

Effects of Variation at the *ALDH2* Locus on Alcohol Metabolism, Sensitivity, Consumption, and Dependence in Europeans

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Background: The low-activity variant of the aldehyde dehydrogenase 2 (*ALDH2*) gene found in East Asian populations leads to the alcohol flush reaction and reduces alcohol consumption and risk of alcohol dependence (AD). We have tested whether other polymorphisms in the *ALDH2* gene have similar effects in people of European ancestry.

Methods: Serial measurements of blood and breath alcohol, subjective intoxication, body sway, skin temperature, blood pressure, and pulse were obtained in 412 twins who took part in an alcohol challenge study. Participants provided data on alcohol reactions, alcohol consumption, and symptoms related to AD at the time of the study and subsequently. Haplotypes based on 5 single-nucleotide polymorphisms (SNPs) were used in tests of the effects of variation in the *ALDH2* gene on alcohol metabolism and alcohol's effects.

Results: The typed SNPs were in strong linkage disequilibrium and 2 complementary haplotypes comprised 83% of those observed. Significant effects of *ALDH2* haplotype were observed for breath alcohol concentration, with similar but smaller and nonsignificant effects on blood alcohol. Haplotype-related variation in responses to alcohol, and reported alcohol consumption, was small and not consistently in the direction predicted by the effects on alcohol concentrations.

Conclusions: Genetic variation in *ALDH2* affects alcohol metabolism in Europeans. However, the data do not support the hypothesis that this leads to effects on alcohol sensitivity, consumption, or risk of dependence.

Key Words: Aldehyde Dehydrogenase, Haplotype Association, Breath Alcohol, Blood Alcohol, Alcohol Dependence.

THERE IS SUBSTANTIAL evidence that alcohol-related problems and risk of alcohol dependence (AD) are related to alcohol and acetaldehyde metabolism (Chen et al., 1999a, 1999b; Crabb et al., 1995; Thomasson et al., 1991; Yin and Agarwal, 2001). A polymorphism of the aldehyde dehydrogenase 2 (*ALDH2*) gene, *ALDH2***Glu487Lys*, reaches a minor allele frequency of about 30%

in East Asian populations and the corresponding ALDH enzyme has low acetaldehyde-metabolizing activity. The *ALDH2***487Lys* allele (*ALDH2***2*) shows dominance for loss of ALDH activity when heterozygous with the *ALDH2***487Glu* allele (Crabb et al., 1987; Farres et al., 1994; Xiao et al., 1996). East Asian carriers of the *ALDH2***487Lys* allele consume less alcohol and have a decreased risk of becoming alcohol dependent. This *ALDH2*-related protection against drinking problems and risk of AD is mediated through the unpleasant side effects of drinking, including nausea and facial flushing (Harada et al., 1981; Higuchi et al., 1994; Yokoyama et al., 2005). Apart from these physiological and behavioral effects, there is evidence that *ALDH2* deficiency causes a reduction in the rate of alcohol metabolism (Lehmann et al., 1986; Luu et al., 1995; Mizoi et al., 1985; Mizoi et al., 1994; Ueno et al., 1990; Yoshihara et al., 2000).

Even in Europeans, where the *ALDH2***487Lys* allele is not found (Oota et al., 2004), the acute effects of alcohol (alcohol sensitivity or the level of response to alcohol) influence alcohol use and dependence (Heath et al., 1999; Schuckit and Smith, 1997; Whitfield, 1997; Wilhelmsen et al., 2003). Variation in alcohol and acetaldehyde metabo-

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Received for publication November 16, 2005; accepted February 14, 2006.

This work was supported by grants from the National Health and Medical Research Council of Australia, NIAAA (AA007535, AA007728, AA011998, AA013321, AA013326, AA014041) and the Australian Brewers Foundation.

*Peter Dickson died on 15 October 2005. This paper is a memorial to him and an acknowledgment of his work.

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DOI: 10.1111/j.1530-0277.2006.00128.x

lism, including genetic variation in alcohol dehydrogenase (*ADH*) and *ALDH*, may contribute to variation in alcohol sensitivity, although receptor effects may also be important (Hu et al., 2005).

