Association Between In Vivo Alcohol Metabolism and Genetic Variation in Pathways that Metabolize the Carbon Skeleton of Ethanol and NADH Reoxidation in the Alcohol Challenge Twin Study

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Background: Variation in alcohol metabolism affects the duration of intoxication and alcohol use. While the majority of genetic association studies investigating variation in alcohol metabolism have focused on polymorphisms in alcohol or aldehyde dehydrogenases, we have now tested for association with genes in alternative metabolic pathways that catalyze the carbon skeleton of ethanol (EtOH) and NADH reoxidation.

Methods: Nine hundred fifty single nucleotide polymorphisms (SNPs) spanning 14 genes (*ACN9*, *ACSS1*, *ACSS2*, *ALDH1A1*, *CAT*, *CYP2E1*, *GOT1*, *GOT2*, *MDH1*, *MDH2*, *SLC25A10*, *SLC25A11*, *SLC25A12*, *SLC25A13*) were genotyped in 352 young adults who participated in an alcohol challenge study. Traits tested were blood alcohol concentration (BAC), breath alcohol concentration (BrAC), peak alcohol concentration, and rates of alcohol absorption and elimination. Allelic association was tested using quantitative univariate and multivariate methods.

Results: A *CYP2E1* promoter SNP (rs4838767, minor allele frequency 0.008) exceeded the threshold for study-wide significance (4.01×10^{-5}) for 2 early BAC, 8 BrAC measures, and the peak BrAC. For each phenotype, the minor C allele was related to a lower alcohol concentration, most strongly for the fourth BrAC ($p = 2.07 \times 10^{-7}$) explaining ~8% of the phenotypic variance. We also observed suggestive patterns of association with variants in *ALDH1A1* and on chromosome 17 near *SLC25A11* for aspects of blood and breath alcohol metabolism. An SNP upstream of *GOT1* (rs2490286) reached study-wide significance for multivariate BAC metabolism ($p = 0.000040$).

Conclusions: Overall, we did not find strong evidence that variation in genes coding for proteins that further metabolize the carbon backbone of acetaldehyde, or contribute to mechanisms for regenerating NAD from NADH, affects alcohol metabolism in our European-descent subjects. However, based on the breath alcohol data, variation in the promoter of *CYP2E1* may play a role in preabsorptive or early hepatic alcohol metabolism, but more samples are required to validate this finding.

Key Words: Alcohol Metabolism, Association, Genetics, CYP2E1, Alcohol Challenge.

INTERINDIVIDUAL VARIATION IN the absorption and elimination of alcohol (ethanol [EtOH]) has been shown to be significantly heritable (Kopun and Propping, 1977; Martin et al., 1985; Vesell et al., 1971). Genes or loci affecting in vivo EtOH metabolism have been identified through linkage and candidate gene association studies. The majority of these studies have focused on enzymes that catalyze the first step of EtOH oxidation to acetaldehyde, namely alcohol dehydrogenases (ADHs) (Birley et al., 2005, 2008, 2009), and the second step in which aldehyde dehydrogenase 2 (ALDH2) converts acetaldehyde to acetate (Kiyoshi et al., 2009; Yoshida, 1992). Genetic variation in *ADH1B* and *ALDH2* is associated with individual and ethnic differences in alcohol consumption patterns (Macgregor et al., 2009; Neumark et al., 1998; Raimondi et al., 2004; Whitfield et al., 1998) and risk for alcohol dependence (Edenberg et al., 2006; Kuo et al., 2008; Luo et al., 2006) and alcohol-related cancers (Hashibe et al., 2008), raising the possibility that genetic variation affecting alcohol metabolism through other enzymes or transporters could also affect these diseases.

Other pathways and genes that catalyze conversion of EtOH to acetaldehyde are the Microsomal EtOH-Oxidizing System (MEOS; via CYP2E1) and catalase (CAT), but they

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have been estimated to account for <10% of total alcohol metabolism in the body (Agarwal, 2001; Deng and Deitrich, 2008; Lieber, 1999). Known variants in *ADH*s and *ALDH2* cannot account for the heritability of metabolism, estimated at 50 to 60% from twin studies (Martin et al., 1985); variation in downstream events may also be significant (see Fig. 1). First, the carbon skeleton of acetate is further metabolized to Acetyl CoA by Acyl-CoA synthetase short-chain family member 1 or 2 (ACSS1, ACSS2) or can be incorporated into the gluconeogenesis pathway as a carbohydrate source by ACN9 (Dennis and McCammon, 1999). Second, the actions of both ADH (in the cytosol) and ALDH2 (in mitochondria) require the transfer of hydrogen atoms to the oxidized form of nicotinamide adenine dinucleotide (NAD^+), resulting in its reduction to NADH. As mitochondria (which regenerate NAD^+ from NADH in oxidative phosphorylation) are not permeable to NADH, the bidirectional malate-aspartate shuttle transports the reducing equivalents of NADH between the cytosol and the mitochondria via aspartate-glutamate transporters (SLC25A12 and SLC25A13) and malate carriers (SLC25A10, SLC25A11) (Borst, 1963). Within the shuttle, cytoplasmic (GOT1) and mitochondrial (GOT2) aspartate aminotransferases catalyze the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate. Similarly, cytoplasmic (MDH1) and mitochondrial (MDH2) malate dehydrogenases catalyze the interconversion of oxaloacetate and NADH to malate and NAD^+. The NADH/NAD ratio is important as excess NADH decreases ADH activity and alters the cellular redox state; reoxidation of NADH has been hypothesized as the rate-limiting step in EtOH oxidation in the liver (Lindros et al., 1972; Meijer et al., 1975). Furthermore, an increased NADH/NAD ratio affects other substrate couples and produces metabolic changes which are linked with disorders including gout and fatty liver (Lieber, 2003).

Rather than limiting our analysis to the well-characterized alcohol metabolizing *ADH* and *ALDH2* genes, we have extended our previous studies on these enzymes (Birley et al., 2008, 2009; Dickson et al., 2006) to systematically analyze genetic variation in other genes (i) involved in the early steps of EtOH oxidation to acetaldehyde and acetate (*ALDH1A1, CAT, CYP2E1*); (ii) that play a role in the metabolism of the carbon skeleton of EtOH (*ACN9*, *ACSS1*, *ACSS2*); and (iii) within the malate-aspartate NADH shuttle (*GOT1*, *GOT2*, *MDH1*, *MDH2*, *SLC25A10*, *SLC25A11*, *SLC25A12*, *SLC25A13*). This paper reports on the associations of 950 single nucleotide polymorphisms (SNPs) with a series of in vivo alcohol metabolism measures in 352 participants from the Alcohol Challenge Twin Study (ACTS; Martin et al., 1985), specifically blood alcohol concentration (BAC) and breath alcohol concentration (BrAC), the rates of alcohol absorption and alcohol elimination in the blood and breath, and the peak BAC and BrAC achieved following alcohol challenge.

MATERIALS AND METHODS

Samples

Four hundred and twelve people (206 pairs of twins) participated in the original ACTS between 1979 and 1981 (Martin et al., 1985). Twin participants were 51.7% female and comprised 85 monozygotic (MZ) twin pairs (43 female and 42 male) and 121 dizygotic (DZ) twin pairs (44 female, 38 male, and 39 opposite sex). Self-reported ancestry of the ACTS participants is predominantly Northern European (87%) with information available on the birthplace of their 4 grandparents. The twins ranged in age from 18 to 34 years (mean age: 23.0 ± 4.6) at the time of testing with 70% of subjects aged <25 years. Twins were recontacted 10 to 20 years after completion of the ACTS to obtain DNA for genotyping and blood samples were collected from 372 twins. Subjects gave written informed consent and genetic studies were approved by the Queensland Institute of Medical Research (QIMR) Human Research

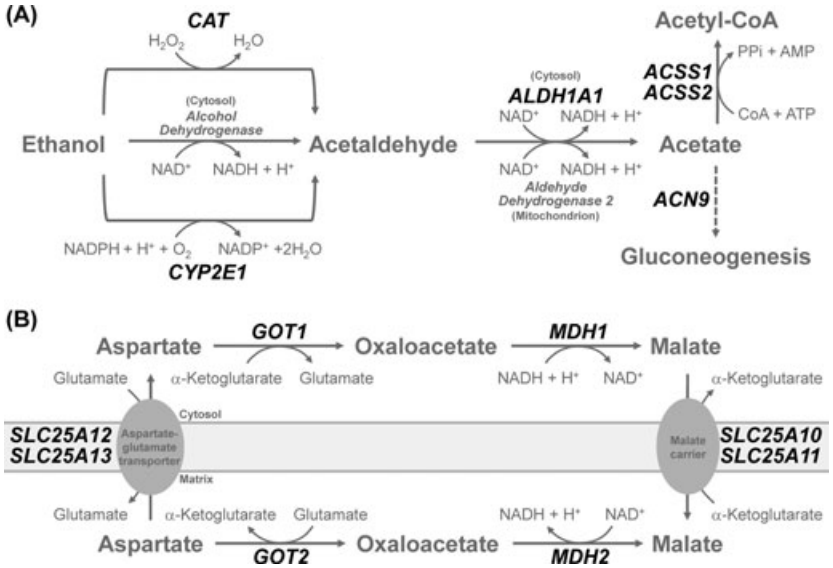

Fig. 1. Alcohol (ethanol [EtOH]) metabolism pathways investigated. (A) The metabolism of the carbon skeleton backbone of EtOH. (B) The bidirectional malate-aspartate NADH shuttle. The 14 candidate genes are represented by italicized gene symbols and are described in Table 2.

