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# Variation in Alcohol Pharmacokinetics as a Risk Factor for Alcohol Dependence

J. B. Whitfield, G. Zhu, D. L. Duffy, A. J. Birley, P. A. F. Madden, A. C. Heath, and N. G. Martin

**Background:** The significant association between alcohol dehydrogenase (ADH)-2 genotype and alcohol-dependence risk, demonstrated in both Asian and non-Asian populations, suggests a link between the metabolism of alcohol (ethanol) and individual differences in susceptibility to dependence.

**Methods:** We tested this hypothesis by following up on subjects who took part in the Alcohol Challenge Twin Study conducted in 1979–1981 and comparing the blood and breath alcohol results in that study between subjects who subsequently did or did not meet diagnostic criteria for lifetime alcohol dependence in 1992–1993.

**Results:** Subjects who met DSM-III-R criteria for lifetime alcohol dependence at follow-up had higher blood and breath alcohol values after alcohol challenge than never-dependent subjects. Multivariate analysis showed independent effects of susceptibility to alcohol dependence and smoking status on blood alcohol concentrations, whereas habitual alcohol intake at the time of the initial study had marginally significant effects. The risk of alcohol dependence was 2-fold higher in men and 3-fold higher in women with blood or breath alcohol concentrations in the highest quartile than in the lowest quartile.

**Conclusions:** In view of this association and the known genetic influences on both alcohol pharmacokinetics and alcohol dependence, it is probable that part of the heritability of dependence is mediated by genes (other than the known *ADH2* and *ADH3* polymorphisms) affecting alcohol metabolism.

**Key Words:** Ethanol, Blood, Breath, Dependence, Twin Study.

POLYMORPHISMS IN TWO alcohol dehydrogenase (ADH) genes, *ADH2* and *ADH3*, have been implicated in variation in susceptibility to alcohol dependence (AD) in Chinese and Japanese populations. This is a robust finding, with multiple reports (Chen et al., 1999; Higuchi, 1994; Maezewa et al., 1995; Muramatsu et al., 1995; Nakamura et al., 1996; Tanaka et al., 1996; Thomasson et al., 1991, 1994), and it has been reinforced by meta-analysis (Whitfield, 1997). The difference in risk associated with *ADH2*11* compared with *ADH2*12* is approximately 3-fold. The *ADH2* effect is independent of aldehyde dehydrogenase (ALDH)-2 type and occurs in people who have *ALDH2*11* with normal ALDH activity (Chen et al., 1999; Higuchi, 1994). The association between *ADH3* genotype and AD is probably due to linkage disequilibrium with *ADH2* (Borras et al., 2000; Osier et al., 1999). Three reports on non-Asian subjects (Borras et al., 2000; Neumark et al., 1998; Whitfield et al., 1998) have confirmed the *ADH2* association in other populations.

More generally, the chromosomal region where *ADH* genes are located has been implicated in two genome-scan studies of AD, one mainly on European Americans (Reich et al., 1998) and the other on Native Americans (Long et al., 1998). More recently, strong indications of linkage close to the *ADH* locus were found when the phenotype was defined as the maximum number of drinks ever taken in 1 day (Saccone et al., 2000).

Because alcohol (ethanol) metabolism is the major known function of class I ADHs, these findings suggest that variation in alcohol metabolism affects susceptibility to AD. Testing for effects of variation in alcohol metabolism on dependence risk in prospective studies requires time and substantial resources, but two ongoing studies can address this issue. These are the Australian Alcohol Challenge Twin Study (Martin et al., 1985a,b) and the studies of Schuckit and collaborators on family-history–positive and family-history–negative men. In each case, young adult subjects were tested with alcohol and have been observed for AD over approximately 15 years.

The articles of Schuckit and colleagues have concentrated on the importance of sensitivity to intoxication in the evolution of AD (Schuckit and Smith, 1996). We too have

*From the Department of Clinical Biochemistry, Royal Prince Alfred Hospital, Sydney, Australia, and the University of Sydney (JBW); Queensland Institute of Medical Research, Brisbane, Australia, and Joint Genetics Program, the University of Queensland (GZ, DLD, AJB, NGM); and Missouri Alcoholism Research Center, Department of Psychiatry (PAFM, ACH), Washington University, St Louis, Missouri.*

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*Reprint requests: John B. Whitfield, PhD, Biochemistry Department, Royal Prince Alfred Hospital, Camperdown NSW 2050, Australia; Fax: 61-2-9515-7931; E-mail: johnwhit@bioc.rpa.cs.nsw.gov.au*

investigated the relationships between sensitivity to intoxication in the initial Alcohol Challenge Twin Study and the subjects' subsequent risk of AD, and the results were in accordance with the hypothesis that increased sensitivity to intoxication is associated with a decreased risk of AD (Heath et al., 1999).