More generally, many theories about alcohol effects, addiction, and the medical consequences of alcohol use propose a key role for acetaldehyde (Eriksson, 2001). These theories are difficult to test directly because even during alcohol metabolism, acetaldehyde concentrations in human blood (except in people with *ALDH2* deficiency) are below the limits of quantitation for current analytical methods. Therefore, genotyping and inferences about alcohol metabolism based on the *in vitro* properties of *ADH* and *ALDH* enzymes have had to supplement or replace direct measurements. If variation in the human *ALDH* gene—apart from *ALDH2*Glu487Lys*—could be shown to affect alcohol metabolism, short-term alcohol effects, alcohol use and dependence, or long-term consequences of alcohol use, this would be strong evidence that acetaldehyde metabolism and acetaldehyde effects are important even at submicromolar concentrations, which we cannot measure reliably. This principle would hold even if the alleles causing such effects are rare. The impact on AD in the population, conversely, will be greater if the alleles are common.

Based on these considerations, we have undertaken single-nucleotide polymorphism (SNP) typing of the *ALDH2* gene in 376 subjects who took part in an alcohol challenge study. A particular strength of this data set is the multiple measurements of blood and breath alcohol, and of sensitivity to intoxication, taken during the alcohol challenge procedure and the longitudinal data on alcohol use and dependence symptoms available for this cohort. Data related to alcohol metabolism, short-term responses to alcohol, and habitual alcohol use and AD have been used to test for *ALDH2* haplotype-associated differences. The expected consequences of a low-activity form of *ALDH2* are shown in Table 1, and these are used to test whether a consistent pattern of effects is observed.

SUBJECTS AND METHODS

Alcohol Challenge Study

Measures of *in vivo* alcohol metabolism, physiological responses to drinking, and self-report evaluation of intoxication were obtained from 412 twins aged 18 to 34 years (mean 23) who took part in the Alcohol Challenge Twin Study (ACTS) in 1979 to 1981 (Martin et al., 1985a, 1985b). Ten timed readings for breath alcohol level and 6 for blood alcohol level were obtained starting 20 minutes after ingestion of alcohol (0.75/kg body weight) (Martin et al., 1985a). Participants gave a subjective assessment of their intoxication at 1, 2, and 3 hours after the start of drinking by giving their response to the question “How drunk are you now?” on a 10-point scale (1 = “quite sober” to 10 = “the most drunk I have ever been”). Physiological measures and tests of motor coordination were also included. Baseline measurements were obtained for heart rate, systolic and diastolic blood pressure, skin temperature, and body sway (eyes open) 40 minutes before alcohol ingestion, and postalcohol measurements

Table 1. Predicted Effects Associated With a Haplotype Containing a Mutation Similar in Its Effects to *ALDH2*2* (*Glu487Lys*).

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1. Aldehyde dehydrogenase 2 (*ALDH2*) gene: insertion/deletion or change in regulatory or coding sequence, occurring on the background of an associated haplotype
 2. *ALDH2* protein: decrease in specific activity or amount leading to decreased enzyme activity
 3. Increased hepatic acetaldehyde concentration during ethanol metabolism
 - 3.1. Effects on *ADH* activity through product inhibition or cofactor availability
 - 3.1.1 *Slower ethanol metabolism and higher blood and breath alcohol concentrations*
 - 3.2. Formation of protein–acetaldehyde adducts
 - 3.2.1 Immune response and tissue damage
 4. Increased systemic acetaldehyde concentration during ethanol metabolism
 - 4.1. *Increase in perceived level of intoxication*
 - 4.1.1 *Decreased alcohol consumption, Lower risk of alcohol dependence*
 - 4.2. *Increase in physiological effects of alcohol*
 - 4.2.1 *Decreased alcohol consumption, Lower risk of alcohol dependence*
 - 4.3. *Alcohol flush reaction*
 - 4.3.1 *Decreased alcohol consumption, Lower risk of alcohol dependence*
-

The predictions that can be tested against our data are italicized.

were obtained at 1, 2, and 3 hours after the start of drinking. Body sway measures were adjusted for height and weight (Martin et al., 1985b).

