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ADH Genotype Does Not Modify the Effects of Alcohol on High-Density Lipoprotein

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Background: Alcohol consumption has beneficial effects on mortality which are mainly due to reduction in cardiovascular disease. These are believed to be due, at least in part, to the increase in plasma high-density lipoprotein (HDL) which is associated with alcohol consumption. It has been proposed that *ADH3* genotype modifies the relationships between alcohol intake and cardiovascular disease by altering the HDL response to alcohol. The aim of this paper was to test for effects of *ADH2* and *ADH3* genotypes on the response of HDL components to habitual alcohol consumption.

Methods: Adult male and female subjects were genotyped for *ADH2* and *ADH3*; and plasma HDL cholesterol, apolipoprotein A-I, and apolipoprotein A-II were measured. Nine hundred one subjects had both *ADH2* and *ADH3* genotypes and HDL cholesterol results, while 753 had both genotypes and all three lipid results. The effect of alcohol intake on the three measured HDL components, and a factor score derived from them, was estimated for each of the *ADH2* and *ADH3* genotype groups.

Results: All the measured components of HDL increased with increasing alcohol consumption over the range of intakes studied, 0–4 drinks per day. There were no significant interactions between alcohol consumption and *ADH2* or *ADH3* genotypes.

Conclusions: The concept that alcohol dehydrogenase genotype and alcohol metabolic rate modify the effects of alcohol on plasma HDL concentration is not supported by our results.

Key Words: High-Density Lipoproteins, HDL, *ADH* Genotype.

THE RELATIONSHIP BETWEEN alcohol use and mortality, particularly cardiovascular mortality, has been the subject of many studies. Mortality among older subjects is lower in drinkers than abstainers, up to an intake around four standard drinks or 40 g of ethanol per day for men (Thun et al., 1997; White, 1999). Above this level, and in younger subjects whose cardiovascular risk is relatively low, alcohol-specific causes of mortality may outweigh cardiovascular benefits. Plasma high-density lipoprotein (HDL), usually measured as HDL cholesterol (HDL-C), confers protection against atherosclerosis (Maron, 2000) and increases with increasing alcohol consumption. This is at least partly responsible for the protective effect of alcohol on cardiovascular disease (Gaziano et al. 1993; van Tol and Hendriks, 2001).

Many authors have considered the possibility that the effect of alcohol on HDL, or on cardiovascular disease, may vary between people. If characteristics which predict benefit or lack of benefit from drinking could be defined, this would provide insight into protective mechanisms and allow improved individual recommendations to be made. Genetic polymorphisms at several loci [cholesterol ester transfer protein (Fumeron et al., 1995; Gudnason et al., 1997), apolipoprotein E (Liinamaa et al., 1997), aldehyde dehydrogenase (Nakamura et al., 2002), and alcohol dehydrogenase (*ADH*) (Hines et al., 2001)] have been reported to modify the alcohol-HDL relationship. The report that *ADH3* (*ADH1C*) genotype affects both cardiovascular mortality and HDL responses to alcohol use (Hines et al., 2001) is of particular interest because it suggests that alcohol metabolism, rather than any particular type of alcoholic beverage or individual characteristics related to drinking habits, leads to the observed effects. However, a more recent paper that classified drinkers by *ADH2* (*ADH1B*) type (Hashimoto et al., 2002) reported no significant difference in HDL by genotype, and the in vitro properties of *ADH2* enzymes suggest that any effect should be larger than for *ADH3*.

In this paper, we consider the relationships between alcohol intake, the *ADH2* and *ADH3* polymorphisms, and plasma HDL concentration in adult male and female sub-

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Table 1. Correlations Between Measures of Alcohol Intake (Log-Transformed) and of High-Density Lipoprotein

	Alcohol intake: Past 7 days	Alcohol intake: Usual quantity × frequency	HDL-C	Apo A-I	Apo A-II	Factor score
Alcohol intake: past 7 days	–	0.80	0.19	0.26	0.38[(a)]	0.33
Alcohol intake: usual quantity × frequency	0.75	–	0.17	0.21	0.35[(a)]	0.30
HDL-C	0.25	0.23	–	0.67	0.36	0.81
Apo A-I	0.24	0.22	0.70	–	0.48	0.89
Apo A-II	0.21	0.22	0.34	0.46	–	0.72
Factor score	0.28	0.27	0.84	0.90	0.68	–

[(a)] Apo A-II correlation with alcohol intake is significantly different from HDL-C correlation, $p < 0.0001$.