Ethics Committee. Zygosity of same-sex twin pairs was assessed using a combination of self-report, blood groups, and a set of 9 polymorphic DNA microsatellite markers (AmpF1STR Profiler Plus Amplification Kit; Applied Biosystems, Foster City, CA).

Measures

BAC and BrAC (expressed as mg per 100 ml of blood) was recorded following ingestion of a weight-related dose of EtOH (0.75 g/kg) over 20 minutes. Full details are given in Martin and colleagues (1985) and a summary of the phenotypes analyzed in the current study is given in Table 1. Six timed measurements of BAC (LP1-LP6; over 3.5 hours) and 10 of BrAC (RP01-RP10; over 5.5 hours) were obtained starting 40 minutes after the commencement of EtOH intake. Owing to changes in the sampling schedule over the duration of the ACTS, occasional delays in taking blood samples and missing samplings, values were predicted for each individual at the 6 mean observation times for BAC and 10 mean observation times for BrAC. A curve was fitted to the predicted BACs and BrACs for each subject; the rate of absorption, peak concentration, and rate of elimination were calculated for blood (BLAP, PKLP, BLEP, respectively) and breath (BRAP, PKRP, BREP) separately. Correlations between observed and predicted BACs and BrACs at all sampling times were >0.92, and variances of predicted BACs and BrACs were lower than those of the corresponding observed readings consistent with a reduction in error variance.

SNP Selection

Data for 950 SNPs from 14 candidate genes were obtained from 2 data sets: a candidate gene study (using Sequenom iPLEX genotyping chemistry) and a genome-wide association study (GWAS) using Illumina BeadChips. The genes and number of SNPs genotyped and analyzed are summarized in Fig. 1 and Table 2, respectively.

The candidate gene study was conceived in December 2007, carried out in 2 stages and completed in 2009. A total of 132 SNPs from 12 genes were selected based on the information available at the time from published literature (Dick et al., 2008; Webb et al., 2011), in addition to common (minor allele frequency [MAF] > 5%) synonymous or nonsynonymous SNPs and tag SNPs selected from genotype data downloaded from the International HapMap Project public database using NCBI Build 35 (Stage 1; *ACN9*, *ACSS2*, *CYP2E1*, *SLC25A13*) and NCBI Build 36 (Stage 2; *ACSS1*, *GOT1*, *GOT2*, *MDH1*, *MDH2*, *SLC25A10*, *SLC25A11*, *SLC25A12*) and Haploview version 4.0 software (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview; Barrett et al., 2005).

Imputed genotypes from the GWAS data set were extracted for all SNPs (N = 936) within a fixed 20 Kb gene border for each of the 12 candidate genes above plus *ALDH1A1* and *CAT* using gene coordinates downloaded from the University of California, Santa Cruz table browser for all RefSeq genes on July 24, 2008.

Table 1. Summary of Predicted Blood and Breath Alcohol Phenotypes

Phenotype	Measurement	Time (minutes)[a]	Abbreviation
Predicted blood alcohol concentration	1	56	LP1
	2	68	LP2
	3	83	LP3
	4	123	LP4
	5	143	LP5
	6	182	LP6
Rate of blood alcohol absorption			BLAP
Peak blood alcohol concentration			PKLP
Rate of blood alcohol elimination			BLEP
Predicted breath alcohol concentration	1	40	RP01
	2	56	RP02
	3	68	RP03
	4	83	RP04
	5	100	RP05
	6	123	RP06
	7	143	RP07
	8	160	RP08
	9	182	RP09
	10	213	RP10
Rate of breath alcohol absorption			BRAP
Peak breath alcohol concentration			PKRP
Rate of breath alcohol elimination			BREP

[a]Minutes following alcohol ingestion.

Genotyping

Sequenom Platform. Assays were designed using the Mass ARRAY Assay Design (version 3.0) software (Sequenom Inc., San Diego, CA) and typed using iPLEX chemistry on a Compact MALDI-TOF Mass Spectrometer (Sequenom Inc.). Primers were purchased from Bioneer Corporation (Daejeon, Korea). Genotyping was carried out in standard 384-well plates with 12.5 ng genomic DNA used per sample and allele calls were reviewed using the cluster tool in the SpectroTyper software (Sequenom Inc.).

Illumina Platform. Genotype data for 275 ACTS twins (352 if including 77 imputed MZ co-twins) were drawn from 4 of 9 GWAS subsamples (N = 17,862 individuals) genotyped for the Genetic Epidemiology laboratory at QIMR using Illumina HumanCNV370-Quadv3 (N = 223), Human610-Quadv1 (N = 36), and 317 K (N = 16) BeadChips. Standard QC filters were applied and have been described elsewhere (Medland et al., 2009). A consensus marker set (N = 269,840 SNPs) was imputed up to 2,380,486 HapMap SNPs (The International HapMap Consortium, 2003) using the Mach (Li and Abecasis, 2006) program, as described by Medland and colleagues (2009). Samples were screened for ancestry outliers and individuals were excluded who were >2 standard deviations from the PC1 and PC2 centroid derived from European populations following Eigenstrat analysis (McEvoy et al., 2009).

Imputation QC measures for 818 of 936 SNPs are summarized in Table S1. Imputed genotypes for 118 SNPs typed in the candidate gene study were not included in the joint data set. On average the genotype discordance rate for the 118 duplicated SNPs was 2.4% and is summarized in Table S2. The genotype data for *ACSS1* and *SLC25A10* SNPs showed the strongest discordance rates on average with 4.39% (range, 0 to 7.9%) and 4.33% (range, 0 to 24.5%), respectively.

Statistical Analysis

We tested whether 950 SNPs were associated with (i) 6 BAC [LP1-LP6] and 10 BrAC [RP01-RP10] measurements; (ii) rate of alcohol absorption (BLAP, BRAP); (iii) rate of alcohol elimination (BLEP, BREP); and (iv) peak alcohol concentration in the blood and breath (PKLP, PKRP). Tests of total association with each quantitative trait at each marker were performed in QTDT (Abecasis et al., 2000). Total association considers transmission within and between families, specifying an additive model against

Table 2. Summary of Study Design

Candidate region (Gene symbol)	Candidate gene	Chr	Band	Transcript position[a]	Gene size (bp)	Number of SNPs				
						Sequenom (N = 132)	Illumina (N = 818)	Total (N = 950)	In Gene (N = 571)[b]	Intergenic (N = 379)[c]
MDH1	Cytosolic malate dehydrogenase	2	2p15	63,669,626–63,687,833	18,208	6	25	31	20	11
SLC25A12	Solute carrier family 25 (mitochondrial carrier, Aralar), member 12	2	2q31.1	172,349,127–172,458,979	109,853	13	63	76	62	14
MDH2	Mitochondrial malate dehydrogenase precursor	7	7q11.23	75,515,329–75,533,865	18,537	9	23	32	20	12
SLC25A13	Solute carrier family 25, member 13 (citrin)	7	7q21.3	95,587,469–95,789,341	201,873	13	101	114	99	15
ACN9	Homolog of the Saccharomyces cerevisiae acetate nonutilizing 9 gene	7	7q21.3	96,584,956–96,649,010	64,055	13	34	47	29	18
ALDH1A1	Aldehyde dehydrogenase 1 family, member A1	9	9q21.13	74,705,408–74,757,789	52,382	0	115	115	74	41
GOT1	Glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1)	10	10q24.2	101,146,618–101,180,336	33,719	13	51	64	46	18
CYP2E1	Cytochrome P450, family 2, subfamily E,	10	10q26.3	135,190,857–135,202,611	11,755	15	82	97	35	62
CAT	Catalase	11	11p13	34,417,054–34,450,180	33,127	0	138	138	70	68
GOT2	Glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2)	16	16q21	57,298,536–57,325,747	27,212	12	33	45	34	11
SLC25A11	Solute carrier family 25 (mitochondrial carrier; oxoglutarate carrier), member 11	17	17p13.2	4,781,349–4,784,063	2,715	4	21	25	0	25
SLC25A10	Solute carrier family 25 (mitochondrial carrier; dicarboxylate transporter), member 10	17	17q25.3	77,289,776–77,298,447	8,672	7	8	15	5	10
ACSS1	Acyl-CoA synthetase short-chain family member 1	20	20p11.21	24,934,874–24,987,616	52,743	20	89	109	59	50
ACSS2	Acyl-CoA synthetase short-chain family member 2	20	20q11.22	32,926,502–32,979,422	52,921	7	35	42	18	24

bp, base-pair; Chr, chromosome; SNPs, single nucleotide polymorphisms.
[a]Base-pair position of the transcription start and stop site of the candidate gene based on NCBI dbSNP build 130.
[b]Number of SNPs physically located in the candidate gene (promoter, intronic, exonic, and 3′ untranslated region SNPs).
[c]Number of SNPs physically located outside of the candidate gene.

the null hypothesis of no linkage and no association (Table S3). The between-family association component is not robust to population stratification. Therefore, additional analyses in QTDT were performed to check for population stratification by using a variant of the orthogonal model which evaluates population stratification by comparing the between- and within-family components of association (Table S4). QTDT takes into account familial relatedness and zygosity, with the trait values of MZ twins averaged across the pair. All quantitative traits were transformed to normality using inverse rank normal transformation. Correction for sex, age, age^2, sex × age, and sex × age^2 was performed by fitting covariates in the regression model. Phenotypes were also adjusted for possible effects of population stratification in our sample by fitting the first 10 eigenvectors (PC1-PC10) from European-only principal components analysis of ancestry in the regression model (McEvoy et al., 2009). Sequenom genotype data and phenotypes were available for 366 twins (40.0% male) from 187 families comprising 77 MZ pairs (42 female and 35 male), 102 DZ pairs (37 female, 27 male, and 38 opposite sex), and 8 unpaired DZ twins. Imputed GWAS data and phenotypes were available for 275 twins (45.5% male) from 191 families comprising 77 MZ twins (40 female and 37 male), 84 DZ pairs (34 female, 23 male, and 27 opposite sex), and 30 unpaired DZ twins. Two SNPs (rs11190090 and rs17850882) were found to be monomorphic.