Alcohol Reactions

A measure of physiological response possibly associated with the effects of acetaldehyde was obtained from a follow-up questionnaire in 1990. Subjects were asked whether they experienced unpleasant reactions after drinking (flushing of the face or body, itching, drowsiness, or palpitations; answered as “always,” “sometimes,” or “never”). A composite score (FA-1, $n = 334$) was constructed that reflected any of 4 symptoms and their frequency in decreasing order of magnitude (“always,” “sometimes,” or “never”).

Data on Alcohol Use and Dependence

Multiple measures of alcohol consumption and symptoms related to AD were obtained over the period 1979 to 1998. We follow the abbreviations previously used by Whitfield et al. (1998), but have extended the set of variables to include additional measures of alcohol consumption or use. The responses to the individual questionnaires were incomplete for these measures, but 86% of the cases had at least 6 measures available. At the time of the initial (I) alcohol challenge study and before drinking the alcohol dose, participants provided 2 measures of consumption (C), namely the number of drinks usually taken in a week (IC-1, $n = 410$) and the frequency and quantity of alcohol use (IC-2, $n = 407$). Alcohol Challenge Twin Study subjects completed a follow-up (F) questionnaire in 1990 to 1992 that provided 3 measures of alcohol consumption: the number of drinks in the previous week (FC-1, $n = 337$), the frequency and quantity of alcohol use (FC-2, $n = 311$), and the number of drinks usually taken in a typical week (FC-3, $n = 338$). They also provided their lifetime symptoms of AD (FD-1, $n = 345$) and the maximum number of drinks ever taken in a day (FC-4, $n = 317$). From 1992 to 1996, most ACTS subjects participated in a larger study that included the structured SSAGA (Semi-Structured Assessment for the Genetics of Alcoholism) interview (Bucholz et al., 1994; Heath et al., 1997);

this provided the number of AD symptoms (SD-1, $n = 337$) and the *Diagnostic and Statistical Manual—Third Edition—Text Revision* (DSM-III-R) diagnosis of AD ($n = 337$). Measures of the quantity and frequency of alcohol use (SC-1, $n = 344$) and the number of drinks consumed in the previous week (SC-2, $n = 247$) were obtained in the 1992 to 1996 study. Two further measures of the weekly number of drinks consumed were obtained for ACTS subjects as part of postal questionnaire studies in 1980 to 1982 (Jardine and Martin, 1984) (FC-5, $n = 261$) and in 1989 (Heath and Martin, 1994) (FC-6, $n = 230$). The data were transformed to $\log_{10}(x+1)$ to reduce skewness in the distributions. All intake and dependence variables were converted to normalized scores for analysis so that the different measures could be compared on a common scale of measurement.

Genotyping

Blood for DNA extraction and SNP genotyping was obtained at the time of follow-up (1990 to 1992) or after the SSAGA interview (1993 to 1995). While ACTS subjects were the main target, available parents and siblings were also asked to provide blood samples to help identify haplotype phase. Genotyping was carried out on DNA from one or both members of 82 monozygotic (MZ) pairs and 106 dizygotic (DZ) complete twin pairs. A further 7 DZ families had only 1 member of the twin pair available for genotyping. The genotypes for MZ pairs were identical and validated the reliability of the genotyping method. Only 25 twin pairs (14 MZ and 11 DZ) were genotyped without either additional nontwin siblings or a parent. In all, 376 of 412 twins (91%) in the original ACTS were genotyped, along with 213 of their nontwin siblings and 228 of 412 parents (55%). Phenotypic data were not obtained from nontwin siblings or parents.

Six SNPs (identified by their dbSNP “rs” number; see Table 2 and Fig. 1) located within and 5' to the *ALDH2* gene (chromosome 12q24.2) were selected for genotyping. The functionally important polymorphism *ALDH2***Glu487Lys* characteristic of East Asian ethnicity was one of them, but the *487Lys* allele was not present in our sample. SNPs were typed with the MassARRAY genotyping platform (Sequenom Inc., San Diego, CA), with an average 0.4% dropout rate and estimated <0.1% genotyping error rate. Tests on parents (where available) and for the entire genotyped population using PEDSTATS (Wigginton and Abecasis, 2005) showed good