The factor score is based on HDL-C, Apo A-I, and Apo A-II results. Values for men ($n = 1026$) are above the diagonal and for women ($n = 2102$) below the diagonal. All correlations are $p < 0.001$ except HDL-C with alcohol intake in men, where $p < 0.01$.

jects of predominantly European descent. Three components of HDL [HDL-C, apolipoprotein A-I (apo A-I), and apolipoprotein A-II (apo A-II)] were measured, and a factor score from combining these three results was calculated.

SUBJECTS AND METHODS

Characteristics of the subjects participating in this study were described in a previous paper (Whitfield et al., 1998a). They completed a postal questionnaire in 1988–89 (Heath et al., 1994), a telephone interview in 1993–94 (Heath et al., 1997), and provided a blood sample in 1991–96. All subjects were twins, born between 1903 and 1964 and living in Australia, but in some cases only one member of a twin pair provided blood. Subjects gave informed consent to the questionnaire, interview, and blood collection and the studies were approved by appropriate Ethics Committees. Blood was collected from 1134 men and 2241 women. The mean age at blood collection was 45 years (range, 30–84; SD ± 11) for men and 46 years (range, 29–92; SD ± 12) for women.

At the blood collection visit, the subjects' height and weight were measured, and body mass index (BMI) was calculated from weight and height as weight(kg)/[height(m)]^2. In eight subjects whose height and weight were not recorded at that time, data supplied by them in the 1988–89 questionnaire were used for calculation of BMI.

Information on alcohol intake was available from two sources. The telephone interview included questions about usual frequency and quantity of alcohol use, from which average weekly consumption was calculated. Immediately before blood collection, subjects filled in a brief questionnaire which included a table asking how many drinks containing alcohol (10 g) they had taken on each of the preceding 7 days, and the numbers of drinks were summed to obtain a total for the past week. The geometric means for men's weekly alcohol intake were five drinks (7-day recall method) and four drinks (usual quantity and frequency method), while the corresponding values for women were two drinks per week by either method.

METHODS

Serum was separated from the blood and stored at −70°C until analyzed. HDL cholesterol (HDL-C) was measured by precipitation of non-HDL lipoproteins with dextran/$MgSO_4$ followed by enzymatic cholesterol assay using Boehringer Mannheim (Indianapolis, IN) reagents and methods on a Roche Cobas Fara analyser (Basel, Switzerland). Apo A-I and apo A-II were measured by immunonephelometry using a Behring nephelometer (Dade-Behring, Marburg, Germany) and Behring reagents. HDL-C was measured within a month of sample collection, as the samples became available, while apolipoproteins were measured in larger batches after the blood collection phase had been completed. The analytical precision of these methods was monitored by inclusion of control samples with each batch, giving standard deviations of 0.028 and 0.030 mmol/liter (mean, 0.94 and 1.42 mmol/liter; coefficients of variation, 2.9% and 2.1%; for low and high controls, respectively) for HDL-C; 0.114 g/liter (mean, 1.494 g/liter; coefficient of variation, 7.6%) for apo A-I; and 0.013 g/liter (mean, 0.365 g/liter; coefficient of variation, 3.7%) for apo A-II.