Analysis of the relationship between blood or breath alcohol values and risk of AD has now revealed an association between alcohol metabolism and AD. This association seems to be as strong as, or stronger than, that with sensitivity to intoxication. In this article, we investigate the relationships between blood or breath alcohol after a test dose and DSM-III-R AD 11 to 14 years later, taking into account potentially confounding variables such as age, sex, habitual alcohol use, and smoking status at the time of initial testing and known *ADH* polymorphisms.

## MATERIALS AND METHODS

### *Alcohol Challenge Twin Study Subjects*

Four hundred twelve male and female subjects (206 twin pairs; 199 men and 213 women) aged between 18 and 34 years participated in the Alcohol Challenge Twin Study between 1979 and 1981 (Martin et al., 1985a,b). They were given a dose of 0.75 g of ethanol per kilogram body weight and drank it over a period of 20 min; blood and breath alcohol readings were obtained by using gas chromatography of capillary blood samples and a Drager breath alcohol analyzer (Dräger, Lübeck, Germany), respectively. Breath alcohol values were expressed in milligrams per 100 ml of blood by using a conversion factor of 1:2100, which is known to result in a slight underestimate of blood alcohol concentration (BAC). The times of sampling were measured from the completion of alcohol consumption; up to 6 blood samples were taken, and 10 breath analyses were performed on each subject. The mean times of blood results were 56, 68, 83, 123, 143, and 182 min after completion of alcohol consumption, and for breath alcohol results, 40, 56, 68, 83, 100, 123, 143, 160, 182, and 213 min. The number of times for which data were available varied between subjects. A small number of results were missing for technical reasons, but most missing values were the result of a change in protocol after the study commenced to obtain a greater density of blood alcohol measurements. On the day of testing, subjects were asked about their usual weekly alcohol consumption (weekly number of drinks summed across categories of alcoholic beverage) and their smoking status (current, past, or never a smoker).

### *Follow-Up Procedure*

These 412 subjects have been invited to participate in a number of subsequent studies. Between 1990 and 1995, 375 of them (91%) provided blood samples for *ADH* genotyping and other work on the genetics of alcoholism. DNA was extracted from white blood cells, and *ADH2* and *ADH3* genotypes were assessed as previously described (Whitfield et al., 1998). In 1992 and 1993, 341 of them completed an interview with the Semi-Structured Assessment for the Genetics of Alcoholism instrument (Bucholz et al., 1994), which allows diagnosis of AD by DSM-III-R criteria. Statistical analyses were performed with data from 341 individual subjects, except where *ADH* genotypes were incorporated; this reduced the available number to 332.

### *Statistical Analysis*

Analysis was conducted with Mx 1.47 (Neale, 1999), which is designed for analysis of twin and family data and takes into account the correlation between members of a twin pair. Models are written simultaneously for the means, in terms of fixed regression effects for age, sex, AD diagnosis, and *ADH* genotypes and also for the covariances between observations. The latter can be parameterized in terms of genetic and environmental variance components, but because the focus of our attention here is on mean differences in BAC between AD-positive (AD+) and AD-negative (AD−) subjects, we have merely estimated empirical covariance matrices for monozygotic (MZ) and dizygotic (DZ) twins—12 × 12 for blood readings and 20 × 20 for breath alcohol concentrations.