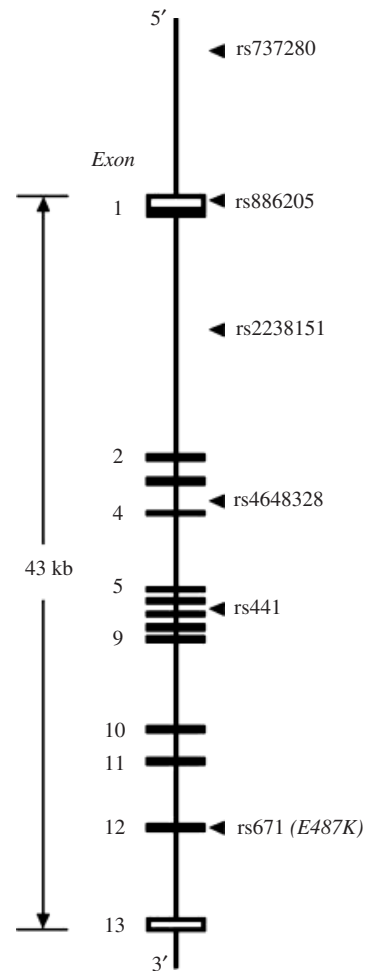


Fig. 1. Intron and exon structure of the aldehyde dehydrogenase 2 (*ALDH2*) gene, showing positions of the typed SNPs.

Table 2. Locations and Population Characteristics of Aldehyde Dehydrogenase 2 (*ALDH2*) Gene Single-Nucleotide Polymorphisms (SNPs).

SNP	Chromosome position	Functional location (bp) ^a	Role	Major allele	Minor allele	Observed minor allele frequency	Heterozygosity	Genotype	AUC ^b	<i>p</i>
rs737280	110,657,696	-9,370	5' Flank	A	G	0.263	0.387	AA AG GG	11928 11911 13194	0.002
rs886205	110,667,147	81	5' UTR	T	C	0.162	0.272	TT TC CC	12002 12039 12774 ^c	0.359
rs2238151	110,674,553	7,487	Intron 1	T	C	0.305	0.424	TT TC CC	11843 11967 13122	<0.001
rs4648328	110,685,508	18,442	Intron 3	C	T	0.157	0.265	CC CT TT	12016 12026 12774 ^c	0.377
rs441	110,691,569	24,503	Intron 6	T	C	0.156	0.264	TT TC CC	12022 12039 12774 ^c	0.176
rs671 (<i>ALDH2</i> *2)	110,704,486	37,420	Exon 12	G	A	0	0	GG	NA	NA

^aPosition relative to transcription start site at 110,667,066 on Chromosome 12 (NCBI Build 35 version 1, June 2004).

^bAUC, area under the concentration–time curve for breath alcohol, for the time interval 40 to 213 min.

^cNote that for SNPs rs886205, rs4648328, and rs441 the values of AUC are identical for the rare homozygotes. This is because of the high linkage disequilibrium: the rare homozygote group comprises the same individuals at all 3 SNPs.

agreement with Hardy–Weinberg equilibrium for all 5 polymorphic SNPs.

Tests of Allelic Effects

A maximum likelihood method, implemented in the software Mx 1.54 (Neale, 1999), was used to estimate jointly the effects of individual SNPs or haplotypes in the means part of the model of the multivariate likelihood equation as well as components of the residual phenotypic covariance. The categorical variables AD and FA-1 were analyzed using the ordinal data option. The expected model for the covariance structure was parameterized for MZ and for DZ twins following the classical model for additive genetic (A) and both familial (C) and individual-specific environmental (E) effects, and hypotheses about means were tested in the context of this random effects model. As the focus of this paper is on the fixed effects of *ALDH2* polymorphisms, random effects variance components are not reported here (but see Martin et al., 1985b). Spurious allelic association due to population admixture was tested for by partitioning the effect for overall association into within-family and between-family effects (Fulker et al., 1999). The effects of the within-sib-pair and between-sib-pair components of genetic association were found to be homogeneous in all tests, suggesting that stratification is not a concern; hence we report only the results of tests for the overall associations.