ADH2 and *ADH3* genotypes were ascertained by amplification of DNA from blood samples, restriction digestion, and electrophoretic separation of the digested amplification products, as previously described (Whitfield et al., 1998b). Of the 901 subjects with both *ADH2* and *ADH3* genotypes and plasma HDL-C values, 850 were *ADH2*11* and 51 were *ADH2*12*. Two hundred seventy-one were *ADH3*11*, 469 *ADH3*12*, and 161 were *ADH3*22*. One *ADH2*22* subject was found but was not included in the analysis, so that the *ADH2* analysis only contrasted the *ADH2**11 homozygotes with the heterozygotes. Numbers of subjects with complete genotype and covariate data are shown for each of the HDL-related variables (HDL-C, apo A-I, apo A-II, and factor score) in Table 2. Information on smoking status, taken from the 1988–89 questionnaire, was not available for all subjects and requiring smoking data reduced the number of subjects with complete data to 669.

Table 2. Results of Testing for Genotype and Genotype-Alcohol Interaction Effects on Plasma HDL-C, Apo A-I, Apo A-II, and the HDL Factor Score

	HDL-C ($N = 901$)		Apo A-I ($N = 779$)		Apo A-II ($N = 780$)		Factor score ($N = 753$)	
	$\Delta\chi^2$	p	$\Delta\chi^2$	p	$\Delta\chi^2$	p	$\Delta\chi^2$	p
Weekly alcohol intake	69.2	<0.0001	46.6	<0.0001	79.7	<0.0001	91.0	<0.0001
ADH2	4.22	0.040	0.57	0.450	0.00	1.000	0.03	0.874
Alcohol × ADH2	0.01	0.933	0.75	0.386	1.63	0.201	0.68	0.409
ADH3	0.03	0.863	1.02	0.312	1.31	0.252	0.42	0.518
Alcohol × ADH3	0.16	0.686	1.12	0.290	1.19	0.275	0.88	0.349

Data adjusted for effects of sex, age and BMI.