Multivariate analyses tested for effects of sex, age, lifetime AD diagnosis, habitual alcohol use at the time of the alcohol challenge study, smoking status at the time of that study, and *ADH2* and *ADH3* genotypes on blood and breath alcohol values. In the case of *ADH2*, the *2* allele is uncommon in Europeans, and only two genotypes (*11* and *12*) were encountered, but for *ADH3* all three (*11, 12,* and *22*) were present. Two way interaction terms for age × sex, age × AD, and sex × AD were also included in the model, but results for these terms are not tabled. To reduce the skewness of the frequency distribution, weekly alcohol consumption was transformed to $\log_{10}(x + 1)$, where $x$ is the reported number of drinks per week. Smoking status was grouped into categories of current smoker and not current smoker (past smoker or never-smoker). Because there were up to 6 blood and 10 breath alcohol results across a period of 3.5 hr, we tested for heterogeneity of effects of each of these variables across time and also for effects of the variables on blood or breath alcohol concentrations across all times. From the results of the full model, including interaction terms, the means and 95% confidence intervals (CIs) for blood and breath alcohol were estimated for each of the 6 blood and 10 breath time points, dividing subjects into those who were lifetime AD+ or AD− at follow-up, with separate estimates for men and women. These estimates thus take into full account the nonindependence of observations, both between twins and between time points in the same individual.

We also estimated odds ratios for the association of AD with quartile of blood or breath alcohol for men and for women, by using STATA (Stata Corporation, College Station, TX) to obtain estimates of the 95% CIs for these odds ratios. STATA uses a robust variance estimation procedure that corrects for the statistical nonindependence of repeat measures that were correlated both across time and between twins.

Having established an association between blood or breath alcohol concentrations and AD, we tested whether this might be due to population stratification. We performed a logistical regression analysis to account for the difference in AD status between co-twins in DZ pairs (whose genes are derived from the same gene pool) as a function of difference in blood or breath alcohol level, while simultaneously adjusting for sex, weekly alcohol consumption, smoking, and *ADH2* and *ADH3* genotypes (both additive and dominant components). Separate analyses were performed for all 6 blood and 10 breath time points, and also for their means and maximum (peak BAC) values. If a difference in blood or breath alcohol between co-twins is associated with a difference in AD, then we conclude that the effect is not due to population stratification. Joint analyses of all pairs were carried out by adding a zygosity × blood alcohol difference or zygosity × breath alcohol difference interaction term, to assess whether any association between difference in alcohol concentration and difference in AD risk was dependent on zygosity.

## RESULTS

Figure 1 shows the mean blood alcohol values by AD status at follow-up, for men and for women. Note that the error bars in this figure show the 95% CIs rather than SEs.

Results of the multivariate analysis on 332 subjects for blood and breath alcohol concentrations are shown in Table 1. For each set of results (blood and breath), the table shows the estimated effects of age, sex, AD at follow-up, habitual alcohol intake at baseline, smoking status at baseline, and *ADH2* and *ADH3* genotypes. The values in each column are deviations from the time-specific mean values

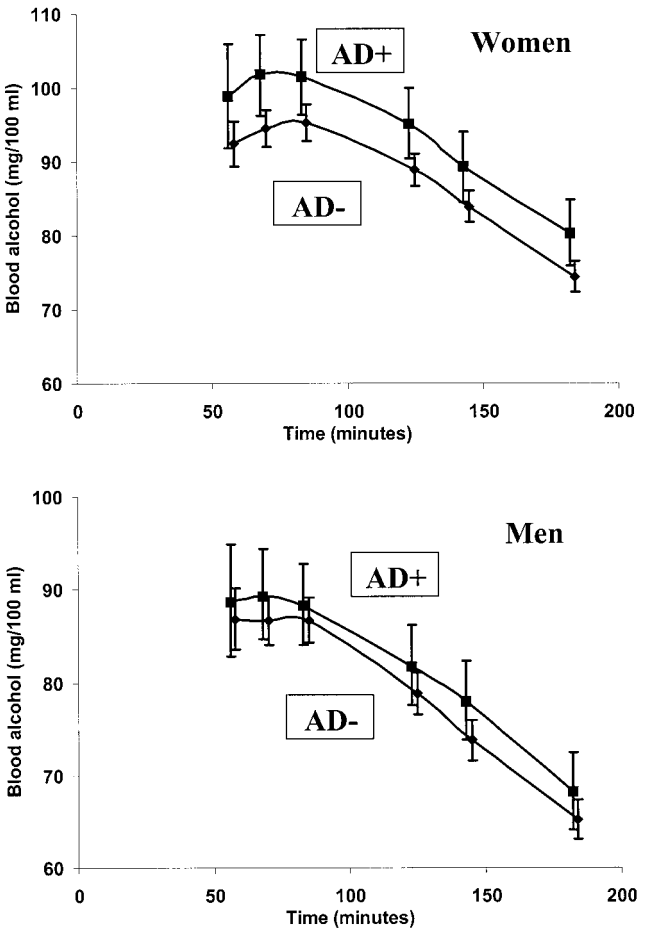


**Fig. 1.** Blood alcohol values in 158 male (bottom graph) and 183 female (top graph) twin subjects, by alcohol dependence status (lifetime dependence status from SSAGA interview, approximately 14 years after the alcohol challenge study: AD+, alcohol dependent; AD−, never alcohol dependent). Error bars take into account the nonindependence of observed measures and show 95% confidence intervals around the means.