To calculate the study-wide threshold of significance, the multiple correlated SNPs and phenotypes tested must first be corrected for. Reflecting moderate linkage disequilibrium (LD) across many of the candidate genes, the effective number of independent SNPs tested was 208, as determined by SNPSpD (Nyholt, 2004). Likewise, the effective number of independent phenotypes was calculated to be 6.0003 in matSpD (http://gump.qimr.edu.au/general/daleN/matSpD/) (Nyholt, 2004). Therefore, a conservative p-value $<4.01 \times 10^{-5}$ (0.05/208 SNPs/6 traits) would be required for study-wide significance. We have 96% and 37.0% power ($\alpha = 0.05$) to detect overall association with an SNP (with MAF above 0.01) which explains 5% and 1%, respectively, of variance in our trait under an additive model and against a background sibling correlation of 0.30 (Purcell et al., 2003). Power is reduced to 33.5% for an SNP explaining 5% of variance when $\alpha = 4.01 \times 10^{-5}$ (the study-wide significance threshold); to achieve 80% power, a sample size of 477 would be required. The sample size required to achieve 80% power increases to 1,304 for an SNP explaining 1% of variance.

To account for the multiple correlated BAC and BrAC phenotypes (see Table S5 for correlations), we also employed the MQFAM multivariate extension in PLINK 1.06 (Ferreira and Purcell, 2009) to test for association between individual SNPs and (i) all BAC levels; (ii) early BACs (LP1-LP3); (iii) late BACs (LP4-LP6); (iv) all BrAC levels; (v) early BrAC levels (RP01-RP05); and (vi) late BrACs (RP06-RP10) (Table S6). For these analyses, standardized residuals adjusting for sex, age, sex × age, age^2, and sex × age^2 and PC1-PC10 eigenvectors for each measurement were calculated before submission to MQFAM. Trait values of MZ twins were averaged across the pair. Permutation testing within MQFAM was used to correct for family structure. For the multivariate analyses, the study-wide level of significance is 4.01×10^{-5} (0.05/208 SNPs × 6 traits).

RESULTS

Descriptive

With a combined set of in-house genotyping and imputed GWAS markers, a total of 950 SNPs spanning 14 candidate genes were analyzed. No marker showed significant departures from Hardy–Weinberg equilibrium ($p < 0.001$). We first conducted analyses of blood alcohol measures and then breath alcohol measures obtained during the alcohol challenge. Total association results for all SNPs and blood and breath phenotypes are presented in Table S3 and Figs S1–S3.

Allelic Effects on Blood Alcohol Concentration

Tests of total association were conducted for each SNP with all 6 BAC levels (LP1-LP6), as well as the rate of alcohol absorption into the blood (BLAP), rate of alcohol elimination (BLEP), and the peak BAC (PKLP). Twenty-three SNPs associated with one or more of these blood alcohol phenotypes at a significance level of $p < 0.005$ are summarized in Table 3 (rs348447 in the *ALDH1A1* promoter was affected by population stratification). The strongest evidence of association with blood alcohol metabolism was observed with SNPs in *CYP2E1* and *ALDH1A1*. One SNP, rs4838767, in the promoter of *CYP2E1* exceeded the threshold for study-wide significance (4.01×10^{-5}) for 2 blood alcohol measures tested (LP1 and LP2). Six study participants (1 male MZ twin pair, 1 female DZ twin pair, and 2 unrelated twins) were observed to be heterozygous (A/C) for this rare *CYP2E1* promoter SNP (MAF = 0.008) that was primarily associated with the earlier times LP1-LP3 ($p < 0.00007$) and the peak BAC ($p = 0.0007$). A second *CYP2E1* SNP (rs4646976) in moderate LD ($r^2 = 0.748$) with rs4838767 was most associated with LP1-LP3 measures ($p < 0.001$) and the rate of blood alcohol elimination (BLEP, $p = 0.0004$). Four intronic *ALDH1A1* SNPs in moderate to high LD (rs2017362, rs348461, rs348463, and rs2210103) were strongly associated with late BAC readings LP4-LP6 ($p < 0.003$). A nonsynonymous coding polymorphism, rs238239, in exon 5 of enolase 3 (*ENO3*), near *SLC25A11*, was associated with LP1 ($p = 0.0043$), as well as the rate of blood alcohol absorption (BLAP; $p = 0.0029$).

Allelic Effects on Breath Alcohol Concentration

Total association analyses were conducted for 10 BrAC levels (RP01-RP10), as well as the rate of alcohol absorption in the breath (BRAP), rate of alcohol elimination (BREP), and the peak BrAC (PKRP). Seventeen SNPs associated with one or more of these phenotypes at a significance level of $p < 0.005$ are summarized in Table 4. The rs238247 SNP in the *SLC25A11* candidate region showed evidence of population stratification for 5 BrAC levels (RP06-RP10).

The most associated SNP (rs4838767) with the breath alcohol metabolism variables is the *CYP2E1* promoter SNP, rs4838767 (Table 4). p-Values for 8 traits (RP01-RP07 and peak BrAC [PKRP]) reached a study-wide level of significance ($p < 4.10 \times 10^{-5}$). The observed effect of rs4838767 was strongest for RP04 ($p = 2.00 \times 10^{-7}$) with the minor C allele relating to a lower BrAC; the beta calculated in QTDT = 2.205 and explained approximately 8% of

Table 3. Blood Alcohol Metabolism Results Where Total Association *p*-Value <0.005 were Observed for at Least 1 Trait

SNP[a]	Chr	Position	MAF	Alleles	Candidate region[b]	Gene[c]	Function	Codon	Predicted blood alcohol concentration						Summary parameters		
									LP1	LP2	LP3	LP4	LP5	LP6	BLAP	BLEP	PKLP
rs2017362	9	74,734,211	0.384	C/T	ALDH1A1	*ALDH1A1*	Intron		0.0079	**0.0035**	**0.0020**	**0.0011**	**0.0006**	**0.0010**			**0.0040**
rs348461	9	74,734,890	0.384	A/T	ALDH1A1	*ALDH1A1*	Intron		0.0079	**0.0035**	**0.0020**	**0.0011**	**0.0006**	**0.0010**			**0.0040**
rs348462	9	74,736,989	0.313	C/G	ALDH1A1	*ALDH1A1*	Intron		0.0096	**0.0045**	**0.0021**	**0.0023**	**0.0019**	0.0100			**0.0030**
rs348463	9	74,737,432	0.291	C/T	ALDH1A1	*ALDH1A1*	Intron		0.0120	**0.0049**	**0.0021**	**0.0012**	**0.0007**	**0.0022**			**0.0041**
rs2210103	9	74,741,953	0.291	C/T	ALDH1A1	*ALDH1A1*	Intron		0.0120	**0.0049**	**0.0021**	**0.0012**	**0.0007**	**0.0022**			**0.0041**
rs1330286	9	74,742,773	0.355	C/G	ALDH1A1	*ALDH1A1*	Intron		0.0212	0.0106	**0.0045**	**0.0028**	**0.0018**	0.0055			0.0074
rs647880	9	74,749,638	0.305	A/G	ALDH1A1	*ALDH1A1*	Intron		**0.0024**	**0.0017**	**0.0010**	**0.0022**	**0.0021**	0.0118	0.0411	0.0802	**0.0012**
rs1424482	9	74,753,377	0.365	C/T	ALDH1A1	*ALDH1A1*	Intron		0.0147	0.0086	**0.0039**	**0.0031**	**0.0022**	0.0086			0.0053
rs348447[d]	9	74,765,983	0.253	C/G	ALDH1A1	*ALDH1A1*	Promoter		**0.0043**	**0.0044**	0.0053	0.0203	0.0300		0.0427	0.0317	0.0072
rs6560309	9	74,769,224	0.327	C/T	ALDH1A1				**0.0018**	**0.0014**	**0.0012**	**0.0034**	**0.0036**	0.0198	0.0241	0.0664	**0.0016**
rs4745204	9	74,771,920	0.327	A/G	ALDH1A1				**0.0018**	**0.0014**	**0.0012**	**0.0034**	**0.0036**	0.0198	0.0241	0.0664	**0.0016**
rs918836	9	74,777,539	0.327	C/G	ALDH1A1				**0.0018**	**0.0014**	**0.0012**	**0.0034**	**0.0036**	0.0198	0.0241	0.0664	**0.0016**
rs4838767t	10	135,183,608	0.008	A/C	CYP2E1	*CYP2E1*	Promoter		**0.00004**	**0.00003**	**0.00006**	**0.001**	**0.0042**	0.0269	**0.0017**	0.0109	**0.0007**
rs4646976	10	135,197,717	0.011	A/G	CYP2E1	*CYP2E1*	Intron		**0.0008**	**0.0007**	**0.0009**	0.0139	0.0622		0.0075	**0.0004**	**0.0034**
rs4756146	11	34,420,315	0.145	C/T	CAT	*CAT*	Intron							0.0196		**0.0023**	
rs2300182	11	34,424,424	0.145	A/T	CAT	*CAT*	Intron							0.0196		**0.0023**	
rs2076556	11	34,429,998	0.145	C/T	CAT	*CAT*	Intron							0.0196		**0.0023**	
rs4755374	11	34,443,180	0.145	A/C	CAT	*CAT*	Intron							0.0196		**0.0023**	
rs16925614	11	34,448,885	0.145	C/T	CAT	*CAT*	Intron							0.0196		**0.0023**	
rs17269847	11	34,467,622	0.124	C/T	CAT	*ELF5*	Intron			0.0495	0.0187	**0.0034**	**0.0031**	**0.0030**			0.0623
rs4784971	16	57,285,181	0.162	C/T	GOT2					0.0535	0.0142	**0.0033**	**0.0038**	0.0105			0.0533
rs366577	17	4,795,225	0.375	C/T	SLC25A11	*ENO3*	Intron		**0.0042**	0.0170	0.0628				**0.0038**	0.0419	0.0196
rs238239	17	4,797,326	0.395	C/T	SLC25A11	*ENO3*	Coding exon	A/V85	**0.0043**	0.0183	0.0693				**0.0029**	0.0155	0.0194

BLAP, rate of alcohol absorption; BLEP, rate of alcohol elimination; LP1-LP6, 6 predicted blood alcohol concentration levels; MAF, minor allele frequency; PKLP, peak blood alcohol concentration; SNP, single nucleotide polymorphism.