Multivariate analysis of genetically correlated phenotypes (the multiple measures of blood or breath alcohol, of physiological responses to alcohol, and of alcohol intake and dependence symptoms) was performed as an extension from the univariate analysis implemented in Mx (Birley et al., 2005). The strength of this approach is that the repeated measures reduce the error variance and increase power to detect allelic or haplotype association. Tests for haplotype effects were carried out in stages by equating their effects across time (for repeated measures), equating effects of haplotypes within 2 major groups (Group 1 and Group 2, defined below), and finally equating values for Group 1 and Group 2. At each stage, the change in goodness of fit between the data and the model was assessed using the chi-square test.

RESULTS

Linkage Disequilibrium and Haplotype Frequencies

Substantial linkage disequilibrium between the typed SNPs was confirmed (Table 3). Seven haplotypes were detected, of which 3 (ATTCT, GTCCT, and GCCTC) had a frequency over 10%. These 3 accounted for 95% of observed haplotypes. Similarly only 3 main haplotypes (at frequencies > 10%) were observed by Oota et al. (2004) using 4 *ALDH2* SNPs in 38 populations excluding East Asians.

The strong linkage disequilibrium for the 5 polymorphic SNPs identifies 2 main haplotypes in our sample, one of which (ATTCT) shows complete association for all 5 com-

Table 3. Pairwise Coefficients (*D'*) of Linkage Disequilibrium for the 5 Aldehyde Dehydrogenase 2 (*ALDH2*) Gene Single-Nucleotide Polymorphisms

	rs886205	rs2238151	rs4648328	rs441
rs737280	0.861	0.869	0.892	0.904
rs886205		0.902	0.979	0.916
rs2238151			0.937	0.860
rs4648328				0.917

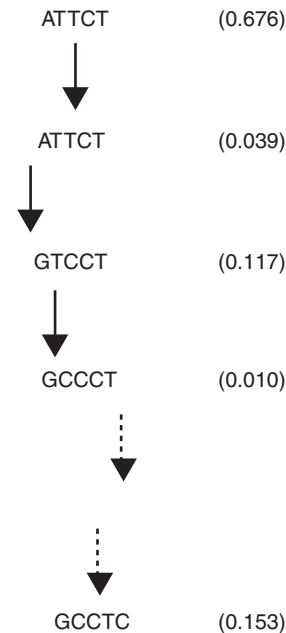


Fig. 2. Haplotype evolutionary pathway representing the most parsimonious transitions from the ATTCT to the GCCTC haplotype. Arrows indicate the positions of single-nucleotide mutational steps of haplotype sequence composition. Haplotype frequencies (in parentheses) in this sample show 3 haplotypes at frequencies > 10%. Very rare haplotypes (frequencies < 0.005) are not shown. Monozygotic twins take identical haplotypes. We were unable to infer 29 of 412 haplotypes because of incomplete family information.

mon alleles and the other (GCCTC) complete association for the rare allele at all 5 loci (Fig. 2). This “yin yang” haplotype architecture is characteristic of 75% to 85% human gene coding regions (Zhang et al., 2003) and was used in tests of the effects of diplotypes that captured the dominance relationships identified by preliminary analysis of individual SNPs. (Diploypes are the haplotype equivalent of a genotype for a single polymorphism and consist, in this case, of the 5-base combination for each of the 2 chromosomes.)

The implied pathway of evolutionary change in haplotype sequence (Fig. 2) was used to construct a series of tests for haplotype effects. Haplotype GCCTC represents the complete association of all rare alleles at the 5 SNP loci. We postulate that a recessive mutation causing variation in ALDH activity has arisen within haplotypes other than ATTCT. This mutation must be in linkage disequilibrium with our set of SNPs and associated with the GCCTC haplotype. Under this model, all diplotypes with 1 copy of the ATTCT haplotype are classified as Group 1 diplotypes. Heterozygous diplotypes involving the GCCTC haplotype but excluding the ATTCT haplotype (classified as Group 2 diplotypes) will be recessive to Group 1 diplotypes in their effects.