for each of the variables. Next, $\chi^2$ tests for heterogeneity across time (5 *df* for the blood results, 9 for the breath results) and associated probabilities are shown. Finally, the estimated deviation averaged across all times, again in milligrams per 100 ml of blood, is shown with the $\chi^2$ test of the null hypothesis of no effect and the associated probability value; this joint estimate is valid only if there is no significant heterogeneity between the estimates for individual time points.

For example, the BACs were on average approximately 3.5 mg/100 ml higher in the subsequently alcohol-dependent subjects ($p = 0.023$), and the difference was homogeneous ($p = 0.45$) across time (individual time estimates ranged from 1 to 4 mg/100 ml). Higher habitual alcohol consumption in 1979–81 was associated with higher blood alcohol values: approximately 8 mg/100 ml higher at time 1 to approximately 2 mg/100 ml higher at time 6, for each log unit of weekly alcohol intake (i.e., a 10-fold difference in the number of drinks per week). However, this effect was not significantly heterogeneous across time and not significant overall ($p = 0.070$ and 0.073, respectively). The subjects who reported themselves to be current smokers had blood alcohol values approximately 5 mg/100 ml below the past or never-smokers ($p = 0.0003$).

*ADH2*12* subjects had blood alcohol values approximately 4 mg/100 ml below those of *ADH2*11* subjects; this was not significant ($p = 0.30$), but the number of *ADH2*12* subjects was very small (32 of 377 subjects genotyped). For *ADH3*, with all three possible genotypes represented among the subjects, two tests were performed. These estimated the deviations of *ADH3*12* and *ADH3*22* genotypes from the baseline *ADH3*11* genotype. The deviation of the *ADH3*12* genotype (−2.9 mg/100 ml) was marginally significant ($p = 0.033$), possibly because of the strong linkage disequilibrium of the *ADH3*2* allele with the *ADH2*1* allele. The *ADH3*22* deviation was smaller and not significant, but it was based on a smaller number of cases ($n = 51$).

Breath alcohol results (Table 1) confirmed the pattern seen for blood alcohol; again there were significant associations with AD and smoking, but not with habitual alcohol intake or *ADH2* type. The difference between *ADH3*11* and *ADH3*12* found for the blood results was not present for the breath results. The effects of age, sex, alcohol intake, and AD were to varying degrees heterogeneous across time.

In view of the significant effect of smoking status on blood or breath alcohol values, we attempted to test whether this reflected current smoking status at the time of the study or lifetime smoking history. The effect of introducing smoking into the model was assessed with two different groupings; in the first, the division was current versus past and never, and in the second, the division was current and past versus never. Improvement in fit between the model and the data was assessed from the change in −2(log likelihood ratio). For the blood alcohol results, the former (current smoker versus not current smoker) was better ($\chi^2_1 = 11.34$ compared with $\chi^2_1 = 8.76$), but for breath alcohol results, the opposite was the case ($\chi^2_1 = 14.59$ compared with $\chi^2_1 = 27.09$). Because of the small number of subjects in the past-smoker group, it was not possible to resolve this issue by statistical testing.

There were 30 twin pairs in total (18 DZ, 12 MZ) who were discordant for AD and had all the variables measured. For blood alcohol, a significant within-pairs association with AD risk was seen for three of six time points and for the mean ($p = 0.037$) and peak ($p = 0.018$) concentrations. In no case was there any interaction of the within-pair difference in AD risk with zygosity, indicating that we cannot say whether this relationship is mediated environmentally or genetically. The breath alcohol results showed a significant within-pairs association with risk at four of ten times and for the mean but not the peak. Significant interaction with zygosity was found at two time points, but not for the mean or peak, so we can neither demonstrate nor exclude a difference between MZ and DZ pairs.