[a]SNPs genotyped in-house end with the letter "t".

[b]Candidate gene that SNPs were originally selected to represent.

[c]Gene that SNP is physically located in (may not be the candidate gene).

[d]Showed evidence of population stratification with BLAP ($p = 0.032$).

The *p*-value of total association for the SNP is given controlling for sex, age, age^2, sex × age, sex × age^2, and the first 10 eigenvectors (PC1-PC10) from European-only principal components analysis of ancestry. *p*-Values <0.001 are highlighted by shading, values <0.005 are in bold, and values >0.10 are not shown.

Table 4. Breath Alcohol Metabolism Results Where Total Association *p*-Values <0.005 were Observed for at Least 1 Trait

SNP[a]	Chr	Location	MAF	Alleles	Candidate region[b]	Gene[c]	Function	Codon	Predicted breath alcohol concentration										Summary parameters		
									RP01	RP02	RP03	RP04	RP05	RP06	RP07	RP08	RP09	RP10	BRAP	BREP	PKRP
rs7804781	7	75,540,655	0.111	C/T	MDH2	*MDH2*	Downstream											0.0762	0.0754	**0.0043**	
rs11787668	9	74,763,412	0.462	A/C	ALDH1A1	*ALDH1A1*	Promoter		0.0315	0.0209	0.0149	0.0174	0.0269	0.0407	0.0669	0.0906			0.0742	0.0574	**0.0046**
rs2298316	10	101,137,682	0.105	A/G	GOT1	*CNNM1*	Coding exon	R/Q454	0.0501	0.0170	0.0099	0.0074	0.0074	0.0089	0.0220	0.0519	0.0927			0.0194	**0.0030**
rs4838767[†]	10	135,183,608	0.008	A/C	CYP2E1	*CYP2E1*	Promoter		**0.000008**	**0.0000007**	**0.0000003**	**0.0000002**	**0.0000005**	**0.000003**	**0.00001**	**0.0001**	**0.0003**	**0.0019**	**0.00009**	0.0172	**0.000001**
rs4646976	10	135,197,717	0.011	A/G	CYP2E1	*CYP2E1*	Intron		0.0011	**0.0003**	**0.0003**	**0.0002**	**0.0005**	**0.0018**	0.0064	0.0302	0.0606		**0.0027**	**0.0023**	**0.0005**
rs11032682	11	34,400,329	0.442	G/T	CAT				0.0186	0.0235	0.0313	0.0338	0.0373	0.0690	0.0697	0.0883			0.0156		**0.0048**
rs7118388	11	34,410,723	0.482	A/G	CAT	*CAT*	Promoter		0.0056	0.0123	0.0262	0.0428	0.0740						**0.0012**	0.0476	**0.0027**
rs4784975	16	57,305,160	0.004	A/G	GOT2	*GOT2*	Intron								0.0582	0.0291	0.0115	0.0024		**0.0039**	
rs4349206	17	4,770,779	0.369	A/G	SLC25A11	*GP1BA*	Promoter		0.0063	0.0110	0.0243	0.0733							0.0085	**0.0024**	0.0142
rs9914087	17	4,772,572	0.288	A/G	SLC25A11	*GP1BA*	Promoter		**0.0043**	0.0064	0.0135	0.0460							0.0099	**0.0013**	0.0078
rs9903826	17	4,774,260	0.288	A/G	SLC25A11	*GP1BA*	Promoter		**0.0043**	0.0064	0.0135	0.0460							0.0099	**0.0013**	0.0078
rs2243102	17	4,779,893	0.365	C/T	SLC25A11	*GP1BA*	Downstream		0.0075	0.0142	0.0329	0.0966							0.0084	**0.0034**	0.0197
rs238247[d]	17	4,786,513	0.377	A/G	SLC25A11	*RNF167*	Intron		**0.0024**	0.0052	0.0150	0.0600							**0.0033**	**0.0036**	0.0107
rs366577	17	4,795,225	0.375	C/T	SLC25A11	*ENO3*	Intron		0.0089	0.0210	0.0538								0.0076	**0.0038**	0.0395
rs238239	17	4,797,326	0.395	C/T	SLC25A11	*ENO3*	Coding exon	A/V85	0.0100	0.0174	0.0387								0.0115	**0.0026**	0.029
rs8184053	20	24,921,005	0.182	C/T	ACSS1	*C20orf3*	Intron		0.0136	0.0395	0.0864								**0.0022**	0.0591	0.0478
rs1985485	20	24,963,492	0.115	C/T	ACSS1	*ACSS1*	Intron						0.0611	0.0227	0.0108	0.0127	0.0063	**0.0047**			

BRAP, rate of alcohol absorption; BREP, rate of alcohol elimination; MAF, minor allele frequency; RP01-RP10, 10 predicted breath alcohol concentration levels; PKRP, peak blood alcohol concentration; SNP, single nucleotide polymorphism.

[a]SNPs genotyped in-house end with the letter "t".

[b]Candidate gene that SNPs were originally selected to represent.

[c]Gene that SNP is physically located in (may not be the candidate gene).

[d]Showed evidence of population stratification with RP06-RP10 series of breath alcohol concentrations (*p*-values range from 0.0187 to 0.0385).

The *p*-value of total association for the SNP is given controlling for sex, age, age^2, sex × age, sex × age^2, and the first 10 eigenvectors (PC1-PC10) from European-only principal components analysis of ancestry. *p*-Values <0.001 are highlighted by shading, values <0.005 are in bold, and values >0.10 are not shown.

variance in the RP04 BrAC. The effect of rs4838767 genotype on BACs and BrACs is illustrated in Fig. 2. This SNP was also associated with later BrAC measurements (RP08, RP09) and breath alcohol absorption (BRAP). A second SNP in *CYP2E1* (rs4646976) was associated with 8 of these traits but to a lesser degree. A grouping of 6 SNPs on chromosome 17 near *SLC25A11* in moderate to complete LD (r^2 = 0.7 to 1.0) was related to early breath alcohol readings and all the breath alcohol summary measures. This grouping included the nonsynonymous rs238239 polymorphism in *ENO3* that codes for an alanine to valine codon change. Two SNPs in or near *CAT* and *ACSS1* and 1 SNP each in *ALDH1A1*, *MDH2*, and *GOT2* were associated with at least 1 breath alcohol trait with a *p*-value <0.005. Finally, the nonsynonymous SNP rs2298316 codes for an arginine to glutamate codon change in *CNNM1* (near *GOT1*) and was most associated with peak BrAC (p = 0.003).

Multivariate Analyses

Given the (i) high correlation between the BAC and BrAC readings throughout the alcohol challenge and (ii) that the strongest allelic associations with variation in BACs or BrACs tended to be either with early or late stages of alcohol metabolism, we used a multivariate approach to search for SNPs that acted on general blood or breath alcohol metabolism (all times, LP1-LP6 or RP01-RP10) or SNPs that acted early (LP1-LP3 or RP01-RP05) or late (LP4-LP6 or RP06-RP10) in the time course of blood or breath alcohol metabolism. The strongest evidence of association with blood alcohol phenotypes was observed with SNPs located near or within *GOT1* on chromosome 10. One SNP upstream of *GOT1* (rs2490286) reached study-wide significance for general blood alcohol metabolism (multivariate p = 0.000040) and was also associated with late blood alcohol metabolism but to a lesser extent (multivariate p = 0.0019). Similar patterns of association were observed with rs2494654 (0.000057 and 0.00026, respectively) and rs2494652 (0.00018 and 0.0011). Two SNPs downstream of *GOT1* (intergenic rs10748774 and rs6584273 in *CNNM1*) and an intronic SNP in *ALDH1A1* (rs647880) were also nominally associated with general blood alcohol metabolism. With respect to breath alcohol metabolism, the most associated SNPs were located in *CYP2E1* and *ACSS1:* rs4838767 in the promoter of *CYP2E1* was associated with general BrAC levels (multivariate p = 0.00075), the intronic *CYP2E1* SNP rs4646976 was most associated with late BrAC levels (multivariate p = 0.00092), and the intronic *ACSS1* SNP (rs4813543) was strongly associated with late BrACs (multivariate p = 0.000041) but not associated with early BrAC levels (multivariate p = 0.714). Multivariate association results for all SNPs and phenotypes are presented in Table S6 and Fig. S4.

