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ERYTHROCYTE ALDEHYDE DEHYDROGENASE ACTIVITY: LACK OF ASSOCIATION WITH ALCOHOL USE AND DEPENDENCE OR ALCOHOL REACTIONS IN AUSTRALIAN TWINS

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Abstract — Aim: Aldehyde dehydrogenase 1 (ALDH1) has been advocated as a marker of alcohol intake. The absence or low levels of ALDH1 may be associated with alcohol-induced flushing or other reactions to alcohol in Europeans and therefore, with reduced alcohol use. This study tested whether variation in erythrocyte ALDH1 activity was associated with alcohol use, alcohol dependence or reactions to alcohol in unselected subjects of European descent, and whether variation in ALDH1 activity was subject to genetic influences. **Methods:** ALDH activity was measured in erythrocytes from 677 men and women who had participated in a twin study of alcohol use and dependence. **Results:** There were no significant effects of sex, alcohol consumption or alcohol dependence on ALDH activity. Subjects who reported reactions to alcohol did not have low activity. Women aged below 45 years had lower ALDH activity than men or older women. The heritability of ALDH activity was 56% (95% confidence interval = 42–67%). **Conclusions:** Previous reports that erythrocyte ALDH activity is low in alcoholics were not substantiated in this community-based sample. Associations with alcohol reactions were not found. ALDH activity varies widely between subjects, largely because of genetic factors.

INTRODUCTION

Aldehyde dehydrogenases (ALDH) catalyse the interconversion of a wide range of aldehydes and carboxylic acids. There are multiple related enzymes in this family (Yoshida *et al*., 1998; Sophos and Vasiliou, 2003), two of which participate in alcohol (ethanol) metabolism by converting acetaldehyde to acetate. Of these two human ALDHs, one (ALDH2) has a higher affinity for acetaldehyde and is found mainly in the mitochondria whereas the other (ALDH1A1 or ALDH1) has a lesser affinity (higher K_m) and occurs mainly in the cytosol. Usually, the activity of ALDHs in the liver is sufficient to keep acetaldehyde concentrations low during alcohol metabolism and acetaldehyde is not detectable in peripheral blood.

A variant of the mitochondrial or low-K_m ALDH2 occurs commonly in North-East Asia, and the consequent accumulation of acetaldehyde after alcohol consumption leads to facial flushing and other symptoms which deter many affected people from alcohol use and reduce the risk of alcohol dependence (Harada *et al*., 1982). The ALDH2 enzyme activity is reduced by ~90% in heterozygotes compared with unaffected homozygotes, owing to reduced specific activity and increased turnover of ALDH2–2 (Xiao *et al*., 1996).

Although ALDH1 has a K_m for acetaldehyde which is much higher than that for ALDH2 [estimated as 320 or 180 μM by two groups (Henehan and Tipton, 1992; Rashkovetsky *et al*., 1994), compared with <1 μM for ALDH2], there are a number of indications that ALDH1 can also play a role in acetaldehyde metabolism *in vivo*. A number of European subjects with complete or partial ALDH1 deficiency have been identified by investigating people who report alcohol-induced flushing (Yoshida *et al*., 1989; Yoshida, 1992; Ward *et al*., 1994). Second, the activity of ALDH in erythrocytes, which contain only ALDH1, was found to correlate strongly and negatively with increases in heart rate after drinking in Japanese men (Inoue *et al*., 1980). Third, a Japanese subject with deficiencies of both ALDH1 and ALDH2 was identified and tested with a small dose of ethanol, and it was shown that blood acetaldehyde concentrations were notably higher than for subjects with ALDH2 deficiency alone (Ueshima *et al*., 1993). There is also evidence that a polymorphism in rat ALDH1 is associated with variation in voluntary alcohol intake and also in circulating acetaldehyde levels after administration of ethanol (Nishiguchi *et al*., 2002), although caution is needed in extrapolating conclusions from rat experiments to humans. Finally, acetaldehyde concentrations in the hepatic vein during alcohol metabolism in humans average ~15 μM (Nuutinen *et al*., 1984) and the concentration in the hepatocyte cytoplasm must be at least this high. This suggests that the high-affinity ALDH2 is saturated and that ALDH1 may also be active.

Therefore, ALDH1 may play a significant role in metabolism of acetaldehyde derived from ethanol despite its high K_m. How far the variation in ALDH1 activity influences alcohol use and dependence is not clear, and the issue is complicated by the probable existence of effects of drinking on ALDH activity.