**Table 1.** Effects of Alcohol Dependence and Other Factors on Blood and Breath Alcohol Values After a Test Dose of 0.75 g/kg of Alcohol

| | Deviations from mean value at each time attributable to | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Blood: Time (min) | Age | Sex | Alcohol dependence | Weekly intake | Smoker | *ADH2* | *ADH3*12* | *ADH3*22* |
| 56 | 0.53 | −7.39 | 1.02 | 7.89 | −3.76 | −2.94 | −2.46 | −0.54 |
| 68 | 0.52 | −9.01 | 2.51 | 5.12 | −3.03 | −2.51 | −2.46 | −1.55 |
| 83 | 0.33 | −10.35 | 1.19 | 5.01 | −2.89 | −3.03 | −3.11 | −1.29 |
| 123 | 0.25 | −11.54 | 2.92 | 3.55 | −4.81 | −1.90 | −1.66 | −0.43 |
| 143 | 0.24 | −11.67 | 3.62 | 2.67 | −4.74 | −0.65 | −2.57 | 0.40 |
| 182 | 0.15 | −10.63 | 4.04 | 1.60 | −5.48 | −2.74 | −3.27 | −0.30 |
| | Test for homogeneity of effects of each variable across time | | | | | | | |
| | Age | Sex | Alcohol dependence | Weekly intake | Smoker | *ADH2* | *ADH3*12* | *ADH3*22* |
| $\chi^2$ (5 *df*) | 8.28 | 7.75 | 4.73 | 10.21 | 5.71 | 4.99 | 4.65 | 2.00 |
| *p* | 0.141 | 0.171 | 0.450 | 0.070 | 0.335 | 0.418 | 0.460 | 0.850 |
| | Test for effects of each variable across all times | | | | | | | |
| Joint | Age | Sex | Alcohol dependence | Weekly intake | Smoker | *ADH2* | *ADH3*12* | *ADH3*22* |
| Deviation | 0.29 | −10.69 | 3.49 | 2.63 | −4.52 | −2.43 | −2.90 | −0.75 |
| $\chi^2$ (1 *df*) | 4.80 | 60.68 | 5.14 | 3.21 | 12.79 | 1.09 | 4.54 | 0.15 |
| *p* | 0.029 | <0.0001 | 0.023 | 0.073 | 0.0003 | 0.297 | 0.033 | 0.696 |
| | Deviations from mean value at each time attributable to | | | | | | | |
| Breath: Time (min) | Age | Sex | Alcohol dependence | Weekly intake | Smoker | *ADH2* | *ADH3*12* | *ADH3*22* |
| 40 | 0.39 | −3.68 | 6.51 | 5.14 | −4.35 | 3.03 | 1.38 | 2.44 |
| 56 | 0.38 | −7.38 | 4.13 | 5.66 | −4.76 | −1.27 | 0.74 | 1.37 |
| 68 | 0.36 | −8.58 | 0.78 | 6.49 | −4.82 | −1.55 | −0.48 | −1.25 |
| 83 | 0.26 | −9.94 | 3.31 | 2.78 | −5.23 | 0.73 | −0.91 | −0.29 |
| 100 | 0.05 | −10.53 | 2.99 | 2.58 | −4.55 | −1.83 | 0.87 | 0.07 |
| 123 | −0.11 | −11.48 | 2.98 | 3.95 | −4.58 | −2.42 | −0.58 | −0.80 |
| 143 | −0.06 | −11.10 | 3.63 | 1.72 | −3.46 | −2.66 | −0.60 | 0.26 |
| 160 | 0.14 | −12.10 | 3.23 | 1.87 | −3.67 | −2.12 | 0.32 | −0.53 |
| 182 | −0.07 | −10.27 | 2.87 | 2.26 | −3.83 | −2.10 | 0.34 | 1.81 |
| 213 | −0.05 | −10.84 | 2.43 | 1.81 | −4.19 | −2.68 | 1.33 | −1.28 |
| | Test for homogeneity of effects of each variable across time | | | | | | | |
| | Age | Sex | Alcohol dependence | Weekly intake | Smoker | *ADH2* | *ADH3*12* | *ADH3*22* |
| $\chi^2$ (9 *df*) | 23.18 | 29.00 | 19.45 | 23.91 | 5.39 | 11.87 | 10.42 | 16.50 |
| *p* | 0.0058 | 0.0006 | 0.022 | 0.005 | 0.799 | 0.221 | 0.318 | 0.057 |
| | Test for effects of each variable across all times | | | | | | | |
| | Age | Sex | Alcohol dependence | Weekly intake | Smoker | *ADH2* | *ADH3*12* | *ADH3*22* |
| Deviation | 0.10 | −9.96 | 2.47 | 2.33 | −4.03 | −2.70 | 0.87 | −0.33 |
| $\chi^2$ (1 *df*) | 0.60 | 53.36 | 4.15 | 2.70 | 14.77 | 1.47 | 0.50 | 0.04 |
| *p* | 0.437 | <0.0001 | 0.042 | 0.101 | <0.0001 | 0.226 | 0.479 | 0.842 |