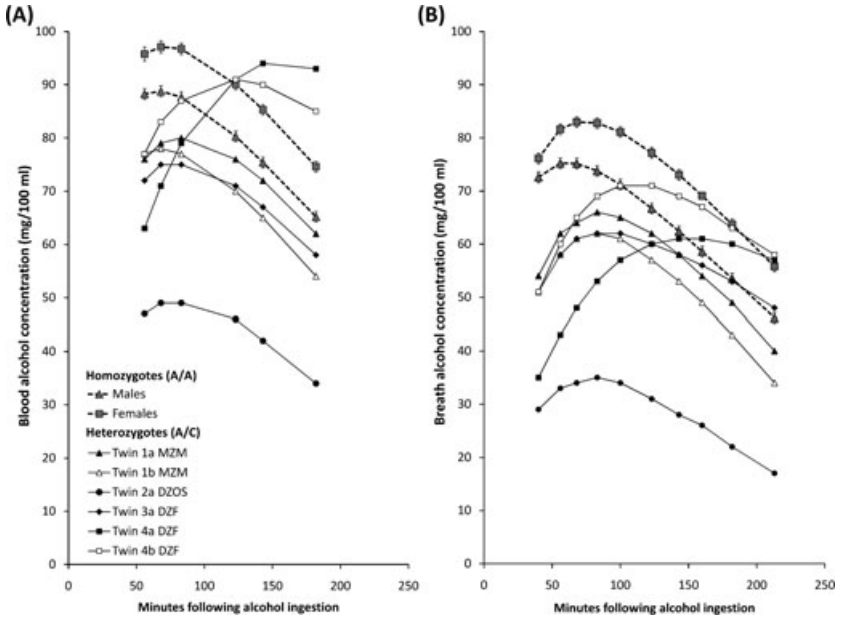

Fig. 2. Effect of *CYP2E1* promoter single nucleotide polymorphisms rs4838767 genotypes on (A) blood and (B) breath alcohol concentration levels following alcohol ingestion. Mean (with standard error bars) alcohol concentrations for male and female homozygous A/A twins plus alcohol concentrations for 6 heterozygous A/C twins are shown. Twins 1a and 1b are a male monozygotic twin pair. Twins 2a and 3a are unpaired female dizygotic twins. Twins 4a and 4b are a female dizygotic twin pair.

DISCUSSION

The object of our study was to identify genetic variants that modulate interindividual variation in the absorption and elimination of EtOH. This is carried out in the context of an alcohol metabolism pathway that includes both the metabolism of the carbon skeleton of EtOH and includes gene products that influence the activity of the malate-aspartate shuttle and rates of mitochondrial NADH reoxidation (Fig. 1). We have previously shown that significant heritability exists for peak blood alcohol (0.62) and rate of elimination (0.49) in our sample (Martin et al., 1985). Linkage and functional studies have pointed toward the role of genetic variation in the ADH cluster and ALDHs (Agarwal and Goedde, 1992; Birley et al., 2005) and association studies have followed up on these findings indirectly by studying the consequences of differing alcohol metabolism, namely subjective feeling of drunkenness, excessive alcohol consumption, or alcohol dependence (Chen et al., 2009; Dickson et al., 2006; Edenberg et al., 2006; Macgregor et al., 2009; Quertemont, 2004). However, most pharmacogenetic studies of alcohol metabolism have focused on a small set of variants in *ADH1B* (Arg48His/rs1229984 and Arg369Cys/rs2066702), *ADH1C* (Ile349Val/rs698 and Arg271Gln/rs1693482), and *ALDH2* (*ALDH2**2/rs671). The role of genes that function in parallel (*CYP2E1* and *CAT*) or downstream of *ADHs* and *ALDH* has, for the most part, not been studied with respect to variation in alcohol metabolism. Therefore, we have comprehensively tested for allelic association between 950 SNPs spanning 14 genes in the alcohol metabolism pathways with a timed series of BAC and BrAC measurements in 352 twins who participated in an alcohol challenge experiment. SNP genotype data were generated from both in-house genotyping and imputation of GWAS genotype data. It is noted that in our data set of 118 SNPs with both in-house generated and imputed GWAS genotypes, discordance rates were on average 2.4% with 4 SNPs (1 in CYP2E1 and 3 in ACSS1) showing discordance rates higher than 10%.

While multiple allelic associations ($p < 0.005$) between the tested SNPs and blood or breath alcohol phenotypes were observed in our sample, the only study-wide significant associations were observed with a promoter SNP in *CYP2E1* (rs4838767). This SNP was genotyped on the Sequenom MassARRAY platform and the assay cluster plot and representative genotype spectrums are given in Fig. S5. Association with rs4838767 was strongest with earlier BrACs (RP01-RP06) and peak BrAC (PKRP), with only suggestive or nominal association with later BrACs and the rate of breath alcohol elimination (BREP). The strongest association was between rs4838767 and RP04 ($p = 2.0 \times 10^{-7}$) explaining ~8% of RP04 phenotypic variance. This SNP was also most strongly associated with early BACs (LP1-LP3) and the peak BAC (PKLP).

The rs4838767 result, however, should be interpreted in the context of several limitations. First, the MAF for rs4838767 is low (~0.9%) in the twin sample where all phenotypes and covariates are available; only 6 heterozygote A/C twins and no homozygote C/C individuals were observed. However, this issue will be encountered in any allelic association analysis of low-MAF SNPs. We therefore obtained empirical *p*-values for rs4838767 at each phenotype by conducting 10,000 or 100,000 simulations in MERLIN (Table S7). The simulate option in MERLIN results in data sets in

which SNP data are simulated under the null hypothesis of no linkage or association with observed phenotypes while phenotypic measurements, including covariates, are preserved. We report study-wide significant empirical *p*-values for RP01, RP02, RP05, and RP06 but *p*-values smaller than those reported for RP03, RP04, and PKRP were not observed following 100,000 simulations. Second, the association finding may be affected by 1 opposite sex DZ female twin ("Twin 2a DZOS" in Fig. 2) who exhibited low alcohol concentrations throughout the course of the experiment. When this twin was excluded, the best *p*-value of association was 0.0002 with RP04. Third, the MAF is higher in non-European populations (ranging from 8 to 17% in the Asian and African HapMap samples). In the ACTS sample, the heterozygote MZ twin pair ("Twin 1a MZM" and "Twin 1b MZM" in Fig. 2) reported Egyptian ancestry for all grandparents. When association analyses were rerun excluding this twin pair, the significance of association remained study-wide significant for RP01-RP05 and PKRP (the *p*-value for RP04 was 2.0×10^{-5}).

Nevertheless, CYP2E1 is a major component of the MEOS which oxidizes up to 10% of EtOH consumed. Furthermore, CYP2E1 contributes up to 60% of EtOH metabolism in heavy drinkers as the enzyme has a higher Km for alcohol than ADH and its activity is induced by chronic alcohol ingestion (Lands, 1998; Lieber, 1994; Tanaka et al., 2000). A recent study (Webb et al., 2011) reported both linkage and association between *CYP2E1* and the level of response to alcohol with the strongest association observed between a promoter SNP, rs10776687, and Subjective High Assessment Scale score. Two other *CYP2E1* SNPs that many association studies have genotyped were included in the current study: CYP2E1*5B (constituting rs2031920 and rs3813867) in the promoter and CYP2E1*1B/rs2070676 in intron 7. CYP2E1*5B has been reported to affect transcription activity of the gene (Hayashi et al., 1991; Watanabe et al., 1994) and enhance both alcohol metabolism and risk for alcohol dependence in Mexican-Americans (Konishi et al., 2003). Similarly, rs2070676 has been associated with increased activity of CYP2E1 in vivo (Haufroid et al., 2001). We did not observe association between the 4 SNPs described above and any phenotypes tested in our study (*p*-values > 0.05), with low LD (rs2070676, $r^2 = 0.116$) or no LD observed between rs4838767 and these SNPs. However, previously reported associations with these SNPs have been inconsistent and inconclusive, possibly due to phenotypic and population differences. It has also been suggested that *CYP2E1* activity is higher in smokers than nonsmokers (Lucas et al., 1995; Schoedel and Tyndale, 2003). While participants were not prevented from smoking during the experimental period, smoking history at the time of the alcohol challenge is available for 411 of the 412 twins: 154 were current smokers, 68 nonsmokers, and 189 ex-smokers. To test for a possible effect of current smoking on alcohol metabolism, secondary analyses of the *CYP2E1* SNPs were performed in which current smoker status (Yes/No) was also included as a covariate. No change in the level or pattern of association was observed (data not shown).

While no other SNP-phenotype associations were study-wide significant, there are suggestive patterns of findings that should be followed up in other cohorts as it would be unwise to dismiss the possibility of a true effect. We have low statistical power to detect an association at the study-wide level in our small sample, and the significance threshold of 4.01×10^{-5} does not take into account the strong prior probability of a true association between some of our genes (in particular *CYP2E1*) and traits of interest. The overall lack of study-wide significant findings may be because there is an upper limit in the effect size associated with any single SNP that can be detected with our comparatively small sample ($N = 352$). While we have 80% statistical power to detect the observed association between rs4838767 and RP04 in our study using $\alpha = 4.01 \times 10^{-5}$ (the study-wide significance threshold), the required sample size almost triples ($N = 804$) when the SNP effect size falls to 3% of the trait variance. Therefore, we cannot exclude associations of smaller effect. However, it should be noted that few alcohol challenge studies currently exist in the literature and also have small sample sizes owing to the practical difficulty in conducting large-scale experiments. Other alcohol challenge studies include 297 adult males (Schuckit et al., 2004), 7 MZ and 7 DZ twin pairs (Vesell, 1972), 19 MZ and 21 DZ male twin pairs (Kopun and Propping, 1977).