Effects on Alcohol Metabolism

An initial inspection of alcohol levels in the time series from breath and blood alcohol levels for genotypes at each

of the 5 SNP loci showed that the effect of the rarer SNP alleles was always recessive and in the direction of higher alcohol levels. This is summarized in Table 2, which shows the mean values for area under the concentration–time curve (AUC) for breath alcohol, by genotype at each SNP. The frequency of the minor allele for SNPs rs737280 and rs2238151 was highest and these SNPs therefore showed the strongest effect.

We expect the effect of Group 1 diplotypes (heterozygous with the common haplotype) to be small and that it can be dropped from the model; i.e., its effect will be similar to that of the ATTCT/ATTCT diplotype. Conversely, we expect the effects for Group 2 diplotypes to be homogeneous and to be an estimate of the effect of the postulated recessive mutation. We have used this model as a test of consistency of gene effects identified by our diplotype groupings in the analysis of all the alcohol-related phenotypes.

The effects of either diplotype group on breath or blood alcohol levels were independent of sample measurement times and the estimated effect sizes could be constrained equal over measurement times (Table 4). There was no evidence of a difference between the homozygous diplotype ATTCT/ATTCT and diplotypes from Group 1 in either breath or blood alcohol levels; effect sizes were -0.33 and -1.08 mg/100 ml, respectively, compared against the homozygous ATTCT/ATTCT diplotype. There was a strong effect of diplotype Group 2 on breath alcohol levels with an effect size of 6.04 mg/100 ml, $p = 0.0005$ (again, compared against ATTCT/ATTCT). The effect of Group 2 diplotypes on blood alcohol concentration was in the same direction, but smaller (2.72 mg/100 ml, averaged across all times) and not statistically significant ($p = 0.103$). For both breath and blood, the Group 2 diplotypes were associated with higher alcohol levels (Fig. 3).

Self-reported Intoxication

The intoxication reports at 1 and 2 hours after alcohol intake corresponded to times when alcohol levels were greatest, and the effects of the haplotype groups on self-report intoxication were largest at 1 hour. Analysis for

Table 4. Model Fits for the effects of Diplotype on the 10 Timed Measurements of Breath Alcohol and 6 Measures of Blood Alcohol Level

Model	Compared with	Breath alcohol			Blood alcohol		
		$\Delta\chi^2$	df	p	$\Delta\chi^2$	df	p
1	–	65.50	80	0.879	53.23	48	0.280
2	1	26.82	18	0.082	7.16	10	0.711
3	2	0.07	1	0.791	0.66	1	0.417
4	3	12.18	1	0.0005	2.66	1	0.103

Model 1: diplotype effects within groups and within measurement times constrained equal; Model 2: diplotype effects within groups constrained equal across measurement times; Model 3: effects of Group 1 diplotypes set to 0; Model 4: Effects of Group 2 diplotypes set to zero.

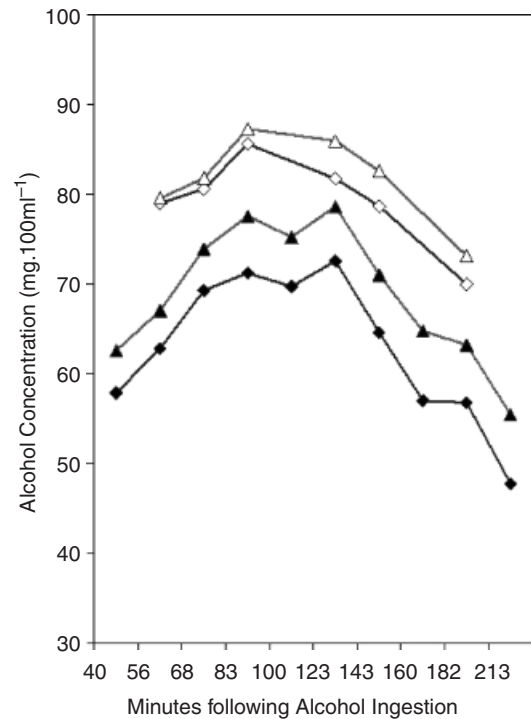


Fig. 3. Mean blood and breath alcohol concentrations by diplotype groups. Breath readings (open symbols) and blood readings (closed symbols) are corrected for the effects of sex and age. Group 2 (see text) diplotypes (Δ) are contrasted against the mean of all other diplotypes (\diamond).