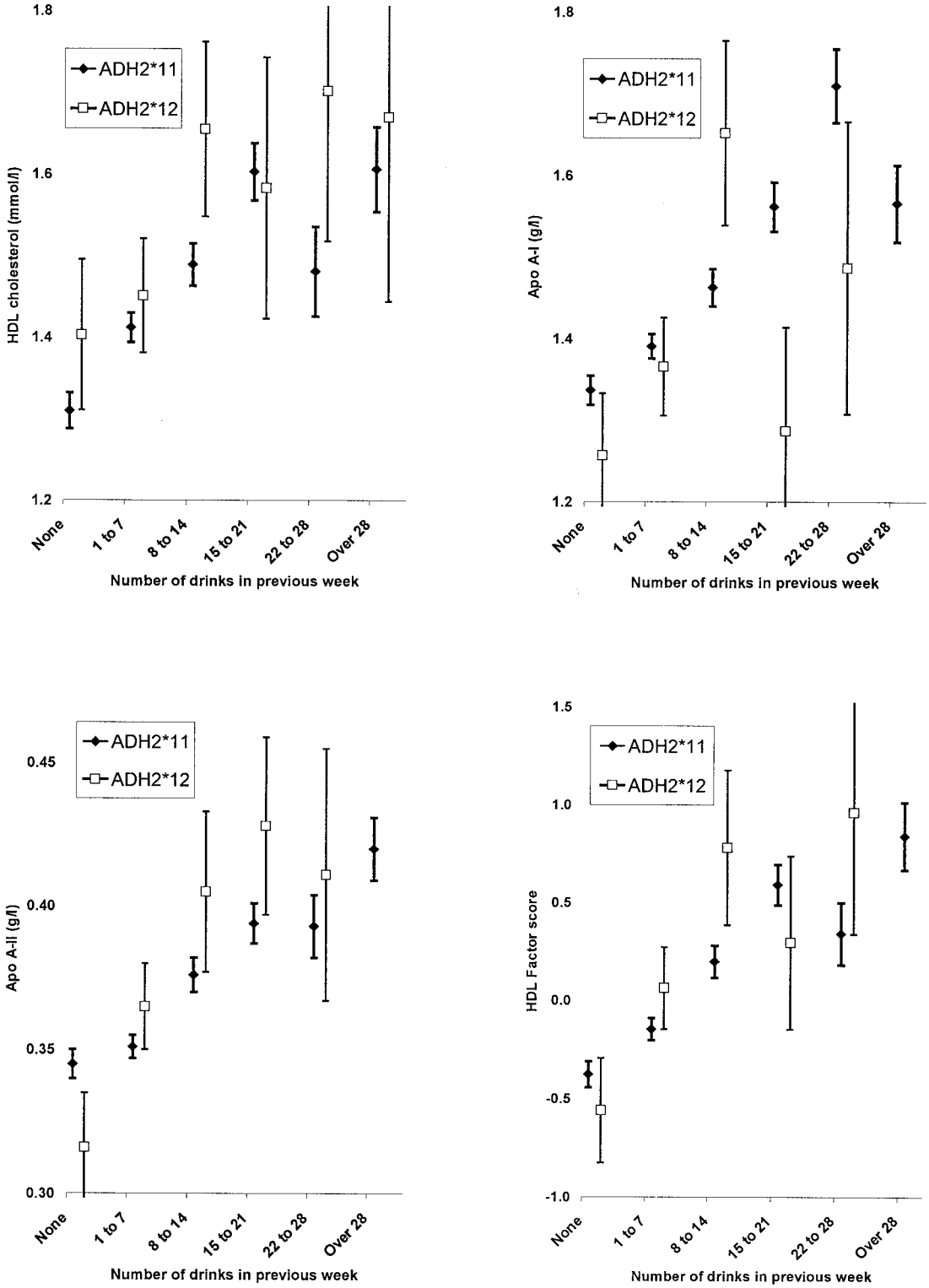

Fig. 1. Response of HDL cholesterol, apo A-I, apo A-II, and the HDL factor score to alcohol intake, grouped by *ADH2* genotype. Error bars show SEM. Note that there were no *ADH2*12* subjects with apolipoprotein results in the highest alcohol intake group. Adjusted for age, sex, and BMI.

Data Analysis

Factor analysis to combine data on HDL particle concentration from the three measured variables (HDL-C, apo A-I, and apo A-II) and estimation of the correlations between each HDL component, the factor score, and alcohol intake were carried out using SPSS (SPSS Inc., Chicago, IL). This approach does not adjust for the familial correlation between twin pairs, which can lead to overestimation of the significance (but not the magnitude) of associations found. Therefore further analysis was performed using Mx (Neale, 1999), which is tailored for analysis of twin data and overcomes the issue of nonindependence of cotwins. Specifically,