Values are shown as deviations from the overall mean for each time point; for the continuous variables age and log-transformed weekly alcohol intake, the deviation is per year or per unit increase in $\log_{10}$(drinks per week + 1). Blood and breath alcohol deviations from the time-specific means, in mg/100 ml, were first tested for heterogeneity across time and then for the joint effect on all times. All effects are tested simultaneously and take account of nonindependence of observed measures.

## DISCUSSION

### *BAC or Breath Alcohol Concentrations and the Risk of AD*

These results show a significant association between alcohol pharmacokinetics, as shown by the BAC and breath alcohol concentrations after a standard dose of alcohol taken by young adults in a laboratory setting, and the subsequent development of AD. This association is independent of alcohol consumption and smoking habits at the time of testing. We have shown that the BACs (Martin et al., 1985b) and AD (Heath et al., 1997) are both subject to significant genetic effects, so it is likely that this relationship is genetically mediated, although with only 30 AD-discordant twin pairs our study does not have the power to show that this is so. Obvious candidates to modulate this association are from known polymorphisms in the genes of the alcohol-metabolizing enzymes, but our analysis of *ADH2* and *ADH3* shows that these are not sufficient to account for the overall association, although *ADH2* type is known to have significant effects on dependence risk.

In a previous analysis of these data (Whitfield and Martin, 1994), we found genetic correlations between alcohol intake at the time of the alcohol challenge study and both peak BAC and the rate of decrease in BAC. This analysis extends this finding to the subsequent course of these subjects' lives and to dependence as well as consumption, makes use of the full range of blood and breath alcohol

data, and explicitly includes the effects of smoking and measured genotypes for *ADH2* and *ADH3.* The results have shown that susceptibility to dependence has an effect that is significant and independent of consumption at the time of testing.

Inclusion of both the 1979–1981 alcohol consumption data and the 1993–1995 AD diagnosis in the current analysis is important because high alcohol consumption can induce alcohol-metabolizing enzymes and cause faster in vivo alcohol metabolism (Mezey and Tobon, 1971; Nomura et al., 1983; Olsen et al., 1989). The results show that the AD effect is not simply due to high alcohol intake at the time of baseline testing of those subjects who reported a history of AD at follow-up. This separation of the two effects is important because we did not obtain an assessment of AD at baseline and cannot in all cases be certain that those subjects reporting a history of dependence at follow-up were free of dependence symptoms at the time of the alcohol challenge testing. The assessment of habitual alcohol use was based on subjects' estimates of the usual number of drinks taken per week, rather than the actual number taken in the preceding week, but we cannot determine whether data on the actual number of drinks would have shown a stronger effect.

The effects of habitual alcohol use at the time of testing and of susceptibility to AD were subtly different. Habitual alcohol use in the period before the alcohol challenge test had its greatest effect on the early blood alcohol results, and this decreased with time (although the heterogeneity was not quite significant), suggesting effects on both peak concentration and the rate of postabsorptive metabolism. Because the rate of consumption of the test dose of alcohol was not regulated (except that it had to be taken within 20 min), more experienced drinkers may have consumed it more quickly and attained a higher peak BAC, with subsequent redistribution. The susceptibility to dependence effect, however, was homogeneous across time, suggesting a difference in early alcohol metabolism perpetuated across subsequent times.