Multiple reports have appeared of decreased erythrocyte ALDH activity in alcoholics compared with control subjects, and use of this measurement as a marker of alcohol intake has been suggested (Agarwal *et al*., 1983; Lin *et al*., 1984; Matthewson and Record, 1986; Towell, *et al*., 1986; Johnson *et al*., 1992; Helander, 1993). Similar decreases have been reported in hepatic ALDH in alcoholics (Jenkins *et al*., 1984), although ALDH1 (Jenkins and Peters, 1980; Thomas *et al*., 1982), ALDH2 (Bohme *et al*., 1991; Panes *et al*., 1989; Takase *et al*., 1989), or both (Meier-Tackmann *et al*., 1988)

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may be affected. It is possible that ALDH1 is more affected by alcohol, and ALDH2 more by liver diseases.

A significant correlation ($r = 0.94$) between erythrocyte and hepatic cytosolic ALDH activity was found in normal subjects (Matthewson and Record, 1986), but not in alcoholics or patients with liver disease (Matthewson and Record, 1986; Zorzano *et al*., 1989). If this reported correlation of ALDH1 activities between organs is a general phenomenon, then investigation of ALDH activity in erythrocytes may allow a more general insight into the expression or activity of ALDH1 and its variation between people.

In addition to their role in alcohol metabolism, ALDHs are active against a number of other physiologically important aldehyde substrates. In particular, ALDH1 is important for the conversion of retinaldehyde to retinoic acid (Ambroziak *et al*., 1999; Molotkov and Duester, 2003), with possible implications for vitamin A metabolism and growth regulation (Duester, 1999) and their interactions with ethanol metabolism (Crabb *et al*., 2001). Second, ALDH1 is abundant in the eye, in both lens and cornea, and it has been suggested (King and Holmes, 1998) that it plays a protective role by metabolising malondialdehyde and long-chain aldehydes produced by exposure to ultra-violet radiation.

We have measured erythrocyte ALDH activity in a group of twin subjects whose alcohol consumption and alcohol dependence histories are well documented. The aims of this study were to test whether variation in cytoplasmic ALDH1 activity is associated with self-reported alcohol intake or the probability of alcohol dependence in a community-based cohort, whether low ALDH1 activity is associated with self-reported reactions to alcohol; and how far variation in this enzyme is subject to genetic effects.

SUBJECTS AND METHODS

Characteristics of the subjects participating in this study were described in a previous paper (Whitfield *et al*., 1998). They were recruited through the Australian Twin Registry for questionnaire- and interview-based studies on alcohol use, alcohol dependence, and co-morbid psychiatric conditions. They participated in a telephone interview in 1993–1994, and subsequently provided a blood sample. All subjects were twins, but in some cases only one member of a twin pair provided blood. They gave informed consent to the questionnaires, interview, and blood collection and the studies were approved by appropriate Ethics Committees.

The telephone interview used the SSAGA interview (Bucholz *et al*., 1994), which allows diagnosis of lifetime alcohol dependence by DSM-IIIR criteria. The interview also contained questions about unpleasant reactions to small quantities of alcohol, including facial flushing, palpitations, nausea, sleepiness, or headache, asking whether each of these had ever occurred and whether they always occurred (Slutske *et al*., 1995).

Information on ethnicity (the place of birth and national origin of each subject's four grandparents) was gathered at the time of blood collection. This showed that the proportion of non-European ancestry among the study participants was ~5%. The Chinese, Japanese, or Korean groups (who might have had ALDH2 deficiency) made up <0.2% of the cohort.

Since information on smoking was not gathered at the time of blood collection, it was taken from self-reports in an earlier survey in 1988 (when smoking status was ascertained for 5538 subjects from this cohort) and in a smaller but near-contemporary one in 1993–1996 (for 1573 subjects). On each occasion, subjects categorized themselves as never having smoked, as being an ex-smoker, or as a current smoker. For the 1432 subjects from whom there was smoking information on both occasions, there was good agreement (kappa = 0.744, Spearman rank correlation = 0.883).

In 1993–1996 blood was collected from 1134 men and 2241 women. Immediately before blood collection, subjects filled in a table asking how many drinks containing alcohol (10 g) they had taken on each of the preceding seven days, divided into beer, wine, spirits, fortified wine, or 'others'. The numbers of drinks were summed to obtain a total for the past week. This total showed a good correlation with a weekly drinking estimate based on habitual quantity and frequency of alcohol use previously provided by the subjects, during the telephone interview ($r = 0.78$ for men, 0.75 for women, on log-transformed estimates).