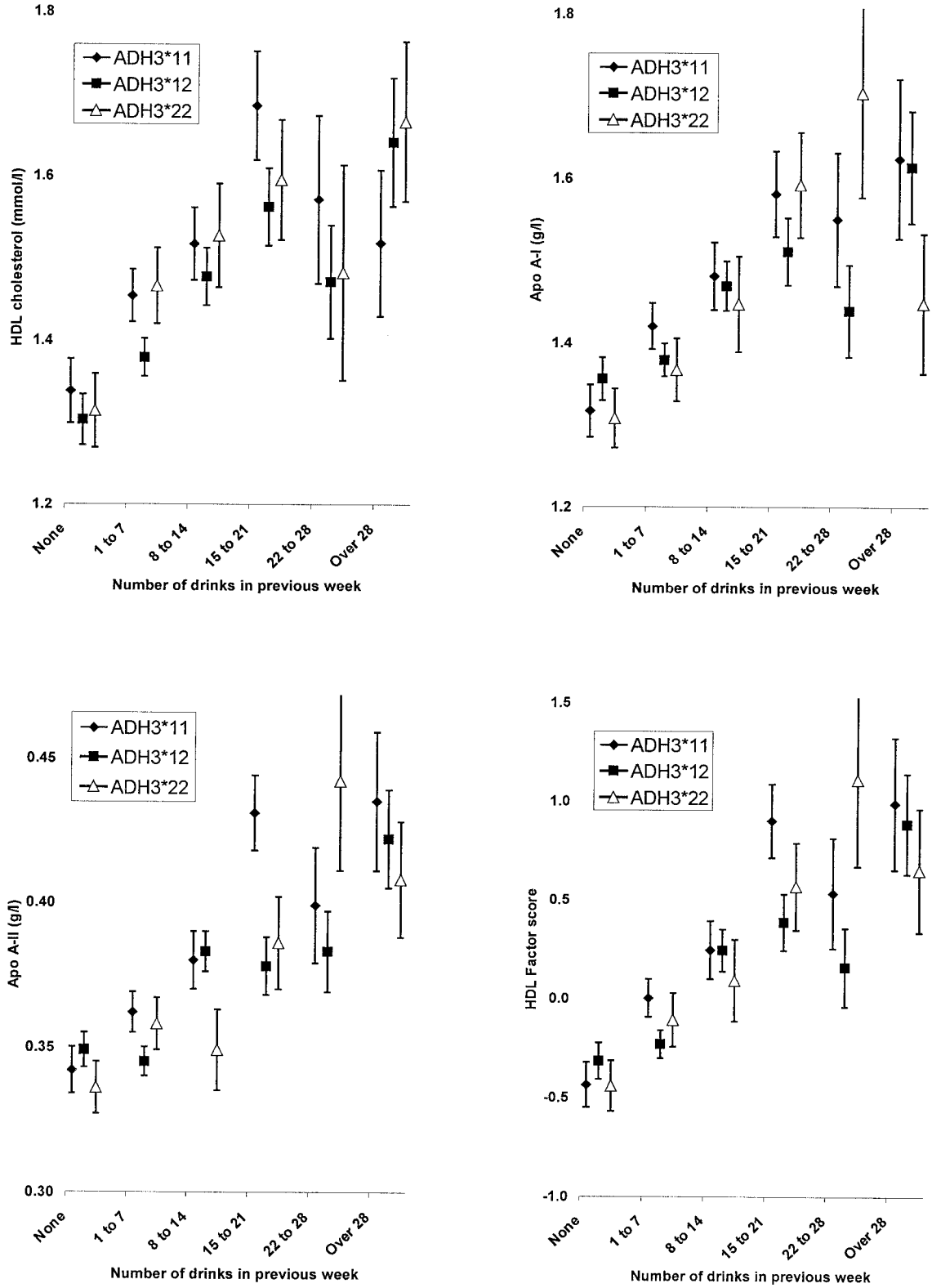

Fig. 2. Response of HDL cholesterol, apo A-I, apo A-II, and the HDL factor score to alcohol intake, grouped by *ADH3* genotype. Error bars show SEM. Adjusted for age, sex, and BMI.

models for mean effects and for residual variances and covariances between twins were simultaneously fitted. Because monozygotic and dizygotic twins are expected to have different covariances, separate groups were allowed for these while at the same time constraining mean effects (regression parameters) to be equal between monozygotic and dizygotic twins. Variables expected or hypothesized to affect HDL concentration

were included in a model of sources of variation, and each variable in turn was dropped from the model and then reinstated. The deterioration in goodness-of-fit for dropping each variable was estimated and tested using the χ^2 statistic. Tests were performed for effects of past-week alcohol intake, *ADH2* and *ADH3* genotype, and intake-genotype interactions, on the three measures of HDL concentration and the factor score. Results were adjusted for effects of sex, age, and BMI. Because of skewed distributions, alcohol intake data were log-transformed, using $\log_{10}$ (number of drinks + 1) to allow inclusion of subjects who reported no drinking or no drinks in the previous week.

RESULTS

The overall correlations between estimates of alcohol intake and the three measured HDL components (HDL-C, apo A-I, and apo A-II) are shown in Table 1. As expected, all three measures increased with increasing alcohol consumption across the range of intake studied. In addition, factor analysis was used to derive a single factor score representing HDL concentration from the three measurements of HDL components. All the measures of HDL concentration increased with alcohol intake, with no evident lower or upper threshold.