Turning to the suggestive associations, there is some evidence for a role of cytosolic ALDH (*ALDH1A1*) in alcohol metabolism, with SNPs in this gene influencing all measurements of BAC and the peak BAC. *ALDH1A1* has previously been reported to be associated with several alcohol-related phenotypes including alcohol consumption levels and alcohol dependence risk in Finns (Lind et al., 2008; Liu et al., 2010) and Southwest California Indians (Ehlers et al., 2004). Next, 5 intronic SNPs in *CAT* (effectively 1 SNP owing to being in complete LD) were the only SNPs tested that achieved a level of significance <0.005 with the rate of blood alcohol elimination in our study. A *CAT* promoter SNP was most associated with the rate of breath alcohol absorption and peak BrAC. Finally, a series of SNPs located near *SLC25A11* (a malate carrier in the malate-aspartate NADH shuttle) on chromosome 17 are associated with aspects of blood and breath alcohol metabolism, including a nonsynonymous coding SNP in *ENO3* that is primarily associated with the rate of blood alcohol absorption and breath alcohol elimination. The role of *ENO3* in alcohol metabolism is unclear as it functions in skeletal muscle and glycogen storage, and it was not selected a priori as a candidate gene for this study (Comi et al., 2001).

Finally, we used a multivariate approach (Ferreira and Purcell, 2009) to search for polymorphisms with effects on multiple blood or breath alcohol traits that could have been missed in our main analysis. One SNP, rs2490286, upstream of the glutamic-oxaloacetic transaminase 1 (*GOT1*) reached study-wide significance for overall blood alcohol metabolism

(Table S6). This SNP was not associated with any univariate BAC or BrAC measure in the main analysis but did show borderline association with the rate of blood alcohol elimination ($p = 0.0068$). *GOT1* is an intriguing candidate gene which has recently been implicated in the level of response to alcohol in a GWAS utilizing 3 variables measured in an alcohol challenge (Joslyn et al., 2010).

In conclusion, to study the genetics of alcohol metabolism it is important to test for effects of variation not only in the well-studied *ADH* and *ALDH2* genes, but in additional genes/pathways involved in the metabolism of EtOH, co-factors (NAD^+/NADH), and breakdown products (acetaldehyde). We suggest that variants that influence alcohol metabolism can be further studied in other alcohol cohorts where their effect on alcohol consumption, and subsequently risk for alcohol dependence, can be investigated. We did not find strong evidence that variation in genes metabolizing the carbon backbone of acetate or in mechanisms for regenerating NAD from NADH or the metabolism of acetaldehyde is rate-limiting in alcohol metabolism in our population. However, we did observe suggestive patterns of association with variants in *ALDH1A1* and on chromosome 17 near *SLC25A11* for aspects of blood and breath alcohol metabolism. Finally, while we lack power to observe small sizes of effect in our sample, we can conclude that regulatory sequences in the promoter of *CYP2E1* may play a role in the preabsorptive metabolism of alcohol; however, more samples are required to validate this finding.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Blood alcohol concentration results. Total association of 950 SNPs spanning 14 candidate genes with a time course of 6 blood (LP1-LP6) alcohol concentration levels following ingestion of a weight-related dose of EtOH.

Fig. S2. Breath alcohol concentration results. Total association of 950 SNPs spanning 14 candidate genes with a time

course of 10 breath (RP01-RP10) alcohol concentration levels following ingestion of a weight-related dose of EtOH.

Fig. S3. Blood and breath summary measure results. Total association of 950 SNPs spanning 14 candidate genes with the rate of alcohol absorption, rate of alcohol elimination and peak alcohol concentration in the blood (BLAP, BLEP and PKLP respectively) and breath (BRAP, BREP, PKRP).

Fig. S4. Multivariate total association of SNPs spanning 14 candidate genes with general blood alcohol metabolism (all times, LP1-LP10), early (LP1-LP3) and late (LP4-LP6) blood alcohol concentration levels, general breath alcohol metabolism (all times, RP01-RP10) and early (RP01-RP05) and late (RP06-RP10) breath alcohol concentration levels following ingestion of a weight-related dose of EtOH.

Fig. S5. Sequenom MassARRAY (a) cluster plot for the *CYP2E1* promoter SNP rs4838767 assay, and (b) spectrums for the heterozygote A/C and homozygote A/A genotypes.

Table S1. Summary of 818 SNPs imputed using genotype data from genome wide association studies.

Table S2. Genotype comparison of 118 SNPs genotyped in-house using the Sequenom MassARRAY platform with imputed genotypes from GWAS data.

Table S3. QTDT total association for all SNPS and all blood and breath phenotypes.

Table S4. QTDT population stratification results for all SNPs and all blood and breath phenotypes.

Table S5. Correlation matrix for all breath and blood phenotypes tested.

Table S6. MQFAM multivariate total association with all SNPs.

Table S7. Empirical *p*-values for association with rs4838767 at each phenotype following genotype simulation analysis in MERLIN.

SUPPLEMENTAL FIGURES

Figure S1. Blood alcohol concentration results. Total association of 950 SNPs spanning 14 candidate genes with a time course of six blood (LP1-LP6) alcohol concentration levels following ingestion of a weight-related dose of ethanol. Results are plotted as $-\log_{10}$(P-value) against the SNP number, with SNPs ordered by chromosome and chromosomal position. Vertical dashed lines separate each candidate gene region, with the location and size of each candidate gene denoted by the solid red horizontal line. The black horizontal dashed line indicates a P-value of 0.005. The red horizontal dashed line indicates the study-wide threshold for significance (P-value = 4.01×10^{-5}).

Figure S2. Breath alcohol concentration results. Total association of 950 SNPs spanning 14 candidate genes with a time course of ten breath (RP01-RP10) alcohol concentration levels following ingestion of a weight-related dose of ethanol. Results are plotted as $-\log_{10}$(P-value) against the SNP number, with SNPs ordered by chromosome and chromosomal position. Vertical dashed lines separate each candidate gene region, with the location and size of each candidate gene denoted by the solid red horizontal line. The black horizontal dashed line indicates a P-value of 0.005. The red horizontal dashed line indicates the study-wide threshold for significance (P-value = 4.01×10^{-5}).

Figure S3. Blood and breath summary measure results. Total association of 950 SNPs spanning 14 candidate genes with the rate of alcohol absorption, rate of alcohol elimination and peak alcohol concentration in the blood (BLAP, BLEP and PKLP respectively) and breath (BRAP, BREP, PKRP). Results are plotted as $-\log_{10}$(P-value) against the SNP number, with SNPs ordered by chromosome and chromosomal position. Vertical dashed lines separate

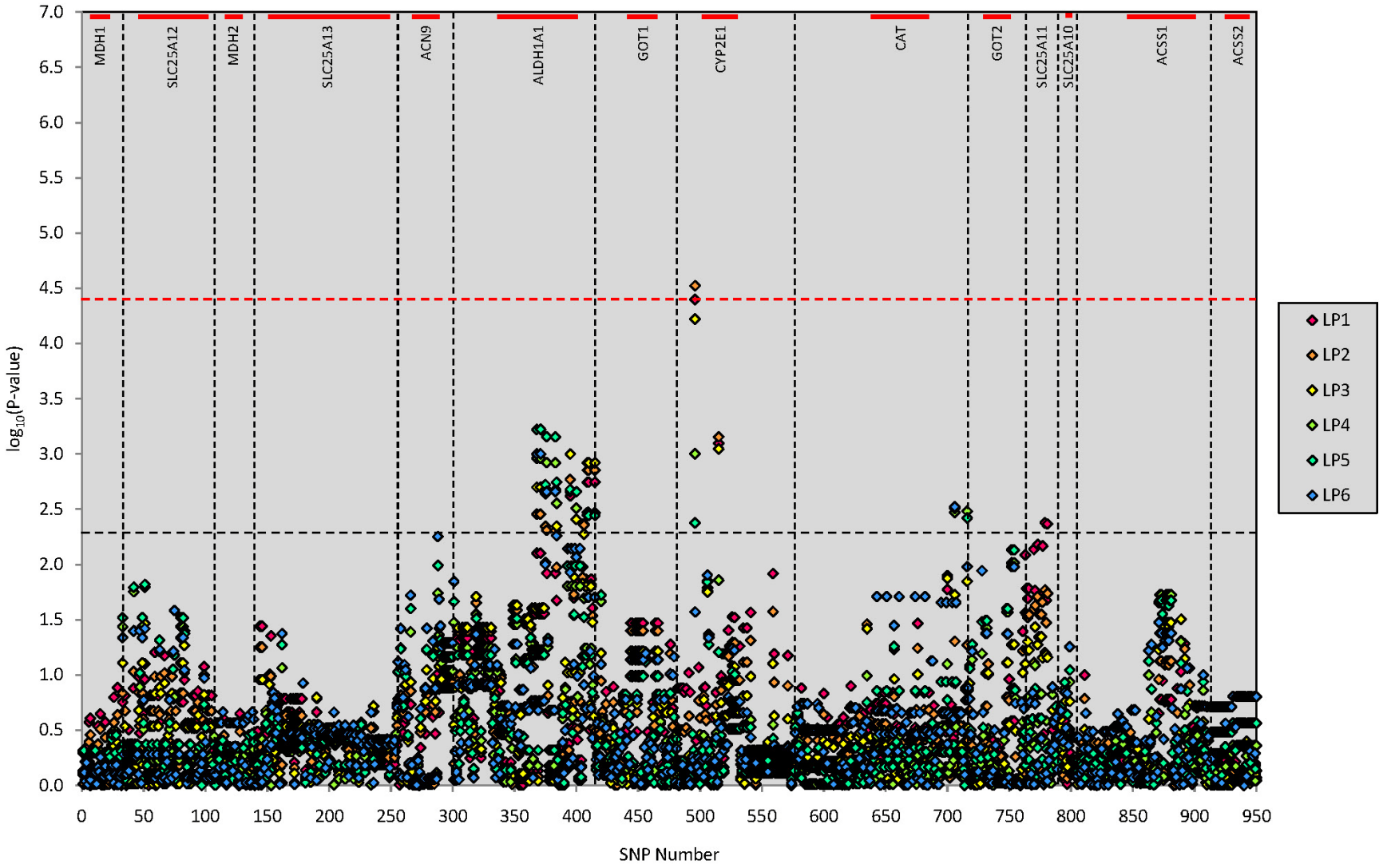
each candidate gene region, with the location and size of each candidate gene denoted by the solid red horizontal line. The black horizontal dashed line indicates a P-value of 0.005. The red horizontal dashed line indicates the study-wide threshold for significance (P-value = 4.01×10^{-5}).

