4	rs1126671	100405592	0.335	0.302	2.66	0.08	1.16(0.97, 1.40)	0.490
	4	rs6836440	100405684	0.976	0.976	0.00	0.80	1.01(0.58, 1.75)	0.976
	4	rs2213035	100421411	0.308	0.285	1.38	0.20	1.12(0.93, 1.35)	0.496
	4	rs3762894	100423262	0.880	0.859	2.08	0.12	1.20(0.94, 1.55)	0.490
	4	rs1984364	100427961	0.313	0.287	1.66	0.16	1.13(0.94, 1.36)	0.496
ADH6	4	rs3857224	100486863	0.732	0.695	3.45	0.051	1.19(0.99, 1.44)	0.434
	4	rs6833176	100488341	0.508	0.491	0.60	0.36	1.07(0.90, 1.27)	0.678
	4	rs4699733	100494712	0.315	0.312	0.02	0.72	1.01(0.84, 1.22)	0.959
	4	rs10008281	100499480	0.814	0.793	1.46	0.19	1.14(0.92, 1.41)	0.496
ADH1A	4	rs3819197	100557687	0.802	0.774	2.61	0.09	1.19(0.96, 1.46)	0.490
	4	rs1229967	100564756	0.767	0.759	0.16	0.57	1.04(0.85, 1.27)	0.859
	4	rs13134764	100569351	0.430	0.385	4.54	0.027	1.21(1.02, 1.43)	0.434
	4	rs904092	100571342	0.817	0.795	1.63	0.16	1.15(0.93, 1.42)	0.496
ADH1B	4	rs1042026	100585644	0.754	0.733	1.30	0.21	1.12(0.92, 1.36)	0.510
	4	rs17033	100586123	0.927	0.909	2.36	0.10	1.27(0.93, 1.73)	0.490
	4	rs1353621	100598753	0.429	0.371	7.50	0.005	1.27(1.07, 1.52)	0.186
	4	rs1159918	100600187	0.664	0.624	3.80	0.042	1.19(1.00, 1.42)	0.434
ADH1C	4	rs1614972	100615333	0.753	0.751	0.01	0.74	1.01(0.83, 1.23)	0.969
	4	rs698	100617967	0.496	0.458	2.97	0.07	1.16(0.98, 1.38)	0.490
	4	rs904096	100620762	0.498	0.456	3.93	0.038	1.19(1.00, 1.41)	0.434

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Gene	Ch	SNP	Position (bp)	Allele frequency		χ^2	p-value[a]	Odds ratio (95%CI)	q-value[b]
				Case	Control				
	4	rs1693482	100621143	0.496	0.455	3.60	0.047	1.18(0.99, 1.39)	0.434
	4	rs1693426	100623508	0.496	0.455	3.61	0.047	1.18(0.99, 1.39)	0.434
ADH7	4	rs729147	100690445	0.193	0.190	0.03	0.70	1.02(0.82, 1.26)	0.959
	4	rs894369	100691024	0.193	0.190	0.02	0.72	1.02(0.82, 1.26)	0.959
	4	rs1154454	100695520	0.855	0.854	0.00	0.78	1.01(0.79, 1.28)	0.969
	4	rs1154456	100696774	0.347	0.335	0.37	0.44	1.06(0.88, 1.26)	0.722
	4	rs1154458	100697700	0.388	0.431	3.91	0.039	1.20(1.00, 1.43)	0.434
	4	rs1154460	100698821	0.466	0.443	1.20	0.22	1.10(0.93, 1.30)	0.529
	4	rs971074	100699039	0.116	0.106	0.52	0.38	1.10(0.84, 1.44)	0.697
	4	rs1573496	100706847	0.896	0.893	0.09	0.63	1.04(0.79, 1.37)	0.918
	4	rs1154469	100713357	0.651	0.646	0.06	0.66	1.02(0.86, 1.22)	0.943
	4	rs1154470	100713515	0.351	0.338	0.40	0.43	1.06(0.89, 1.26)	0.722
ALDH1A1	9	rs3764435	72746430	0.493	0.475	0.69	0.41	1.07(0.91, 1.27)	0.669
	9	rs1888202	72748805	0.502	0.481	0.90	0.34	1.09(0.92, 1.29)	0.627
	9	rs63319	72754338	0.520	0.489	2.09	0.15	1.13(0.96, 1.34)	0.490
	9	rs8187974	72756420	0.991	0.985	1.65	0.20	1.68(0.75, 3.77)	0.496
	9	rs348457	72760108	0.454	0.421	2.32	0.13	1.14(0.96, 1.35)	0.490
	9	rs2303317	72771496	0.488	0.469	0.79	0.37	1.08(0.91, 1.28)	0.642
	9	rs2773806	72780854	0.019	0.018	0.01	0.94	1.03(0.55, 1.92)	0.969
	9	rs1424482	72793111	0.340	0.324	0.67	0.41	1.08(0.90, 1.29)	0.669
	9	rs8187876	72794508	0.947	0.937	0.90	0.34	1.19(0.83, 1.71)	0.627
	9	rs11143429	72837438	0.417	0.409	0.13	0.72	1.03(0.87, 1.23)	0.879
	9	rs1364451	72838314	0.891	0.880	0.58	0.45	1.11(0.85, 1.45)	0.678
	9	rs6560311	72841736	0.307	0.289	0.80	0.37	1.09(0.90, 1.31)	0.642
	9	rs2249978	72856652	0.706	0.703	0.03	0.86	1.02(0.84, 1.22)	0.959
	9	rs1418187	72867214	0.764	0.757	0.17	0.68	1.04(0.86, 1.27)	0.859
	9	rs4745209	72869659	0.766	0.754	0.38	0.54	1.06(0.87, 1.30)	0.722
	9	rs7862749	72873389	0.964	0.959	0.36	0.55	1.14(0.74, 1.77)	0.722
	9	rs4406477	72882012	0.367	0.365	0.01	0.93	1.01(0.85, 1.20)	0.969
ALDH2	12	rs2238151	110674553	0.327	0.302	1.58	0.21	1.12(0.94, 1.35)	0.496