Blood was collected by venepuncture and centrifuged. After removal of plasma the erythrocytes were washed twice with phosphate-buffered saline, lysed by addition of distilled water (3.0 ml of distilled water to 1.0 ml of erythrocytes), and mixed with 1.0 ml of sucrose solution (200 g/l) as described by Johnson *et al*. (1992). The haemolysate was stored at −70°C until analysis. ALDH1 activity was estimated by kinetic spectrophotometry with acetaldehyde (10 mM) and NAD (2.1 μM) as substrates (Johnson *et al*., 1992), using a Roche Cobas FARA analyser, and enzyme activity was expressed as mU/g of haemoglobin (mU/g Hb).

ALDH1 activity was measured in 677 individuals. Results for 30 individuals were dropped as their blood was not processed until the day following collection and this paradoxically resulted in higher ALDH1 activity (mean activity = 246 vs 167 mU/g Hb for individuals with blood processed on the same day as collection). The remaining individuals came from 435 families and data were obtained from both co-twins in 212 families [comprising 103 monozygotic (MZ) female pairs, 33 MZ male pairs, 37 dizygotic (DZ) female pairs, 8 DZ male pairs, and 31 opposite sex (DZO) pairs] and from one twin only in 223 families. The mean age of participants with ALDH1 results was 46.6 years (SD = 12.1) with a range of 30.5–83.9 years.

Zygosity was determined from responses to a self-report questionnaire containing items on physical similarity and the degree to which the twins were mistaken for each other when young. A recent analysis (Heath *et al*., 2003) comparing the best-estimate zygosity diagnosis from these questions with latent class analysis, gave ~0.2% error rate when the estimate was MZ and 1.1% when the estimate was DZ. The latent class analysis in turn gave <1% error rate when compared with genotyping; so we estimate that the zygosity assignments are >98% correct.

Statistical procedures

Analyses utilized the method of maximum-likelihood estimation from raw data observations (Neale and Cardon, 1992). Assumptions that means and variances for ALDH1 did not differ based on the birth order, zygosity, or sex of the

participants were tested. Increasingly constrained models in which means, and then variances, were set equal for first- and second-born same-sex co-twins, and then for MZ and DZ twins of the same sex, were compared with a fully saturated model in which all means and variances were free to vary. The variance for males was set equal to that of females, and to examine whether the means for males and females differed, a deviation for maleness was dropped from the model. The fit of each model was compared with that of the model within which it was nested by likelihood ratio test. Mean effects were also examined for age, sex vs age, number of alcoholic drinks consumed in the week before blood collection, alcohol dependence, flushing in relation to alcohol consumption, and smoking status.

Twin correlations were examined for the influence of sex by setting the correlation between MZF co-twins to be equal to that of MZM co-twins and by setting correlations between DZF and DZM co-twins to be equal and comparing with a model in which twin correlations were free to vary. Further models were compared to determine whether the correlation for DZ opposite-sex pairs could be set equal to the same-sex DZ correlation. Collapsing across sex would allow one MZ and one DZ group to be examined in the univariate genetic analyses.

Variance in ALDH1 activity owing to individual differences was decomposed into additive genetic (A), common environmental (C), and unshared environmental (E) sources of influence. Constrained models containing A and E, C and E, or only E sources of influence were compared with a fully saturated model containing A, C, and E, with the best-fitting model being determined by both fit and parsimony.

Analyses, with the exception of conventional analysis of variance, which was performed in SPSS for Windows Version 11.5 (SPSS Inc., 1989–2002), were performed using the structural equation modeling software Mx [User Interface Version 1.1 (Neale, 1999)]. Familywise outliers were identified using the *%P* option in Mx. The criterion for dropping outlying family data was a *Z*-score of >±3. Outliers accounted for <1% of the dataset and analyses were rerun after they were dropped.

RESULTS

ALDH1 was normally distributed among the sample population with a mean of 166 mU/g Hb (SD = 59) and a range of 3.3–327. This was comparable with the range reported by the originators of this method (23–465 mU/g Hb overall, in two groups of teetotalers and alcoholics).

No differences in mean or variance were found for birth order ($\Delta - 2LL = 1.71$ and 0.00, df = 4, $P > 0.05$), zygosity ($\Delta - 2LL = 0.21$ and 0.00, df = 4, $P > 0.05$) or sex of participant ($\Delta - 2LL = 0.00$ for both, df = 1, $P > 0.05$). Furthermore, recent alcohol consumption, alcohol dependence, flushing in relation to alcohol consumption, and smoking status were found to have no mean effect on ALDH1 (Table 1).