Figure S4. Multivariate total association of SNPs spanning 14 candidate genes with general blood alcohol metabolism (all times, LP1-LP10), early (LP1-LP3) and late (LP4-LP6) blood alcohol concentration levels, general breath alcohol metabolism (all times, RP01-RP10) and early (RP01-RP05) and late (RP06-RP10) breath alcohol concentration levels following ingestion of a weight-related dose of ethanol. Results are plotted as $-\log_{10}$(P-value) against the SNP number, with SNPs ordered by chromosome and chromosomal position. Vertical dashed lines separate each candidate gene region, with the location and size of each candidate gene denoted by the solid red horizontal line. The black horizontal dashed line indicates a P-value of 0.005. The red horizontal dashed line indicates the study-wide threshold for significance (P-value = 4.01×10^{-5}).

Figure S5. Sequenom MassARRAY (a) cluster plot for the *CYP2E1* promoter SNP rs4838767 assay, and (b) spectrums for the heterozygote A/C and homozygote A/A genotypes. The cluster plot graphs each assay result by low mass allele (A) and high mass allele heights (C) on a logarithmic scale. An ideal cluster plot shows homozygous calls clustered along each axis, and heterozygous calls clustered along the diagonal running through the center of the graph. Assay spectra are displayed using an intensity scale (y-axis) and mass (x-axis). The rs4838767 spectrum is annotated with the expected location of allele peaks (both the A and C alleles are coloured yellow in the heterozygote A/C spectrum while the A allele is yellow and C allele is red in the homozygote A/A spectrum) and the

unextended-primer peak (UEP-rs4838767 in red). Note that the unextended-primer and allele peaks from all assays (within the representative mass window) for one DNA sample are shown in the spectrum.

FIGURE S1.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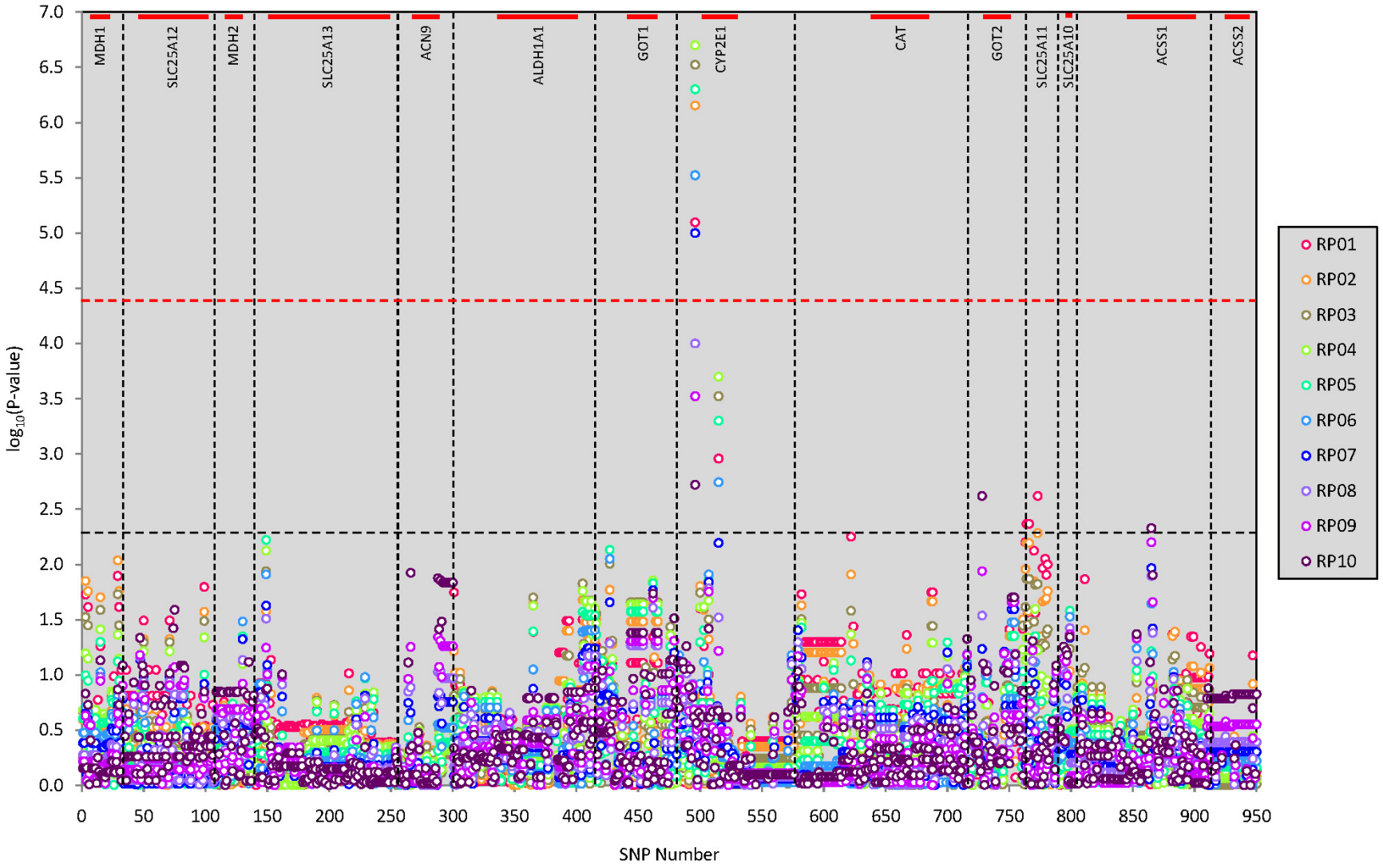
ACN9
ALDH1A1
GOT1
CYP2E1
CAT
GOT2
SLC25A11
SLC25A10
ACSS1
ACSS2
log10(P-value)
SNP Number
LP1
LP2
LP3
LP4
LP5
LP6

FIGURE S2.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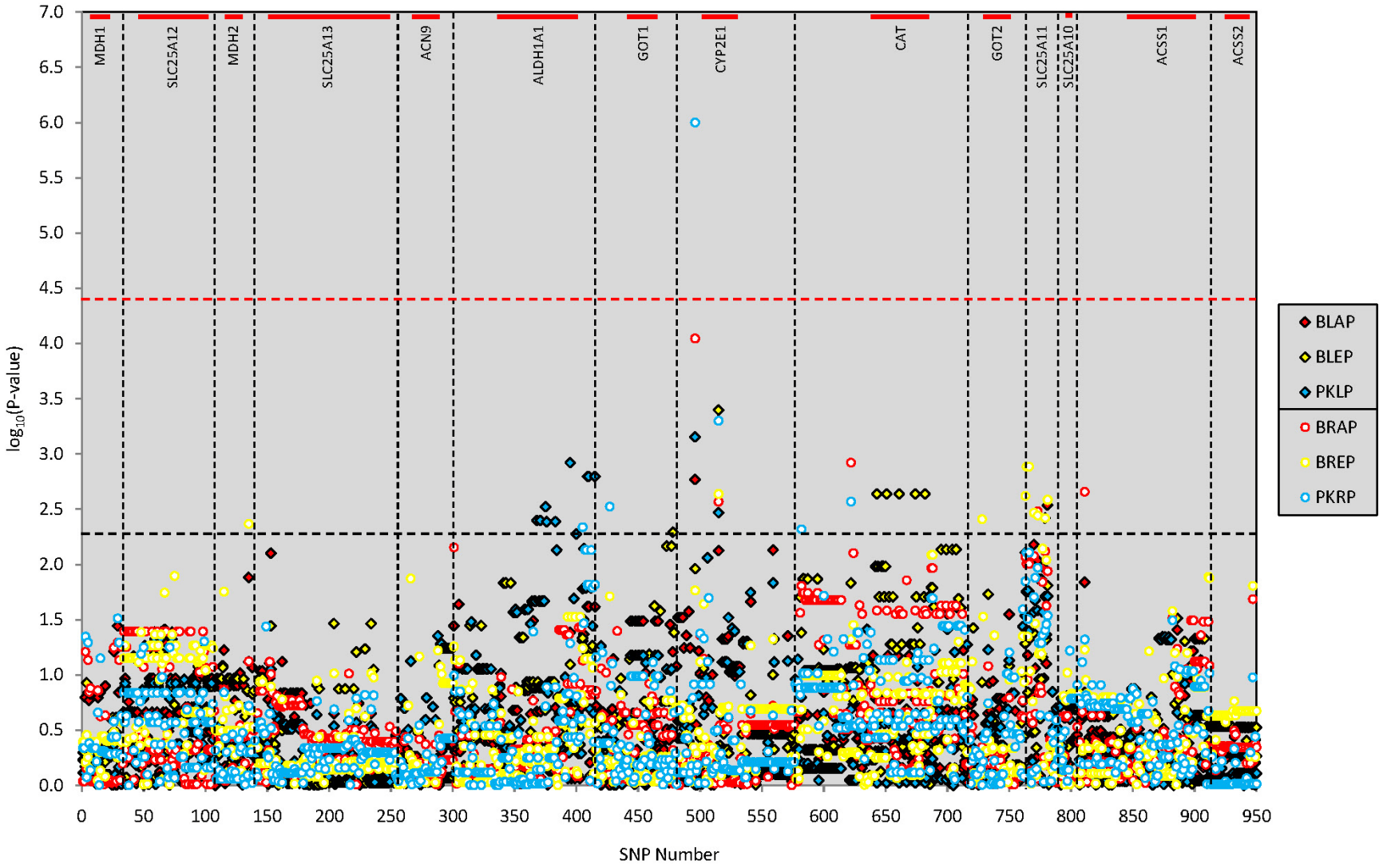
ACN9
ALDH1A1
GOT1
CYP2E1
CAT
GOT2
SLC25A11
SLC25A10
ACSS1
ACSS2
log10(P-value)
SNP Number
RP01
RP02
RP03
RP04
RP05
RP06
RP07
RP08
RP09
RP10