Smoking history also showed significant associations with BAC and breath alcohol concentrations. The subjects were not asked to refrain from smoking, and we assume that those who normally smoked did so during the study. It is known that smoking is strongly associated with AD risk in the Australian twin cohort, of which these subjects are a subset (Whitfield et al., 2000), and such associations have a common genetic component (Koopmans et al., 1997; Swan et al., 1996, 1997), but inclusion of smoking as a term in the multivariate analysis showed that the association between blood or breath alcohol values and subsequent AD is independent of smoking history. We consider that the smoking effect is probably due to smoking during the study or in the preceding 24 hr, rather than being due to an association between smoking and alcohol use or dependence, which themselves affect blood and breath alcohol values. We were not able to resolve this question by statistical testing. It should be noted that the effect of smoking was to decrease alcohol concentrations, whereas higher habitual alcohol consumption or DSM-III-R AD increased them.

Inspection of the results in Fig. 1 and Table 1 shows that the difference in alcohol concentrations between AD+ and AD− subjects starts early and probably represents either gastric or early hepatic (presteady state) alcohol metabolism. There are many articles on the phenomenon of first-pass metabolism (usually measured from the difference between BACs after oral and intravenous administration of alcohol), and it is reported to be lower in alcoholics than controls. However, the first-pass metabolism increased with abstinence in those alcoholic subjects who were re-tested (DiPadova et al., 1987). First-pass metabolism represents a higher proportion of overall alcohol metabolism at low doses and in the fed state and may therefore be less relevant to the conditions of the Alcohol Challenge Twin Study, in which subjects had a high dose 1 to 2 hr after a light, nonfatty breakfast (Martin et al., 1985b).

The effects of *ADH2* and *ADH3* variation on BAC or breath alcohol concentrations were generally small and not statistically significant. The significant *ADH3* effect for BAC was not confirmed from the breath alcohol data. This lack of effect of *ADH* genotype on in vivo alcohol metabolism is important, because many authors have assumed that the effect of *ADH2* genotype on AD risk is mediated by differences in the rate of alcohol metabolism. There are notable in vitro differences in $V_{max}$ between the *ADH2*1* and *ADH2*2* gene products, but they do not seem to affect alcohol pharmacokinetics *in vivo*. Given the small number of *ADH2*12* subjects, we cannot exclude effects of this *ADH* polymorphism on alcohol concentrations after drinking, but neither *ADH2* nor *ADH3* variation can account for the association between alcohol metabolism and dependence risk.

### *Population Stratification and the Within-Pair Tests*

Having found an association between blood or breath alcohol results and AD, we proceeded to check whether this association could be due to population stratification and, if possible, whether the pattern of within–discordant-pair differences by zygosity suggested a genetic cause for the association. Taking all dependence-discordant twin pairs, the higher-than-expected number of significant differences between blood alcohol values ($p < 0.05$, 4 of 6 times) or breath alcohol values ($p < 0.05$, 4 of 10 times) within the pairs indicates that population stratification is not the cause of the association between alcohol pharmacokinetics and dependence risk.

Because we have shown previously (Martin et al., 1985b) that BAC variation is primarily genetically determined, we should expect that the relationship is driven by genes determining differences in alcohol metabolism, which in turn influences dependence risk, with slower metabolizers at greater risk. Our failure to detect a significantly larger

within-pair association in DZ than MZ pairs is most likely due to the small number of informative pairs available for this analysis and the consequent low power. The situation is not clear, because for blood results we apparently observe the relationship in both MZ and DZ twins, whereas for breath results, the within-pair association was stronger for DZ than for MZ pairs. This suggests a genetic effect, but we cannot exclude environmental or epigenetic effects on alcohol metabolism when the subjects were young that have long-term consequences for AD risk, although we consider these unlikely.

### *How Large is the Influence of Alcohol Pharmacokinetics on AD Risk?*

The difference between lifetime alcohol-dependent and never-alcohol-dependent subjects can be assessed as an effect size by dividing the difference in means for the two groups by the pooled SD. From a simple comparison of the AD+ and AD− groups, this is approximately 0.4 SD units, for both men and women, blood or breath, and across all the time points. This effect size is comparable to those for individual measures of body sway or self-reported intoxication in men and considerably greater than for these measures of sensitivity to intoxication in women (Heath et al., 1999; Whitfield, 2001). However, adjustment of the blood and breath alcohol values for multiple effects (Table 1) leads to a smaller estimate of the dependence susceptibility effect, whereas the combination of sway and self-report data gives a stronger association with dependence (Heath et al., 1999).