Gene	Ch	SNP	Position (bp)	Allele frequency		χ^2	*p*-value[a]	Odds ratio (95%CI)	*q*-value[b]
				Case	Control				
	12	rs2238152	110677179	0.159	0.138	1.90	0.17	1.18(0.93, 1.50)	0.496
	12	rs4648328	110685508	0.157	0.138	1.51	0.22	1.16(0.92, 1.47)	0.496
	12	rs4646778	110698503	0.157	0.135	2.20	0.14	1.20(0.94, 1.52)	0.490
	12	rs7296651	110709674	0.158	0.137	1.88	0.17	1.18(0.93, 1.50)	0.496

SNP, single nucleotide polymorphism.

[a] *p*-value using weighted FDR approach for *ADH* genes cluster and unweighted for *ALDH* genes

[b] *q*-value was calculated assuming P_0 equals 1.

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Table 2

Haplotypic Association Results for the *ADH* Genes

Gene	Haplotypes	Haplotype frequency		χ^2	*p*-value
		Case	Control		
ADH5	1111111	0.551	0.551	0.000	0.998
	1111222	0.180	0.163	1.161	0.281
	2212222	0.119	0.109	0.499	0.480
	1121111	0.084	0.117	6.382	0.012
	1111122	0.046	0.042	0.180	0.672
	1111121	0.021	0.019	0.084	0.772
ADH1A-ADH1B	11211121	0.426	0.380	4.411	0.036
	21112111	0.188	0.211	1.817	0.178
	12121112	0.183	0.200	0.963	0.326
	11111212	0.073	0.082	0.673	0.412
	11111112	0.061	0.053	0.484	0.487
	12112111	0.050	0.041	1.022	0.312
	11211112	0.011	0.010	0.043	0.836
	21112112	0.009	0.021	5.381	0.020
ADH1C	12	0.495	0.456	3.233	0.072
	11	0.258	0.295	3.784	0.052
	21	0.248	0.249	0.004	0.947

Markers in *ADH5*, *ADH1A-ADH1B*, and *ADH1C* gene blocks are as follows:

ADH5: rs7684986-rs17595424-rs1154414-rs7683704-rs1154405-rs1154401-rs1154400; *ADH1A-ADH1B*: rs3819197-rs1229967-rs13134764-rs904092-rs1042026-rs17033-rs1353621-rs1159918; *ADH1C*: rs1614972-rs1693482.

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Table 3

Pairwise Interactions Among Markers in the *ADH* and *ALDH* Genes ($p < 0.01$)

Chromosome 1	Chromosome 2	SNP1	SNP2	OR_int	χ^2	*p*-value	*q*-value[a]
4	12	rs3857224	rs7296651	2.320	16.500	0.00005	0.092
4	4	rs3762894	rs904092	3.797	13.770	0.00021	0.195
4	9	rs1154460	rs3764435	1.533	10.300	0.0013	0.805
4	9	rs1154470	rs3764435	1.499	9.243	0.0024	0.805
4	4	rs904092	rs1154469	1.712	9.179	0.0024	0.805
4	4	rs1154405	rs1984364	1.596	8.875	0.0029	0.805
4	4	rs2213035	rs3762894	2.222	8.309	0.0039	0.805
4	4	rs1984364	rs6833176	1.549	8.302	0.0040	0.805
4	9	rs1042364	rs2773806	6.532	7.898	0.0050	0.805
4	4	rs7684986	rs1159918	2.096	7.887	0.0050	0.805
4	4	rs1154414	rs1154458	1.778	7.632	0.0057	0.805
4	4	rs1126671	rs894369	1.633	7.485	0.0062	0.805
4	4	rs10008281	rs3819197	1.749	7.467	0.0063	0.805
4	4	rs4699733	rs1159918	1.510	7.141	0.0075	0.805
4	9	rs1984364	rs4745209	1.571	7.075	0.0078	0.805
4	9	rs1154414	rs1418187	1.939	7.033	0.0080	0.805
4	9	rs971074	rs348457	1.784	7.013	0.0081	0.805
4	4	rs7684986	rs1693482	1.856	6.969	0.0083	0.805
4	12	rs894369	rs7296651	1.813	6.927	0.0085	0.805
4	9	rs971074	rs3764435	1.685	6.921	0.0085	0.805
4	4	rs1126671	rs1159918	1.508	6.683	0.0097	0.840
9	9	rs1888202	rs4406477	1.405	6.676	0.0098	0.840

OR_int: odds ratio of interaction term; SNP, single nucleotide polymorphism.

[a] *q*-value was calculated based on a false discovery rate approach (van den Oord, 2005) using maximum likelihood estimator to first derive P_0 (i.e., the number of tests with no effects), and then utilize the P_0 to obtain the *q*-value for each interaction test. The P_0 estimate was 0.9994698 (i.e., about one true positive among the 1891 interaction tests based on data distribution).