In women, but not men, ALDH activity was clearly lower in those aged <45 years but the maximum-likelihood test for age vs sex interaction was not significant. When only women were considered, and the age effect (<45 years against ⩾45 years) was subject to a conventional analysis of variance, we found a highly significant difference ($F_{1,438} = 12.13$, $P = 0.0005$). Halving of the number of degrees of freedom to take account of the fact that the data were from pairs of twins (which is a very conservative assumption), gave $F_{1,219} = 12.13$, $P = 0.0006$. Since the difference between older and younger women occurred at about the expected age of menopause, we tested whether a division into premenopausal and post-natural menopause groups showed a greater difference in ALDH1 activity than the division by age (< 45 against ⩾45). However, menopausal status gave a less significant result than that for age ($F_{1,305} = 4.80$, $P = 0.029$).

Table 1. Mean effects on erythrocyte aldehyde dehydrogenase activity owing to age, sex vs age, number of alcoholic drinks consumed in the week before blood collection, lifetime alcohol dependence, flushing because of alcoholic drink consumption (ever/always) and smoking status

	Δ − 2LL	Δdf	*P*	Group	Mean (SD), *N*
Age[a]	0.98	1	0.32	<45 years ⩾45 years	160.4 (56.9), 361 172.6 (61.1), 284
Sex vs age	2.32	1	0.13	M < 45 years M ⩾ 45 years F < 45 years F ⩾ 45 years	169.7 (60.1), 128 166.2 (51.9), 77 155.3 (54.5), 233 175.0 (64.1), 207
Drinks in past week[a]	1.99	1	0.16	1–7 ⩾8	166.8 (60.2), 457 162.5 (56.0), 186
Alcohol dependence	0.11	1	0.74	Yes No	175.8 (59.3), 85 164.4 (59.0), 552
Ever flush with drink	1.17	1	0.28	Yes No	164.9 (57.2), 162 164.9 (59.1), 465
Always flush with drink	2.21	1	0.14	Yes No	169.7 (62.6), 43 164.5 (58.3), 584
Smoking status	0.26	1	0.61	Current Non/ex	158.0 (63.9), 89 169.2 (58.6), 507

[a]Treated as continuous variables in this analysis.

Table 2. Fit indices and estimates for alternative models of sources of variation in erythrocyte aldehyde dehydrogenase activity

Model	Tested against	−2LL	df	Δ − 2LL	Δdf	*P*	a^2	c^2	e^2
1. ACE		995.3	370				0.56	0.00	0.44
2. **AE**	**1**	**995.3**	**371**	**0.0**	**1**	**1.00**	**0.56**	**0.00**	**0.44**
3. CE	1	998.4	371	3.1	1	0.08	0.00	0.51	0.49
4. E	1	1035.1	372	39.8	2	0.00	0.00	0.00	1.00

A, variance owing to additive genetic factors (a^2 = % of variance influenced by A, heritability); C, variance owing to common environmental factors (c^2 = % of variance influenced by C); E, variance owing to unshared environmental factors (e^2 = % of variance influenced by E). The best-fitting model is shown in bold.

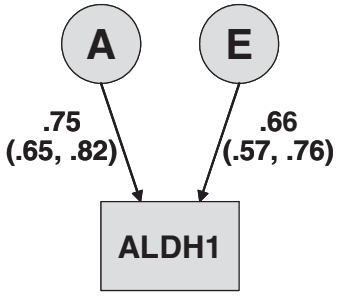

Fig. 1. Best-fitting model of additive genetic (A) and unshared environmental (E) influence on erythrocyte aldehyde dehydrogenase (ALDH1) activity. Path coefficients are standardized such that when squared they indicate the percentage of variance accounted for. CI (95%) are shown in brackets.

Twin correlations were examined and the correlation between MZF co-twins could be set equal to that of MZM co-twins, and similarly, the correlation between DZF co-twins could be set equal to that of DZM co-twins (Δ − 2LL = 2.95, df = 2, $P = 0.23$). In addition, the correlation between DZO co-twins could be set equal to that of the same sex DZ co-twins (Δ − 2LL = 1.52, df = 1, $P = 0.22$). Thus, data from MZ and DZ groups could be collapsed across sex, resulting in an MZ twin correlation of 0.53 [95% confidence intervals (CIs) = 0.39–0.63] and a DZ twin correlation of 0.36 (CI = 0.14–0.53). These correlations (i.e. $r_{MZ} > r_{DZ}$) suggest that ALDH1 is influenced by genes, although the broad confidence intervals owing to small numbers, particularly for the DZ correlation, indicate a lack of power to discriminate between genetic and shared-environmental hypotheses for familial aggregation.