effects of haplotypes, following the procedure described for alcohol levels, did not reveal any heterogeneity of effects within diplotype groups and measurement times. The results showed a clear reduction of mean level of self-reported drunkenness with measurement time (as expected) and some effects associated with Group 2 diplotypes. As both effect size and alcohol level were greatest at the first measurement time, we consider this to be the most appropriate time for the measurement of diplotype effects. The effect size for Group 2 diplotypes at 1 hour postalcohol was -0.78 (lower intoxication ratings in Group 2), with an associated $\chi^2_1 = 2.85$ ($p = 0.09$).

Skin Temperature, Pulse Rate, Systolic and Diastolic Blood Pressure, and Body Sway

The physiological effects of alcohol were measured through skin temperature, systolic and diastolic blood pressure, and standing steadiness or body sway. The effect of sex was included in the means model. Diplotype effects were again investigated by testing for homogeneity of effects within the 2 diplotype groupings and within times and also between diplotype groups. None of these comparisons was statistically significant. The effect of changes of diplotype effect with time was tested by equating the estimates of diplotype association across times within diplotype groupings and between diplotype groupings. Once again there was no evidence of an effect of diplo-

types. The pooled effect sizes for Group 2 diplotypes were all small and only 1 comparison of 60 tests was nominally significant, namely that for homogeneity of haplotype groups for body sway ($\chi^2_1 = 4.68, p = 0.03$).

Alcohol Reactions

Haplotype groupings were internally homogeneous in their effect on alcohol reactions. Although the mean score for subjects with Group 1 diplotypes was not statistically significantly different from that for the ATTCT/ATTCT reference group (effect size = -0.02), the effect of Group 2 diplotypes (effect size = 0.48) was at borderline significance ($p = 0.052$). Hence Group 2 diplotypes were associated with a slightly increased prevalence of alcohol-related reactions.

Effects on Alcohol Consumption, Symptoms, and AD

Phenotypic correlations among the 12 measures of alcohol intake and 2 measures of dependence-related symptoms were generally within the range 0.5 to 0.7, consistent with findings from a larger study (Whitfield et al., 2004). The effects of diplotypes were homogeneous across the multiple occasions of assessment and between the various measures of alcohol intake and dependence symptoms. Group 2 effects were slightly greater than those for Group 1 (i.e., higher intake and more symptoms), but the difference was not significant ($p = 0.292$). There was no significant effect of the *ALDH2* diplotype groups on AD.

DISCUSSION

We investigated the effects of variation in *ALDH2* because it is a clear candidate gene for AD and sensitivity to alcohol's effects. It codes for the second enzyme in the alcohol metabolic pathway, and the enzyme deficiency resulting from the *ALDH2**2 allele is associated with protection from AD in East Asians. We hypothesized that *ALDH2* genetic variation in other populations (and, in principle, among *ALDH2**1 homozygotes in Asia) may produce lesser degrees of activity reduction and acetaldehyde accumulation, which would affect subjective and objective responses to alcohol, and ultimately alcohol use and dependence. Because of reports of *ALDH2* deficiency affecting blood alcohol as well as blood acetaldehyde concentrations, we investigated *ALDH2* haplotype and diplotype effects on blood and breath alcohol. The postulated effects of an *ALDH2* haplotype associated with decreased *ALDH2* activity are shown in Table 1 and form the basis for evaluation of our results.

Our major finding is that a group of diplotypes, characterized by the GCCTC pattern of SNP types and the absence of ATTCT, are associated with higher breath alcohol, and possibly with higher blood alcohol, concentrations after a standardized dose of alcohol. We propose that a variant of *ALDH2* associated with these Group

2 haplotypes has an effect on ethanol oxidation. It is important to note that this *ALDH2* effect is homogeneous across time (see Fig. 3); hence it must affect the early phase of alcohol metabolism in the stomach or in the first pass through the liver, rather than the subsequent linear decline in alcohol concentrations. It should also be noted that because of substantial linkage disequilibrium between the SNPs typed, differences in the p values (Table 2) cannot be used to infer the exact location of the causative variant.