Looking at the results in another way, the difference in AD risk between the first and fourth quartiles of BACs (Fig. 2) is approximately 2-fold in men and 3-fold in women; this is similar to the risks associated with having *ALDH2*11* rather than *ALDH2*12* (Chen et al., 1999; Higuchi et al., 1996) or *ADH2*11* rather than *ADH2*12* (Whitfield, 1997), or having a same-sex DZ twin who is alcohol dependent (Heath et al., 1997).

Many authors who have worked on ADH polymorphisms have proposed or assumed that these polymorphisms produce differences in alcohol metabolism, which in turn has an effect on the risk of alcohol dependence. This seems to be the first study that has tested and validated this general proposition, even though the specific example that generated this hypothesis (*ADH2* or *ADH3* genotypes) does not explain the relationship.

It is by no means clear how differences in BAC or breath alcohol concentration produce differences in dependence risk. The mean difference in BAC between the dependent and never-dependent groups was 3.5 mg/100 ml, and it may seem unlikely that such a small difference would produce a difference in the reward from drinking alcohol, which could lead to increased consumption and eventual dependence. However, it must be remembered that this is a mean difference that may be averaged across heterogeneous subgroups. In some individuals, this may be the major predisposing factor, whereas in others it has no influence on risk. It would be useful to correlate peak BAC with age of onset or severity of AD, but our sample is probably not large enough to do so. Another possibility is that the lower blood alcohol values in the never-dependent group indicate a greater degree of early (gastric or hepatic) metabolism and a greater early production of acetaldehyde, making drinking a less pleasant experience. There is no direct evidence for this mechanism, because blood acetaldehyde is essentially undetectable in subjects with normal ALDH2 activity, but it cannot be ruled out.

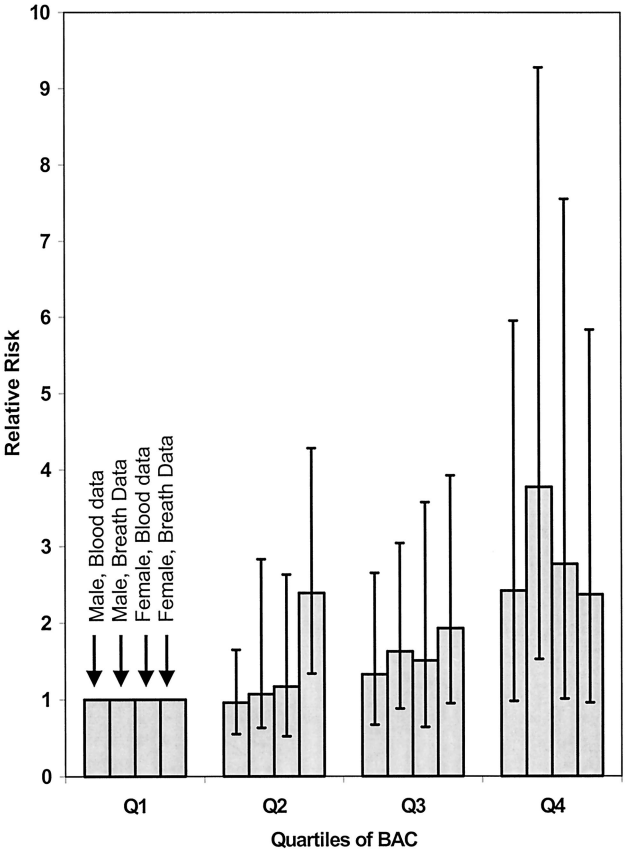


**Fig. 2.** Risk of alcohol dependence by quartiles (Q1 to Q4) of blood and breath alcohol in male and female twins. Within each quartile, the four bars represent estimates for men from blood data, men from breath data, women from blood data, and women from breath data, in that order, and include data from multiple times. Error bars take account of nonindependence of observed measures and indicate 95% confidence intervals on the estimates of relative risk.

Future work can usefully address the mechanisms of alcohol metabolism's effects, or *ADH2* genotype effects, on dependence risk and the location and nature of the other genes that affect alcohol metabolism and thereby influence dependence risk. In view of the genome-scan studies and the possibility of *ADH* expression–modifying genes on chromosome 4 near the *ADH* gene cluster, more detailed examination of chromosome 4 variation is justified.

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