Univariate genetic models were examined to determine the sources of variance influencing ALDH1. A full model containing additive genetic (A), common environmental (C), and unshared environmental (E) sources of influence was compared with models containing only AE, CE, and E influences (Table 2). Familial influence was found to be a significant contributor to variance, as a model containing only E influences was a significantly worse fit to the data when compared with the full ACE model. However, neither the AE nor the CE model showed significant loss of fit when compared with the full ACE model. Therefore, to determine the appropriateness of the AE and CE models, parameter estimates for the ACE model were examined. These estimates indicated that C was not influential (i.e. $c^2 = 0$) and consequently, the AE model (Fig. 1) was chosen as the best-fitting model. Genetic influences were found to account for 56% (CI = 42–67%) of the variance in ALDH1, whereas environmental influences unique to the individual accounted for the remaining 44% (CI = 32–58%) of the variance.

DISCUSSION

Many previous studies (cited above) have found that high alcohol intake or 'alcoholism' is associated with lower erythrocyte ALDH activity. This might be owing to alcohol, or perhaps acetaldehyde formed during the metabolism of alcohol, inactivating ALDH1. Although this may be the case when alcohol-dependent and control groups are compared, we did not find this association among our subjects. The lack of association with current alcohol intake may be owing to the small number of very heavy drinkers or active alcoholics among these subjects, but it weakens the case for the use of erythrocyte ALDH as a 'state marker' of alcohol intake.

Moreover, there was no evidence that low ALDH1 activity is associated with either alcohol-related flushing or a reduction in the prevalence of alcohol dependence. Alcohol-related flushing was reasonably common in this cohort, as previously reported (Slutske *et al*., 1995), and of the subjects with ALDH results, 7% reported that they always flush after alcohol and 26% reported that this had occurred at some time. The previous reports of European subjects with alcohol-related flushing and low ALDH1 activity, or variation in ALDH1 kinetic properties, may have arisen from a strategy of screening only subjects with known alcohol-induced flushing for ALDH1 activity. The causes of alcohol-related flushing among Europeans are largely unknown but are probably not ALDH-related in most cases (Whitfield, 1997).

The lower ALDH1 activity in younger women was statistically significant in a one-way analysis of variance on female subjects only, but not in the maximum-likelihood analysis where a test for sex vs age interaction was used. This discrepancy is ascribed to the lack of power to test for interaction compared with tests for main effects, particularly because of the small number of male subjects. This age effect in women, occurring at about the age of menopause, might be associated with hormonal effects on ALDH1 activity and it may be relevant that estrogen effects on acetaldehyde concentrations after alcohol consumption have been reported (Eriksson *et al*., 1996). However, we did not find that menopausal status provided any stronger association than age alone, and this age effect in women remains unexplained. Owing to the wide range of oral contraceptive preparations which may have been used by the younger women, and because of lack of good information

on this point, we did not test for possible effects of oral contraceptives on ALDH1 activity.

A wide range of ALDH1 values was encountered. The 95% range was from 48 to 284 mU/g Hb, a 5- to 6-fold range of activity. Part of this variation in ALDH1 activity could be owing to varying length of storage or variation in the change of activity over time. However, previous authors (Johnson *et al*., 1992) have also described a wide range of ALDH1 activity values within control or alcoholic groups, and the range of activities we found was similar to that reported by the originators of this method.

Since the ALDH1 activity expressed is relative to the haemoglobin concentration in the stored samples, variation in erythrocyte haemoglobin concentration (MCHC) could affect the calculated ALDH activity. However, the range of MCHC values in normal subjects is narrow (~320–360 g/l) and so this factor is unlikely to contribute significantly to the wide range of ALDH values observed.

Just over half the variation appears to be genetic in origin. If this can be applied to variation in ALDH1 activity in other organs, as suggested in one report (Matthewson and Record, 1986), the metabolism of physiological substrates of ALDH1 could be substantially affected by genetic variation. The observed variation in ALDH1 activity could, therefore, affect such diverse areas as growth regulation and the foetal alcohol syndrome (Zachman and Grummer, 1998), protection of vision against ultraviolet radiation and oxidative stress (King and Holmes, 1998), cyclophosphamide metabolism (Dockham *et al*., 1997), and perhaps even the macrovascular complications of diabetes (Jerntorp *et al*., 1986).

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