This effect on blood and breath alcohol concentrations is consistent with data on the effects of the *ALDH2***Glu487Lys* polymorphism in Asians (Lehmann et al., 1986; Luu et al., 1995; Mizoi et al., 1985; Mizoi et al., 1994; Ueno et al., 1990; Yoshihara et al., 2000). Although some of these reports are on few subjects, and there is diversity in the measures of alcohol metabolism reported (blood and breath alcohol concentrations, peak blood alcohol, AUC, β_{60} , or whole-body ethanol metabolic rate), they are consistent in finding that *ALDH*-deficient states are associated with a decrease in alcohol metabolism. In addition, pharmacological inhibition of *ALDH* in people without a genetic deficiency in enzyme activity has been shown to produce a small ($\sim 10\%$) but significant decrease in the rate of alcohol metabolism (Jones et al., 1988). Therefore, the association that we have found between *ALDH* haplotype or diplotype and breath or blood alcohol concentrations is consistent with an *ALDH* variant occurring in Europeans and affecting acetaldehyde metabolism. The mechanism leading to an influence of *ALDH2* upon the action of *ADH* in the first step of ethanol oxidation is unclear, but may involve product inhibition of *ADH* by acetaldehyde or a mass-action effect on the rate of the forward (ethanol to acetaldehyde) reaction (Umulis et al., 2005).

Raised acetaldehyde levels due to a variant of *ALDH2* should lead to an increased perception of acetaldehyde through flushing and related characteristics and (if the concentration is high enough to be aversive) to reduction in alcohol consumption and risk of AD. These were tested through physiological and psychomotor measurements during the alcohol challenge and by the participants' subsequent reports of alcohol reactions, alcohol intake, and AD. We therefore have a series of predictions based on the known effects of the Asian *ALDH2**2 polymorphism and with the direction of effects for each haplotype group predicted by the observed significant effects on breath alcohol. However, the effects on these downstream measures of *ALDH2* variation (summarized in Table 5) were at best marginally significant and were sometimes in the opposite direction to our predictions. We have to conclude that *ALDH2* variation in Europeans does not have a major effect on alcohol sensitivity, use, or dependence.

Although our 2 findings may appear to be in conflict, consideration of the probable relationship between intracellular acetaldehyde concentration and the rate of alcohol metabolism suggests that a decrease in rate of 10% could

Table 5. Estimated Effect Sizes for Group 1 and Group 2 Diplotypes at the Aldehyde Dehydrogenase 2 (*ALDH2*) Gene Locus and Their Contribution to the Genetic Variance for Each of the Listed Phenotypes

Alcohol-related phenotype	Direction of Group 2 effect		Effect size (Group 1)	Effect size (Group 2)	% of genetic variance
	Predicted	Observed			
Breath alcohol concentration ^a	+	+	+0.02 mg/100 ml	+6.04 mg/100 ml	4.5
Blood alcohol concentration ^a	+	+	-1.08 mg/100 ml	+2.72 mg/100 ml	3.2
Self-report intoxication ^b	+	-	+0.18	-0.78	0.9
Flushing-related symptoms	+	+	-0.02	+0.48	7.2
Drinking habits and problems ^a	-	+	-0.02	+1.16 ^c	0.8
Alcohol dependence ^d	-	+	-0.18	+0.25	16.7

The effect sizes for Groups 1 and 2 are calculated as the differences from the reference diplotype ATTCT/ATTCT.

^aMean effect size and percentage contribution to the total genetic variance for all timed measures of either breath or blood alcohol concentration and over all 12 measures related to alcohol consumption or symptoms of alcohol dependence.

^bEffects at the time of peak alcohol concentration (see text).

^cMean standardized deviation of 12 variables showing consistent direction of effect.

^dNumbers (AD+, AD-) are baseline diplotype (30, 123); Group 1 diplotypes (23, 115); and Group 2 diplotypes (9, 31).

be caused by an increase in acetaldehyde concentration of around 10%. Such an increase would have only small effects on subjective perceptions of intoxication, physiological measurements, alcohol use, and dependence risk. The change in alcohol metabolism is however large enough to be detected by this experimental protocol and our methods of data analysis.

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