FIGURE S3.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ACN9
ALDH1A1
GOT1
CYP2E1
CAT
GOT2
SLC25A11
SLC25A10
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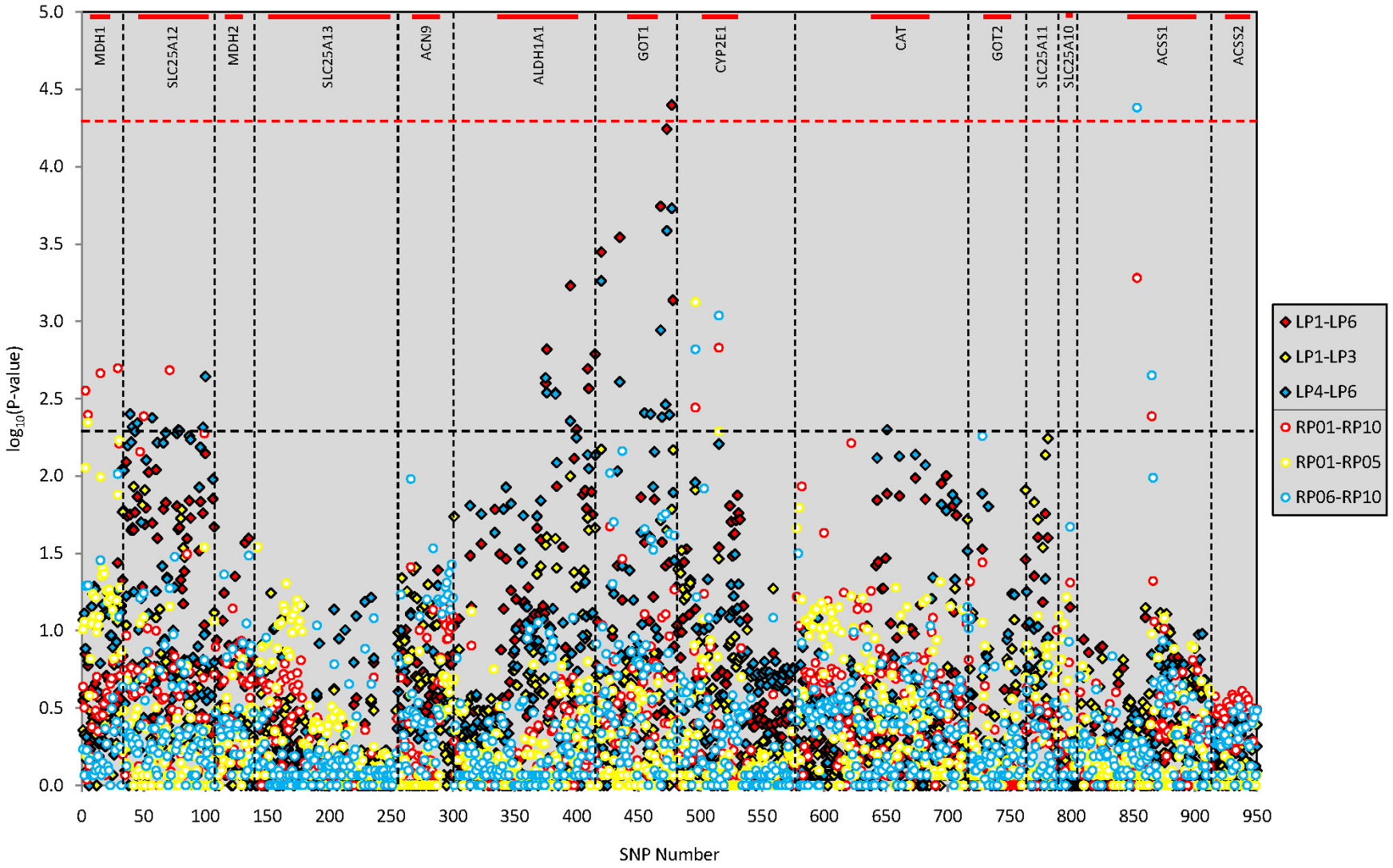
ACSS2
log10(P-value)
SNP Number
BLAP
BLEP
PKLP
BRAP
BREP
PKRP

FIGURE S4.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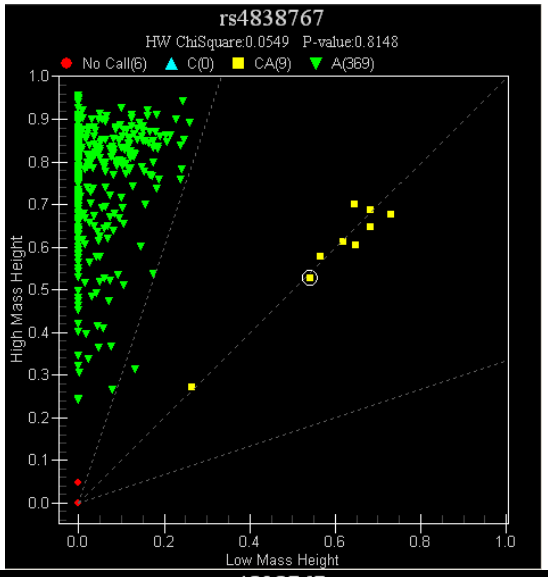
GOT1
CYP2E1
CAT
GOT2
SLC25A11
SLC25A10
ACSS1
ACSS2
log10(P-value)
SNP Number
LP1-LP6
LP1-LP3
LP4-LP6
RP01-RP10
RP01-RP05
RP06-RP10

FIGURE S5.

(a)


rs4838767
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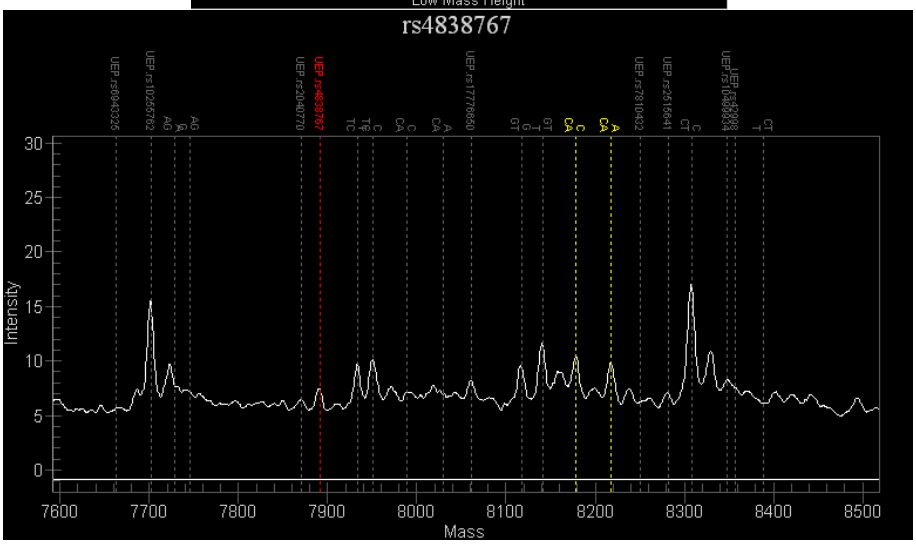
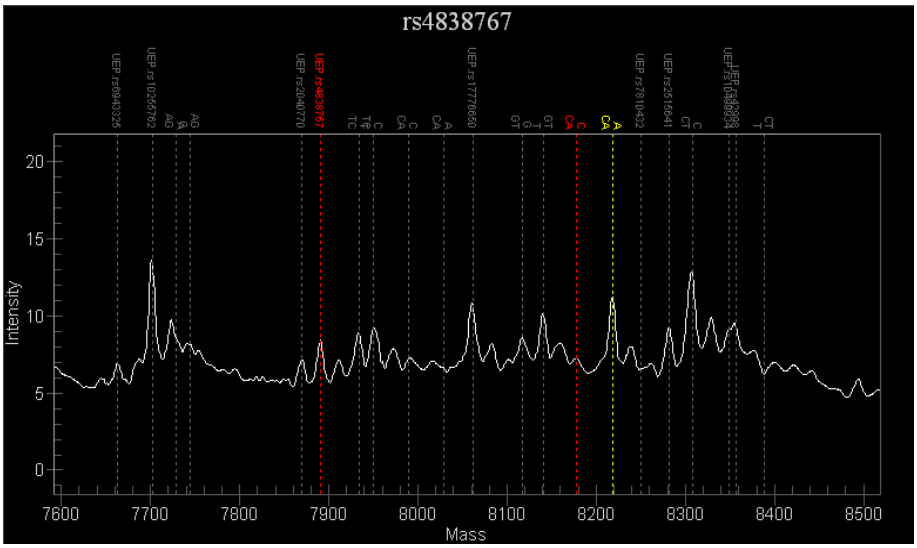
No Call(6)
C(0)
CA(9)
A(369)
High Mass Height
Low Mass Height

(b)


rs4838767
Intensity
Mass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Intensity
Mass