Genetic variation at *ADH2* or *ADH3* showed no significant alcohol-by-genotype interaction effects (Table 2). The marginally significant main effect of *ADH2* genotype on HDL-C was not supported by results for apo A-I, apo A-II, or the HDL factor score. Inclusion of smoking status as a covariate lowered the number of subjects with full data and did not alter the conclusion that genotype and genotype-alcohol interactions had no effects on HDL components (data not shown).

The results were examined in more detail by plotting the measured HDL components and the HDL factor score against alcohol intake by genotype groups. The relationship between alcohol intake and HDL for the subjects genotyped for *ADH2* is shown in Fig. 1 and for *ADH3* in Fig. 2. Despite variation in the estimated means at the higher levels of alcohol intake because of small numbers of subjects, it can be seen that there were no obvious patterns of variation by genotype and certainly none which applied to all three measured HDL constituents and the factor score derived from them.

DISCUSSION

The hypothesis to be tested was that *ADH* genotype affects the relationship between alcohol intake and plasma HDL concentration, with the presumed mechanism of any such effect being variation in the in vivo rate of alcohol metabolism. The initial report (Hines et al., 2001) was that HDL-C concentration was not affected by *ADH3* genotype in nondrinkers but, in men averaging one drink per day or more and in women averaging half a drink per day or more, HDL-C was highest in *ADH3*22* subjects, lowest in *ADH3*11* subjects, and intermediate in the heterozygotes. Because the *ADH3*2* allele codes for the enzyme with less in vitro activity, these results were consistent with the possibility that a slower rate of clearance of alcohol in vivo leads to a greater effect on HDL and hence lower cardiovascular mortality.

This reasoning should also apply to genetic variation at the *ADH2* locus, where the in vitro enzyme activities associated with *ADH2*2* and *ADH2*3* are substantially greater than for *ADH2*1*. In another report (Hashimoto et al., 2002) on *ADH* effects on several cardiovascular risk factors in moderate to heavy drinkers, the effects of *ADH2* variation were studied. Among these Japanese subjects, there was a comparatively high frequency of the *ADH2*2* allele. The *ADH2*11* genotype was associated with approximately 10% higher HDL-C than either the **12* or **22* genotypes, consistent with the slower alcohol metabolism–greater effect hypothesis. However, this effect was not significant.

We have not been able to reproduce the reported *ADH* effect. Despite the highly significant effect of alcohol on all three measured variables and on the HDL factor, across the range of alcohol intakes in this population-based cohort, there is no evidence from our results to support the concept that polymorphic *ADH* variation affects the HDL response to alcohol. Neither *ADH2* nor *ADH3* variation showed consistent effects on the dose-response curves, contrary to the original report on *ADH3*. The results of Hines et al. (2001) are intriguing and extend to clinical events about which we have no data, but we could not replicate their results for effects on plasma HDL. The explanation which they put forward, that *ADH3* variation affects ethanol metabolism, is also open to question because the available in vivo data point to a lack of any difference in metabolic rate by either *ADH2* or *ADH3* type (Mizoi et al., 1994; Whitfield, 1994; Whitfield et al., 2001).

Genetic association studies have often shown lack of consistency (Ioannidis et al., 2001) and the reasons are not always clear. Our study is of comparable size to that of Hines et al. (2001), who measured both *ADH3* genotype and HDL-C concentration in 1093 subjects and, although we had comparatively few subjects with consistently high alcohol intake (averaging over four drinks per day), there were substantial numbers consuming alcohol regularly. The population studied was in both cases of predominantly northern European origin. Further studies are needed to resolve the question of whether the initial positive result, or our negative one, better represents the true situation.

Although we cannot find any effect of *ADH* variation, it is still likely that other genes, or nongenetic differences, do alter the response of HDL cholesterol, apo A-I, and apo A-II to alcohol intake. Further gene-specific studies, perhaps with a focus on loci more directly affecting HDL, and further analysis of our twin data to determine whether general gene × alcohol interaction effects on HDL can be demonstrated, are required.

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