Towards a molecular epidemiology of alcohol dependence: analysing the interplay of genetic and environmental risk factors

A. C. HEATH, J. B. WHITFIELD, P. A. F. MADDEN, K. K. BUCHOLZ, S. H. DINWIDDIE, W. S. SLUTSKE, L. J. BIERUT, D. B. STATHAM and NICHOLAS G. MARTIN

Background Progress in identifying genetic factors protective against alcohol dependence (AlcD) requires a paradigm shift in psychiatric epidemiology.

Aims To integrate analysis of research into the genetics of alcoholism.

Method Data from prospective questionnaire and interview surveys of the Australian twin panel, and from a subsample who underwent alcohol challenge, were analysed.

Results In men, effects of alcohol dehydrogenase *ADH2*1/*2* genotype or high alcohol sensitivity (risk-decreasing), and of history of childhood conduct disorder, or having monozygotic co-twin or twin sister with AlcD (risk-increasing) were significant and comparable in magnitude. Religious affiliation (Anglican versus other) was associated with the *ADH2* genotype, but did not explain the associations with AlcD symptoms. No protective effect of the *ADH2*1/*2* genotype was observed in women.

Conclusions The early onset and strong familial aggregation of AlcD, and opportunity for within-family tests of genetic association to avoid confounding effects, make epidemiological family studies of adolescents and young adults and their families a priority.

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Epidemiological studies on local or national probability samples of unrelated individuals such as those by Regier *et al* (1990), Kessler *et al* (1996) and Grant (1997) have helped characterise the population distribution of alcohol dependence, its socio-demographic correlates and associations with other psychiatric disorders. Such studies have, however, largely ignored the most striking feature of alcohol dependence: its strong and apparently genetically determined familial aggregation (reviewed in McGue, 1994; Heath *et al*, 1997*b*). Positive reports are beginning to emerge from genetic linkage studies of alcohol dependence, albeit without strong replication of findings as yet (Long *et al*, 1998; Reich *et al*, 1998), which may ultimately lead to the identification of new genetic risk or protective factors. As in other areas of psychiatry, alcoholism researchers face the challenge of developing new paradigms for 'molecular epidemiology', which seeks to incorporate assessment of genetic risk into epidemiological studies on general population samples.

REVIEW OF PAST PROGRESS

Aldehyde dehydrogenase gene (*ALDH2*)

Unlike most other areas of psychiatric research, the subject of alcoholism benefits from the identification of at least two genetic polymorphisms which, in samples of Asian ancestry, have been found to be associated with differences in alcohol dependence risk. These can serve as a model system for understanding some of the challenges that will be faced in molecular epidemiological research on alcoholism. For example, Table 1 summarises ratios of the penetrances (i.e. probabilities of being an alcoholism case) of the different genotypes for two genes coding for enzymes involved in the metabolism of alcohol, aldehyde dehydrogenase (ALDH) and alcohol dehydrogenase (ADH), based on reanalyses of data on Japanese alcoholism case–control series published by Higuchi (Higuchi, 1994; Higuchi *et al*, 1994). Ethanol is converted by the enzyme alcohol dehydrogenase to the toxic metabolite acetaldehyde, which is in turn converted by the enzyme aldehyde dehydrogenase to acetate.

Possession of a single *ALDH2*2* allele (a variant form that is rarely observed in samples of European or African–American ancestry) is associated with impaired conversion of aldehyde dehydrogenase to acetate, leading to substantially elevated blood acetaldehyde concentrations after ingestion of alcohol (Yamamoto *et al*, 1993; Wall *et al*, 1997) and a characteristic flushing response, which is associated with a decreased risk of alcohol dependence (Goedde *et al*, 1992). Individuals who are *ALDH2*2/*2* homozygotes (i.e. have two copies of the variant allele) have such an extremely adverse reaction to even moderate doses of alcohol that not a single occurrence of this genotype has been found in large series of alcoholic subjects in Japan (see Table 1; Higuchi *et al*, 1994; Nakamura *et al*, 1996; Tanaka *et al*, 1996), China (Shen *et al*, 1997) and Taiwan (Chen *et al*, 1996). Other studies have shown that the effects of a single *ALDH2*2* allele may be dependent upon drinking course: while the allele serves as a protective factor against development of alcohol problems, in those who nevertheless progress to heavier drinking, the impaired metabolism of alcoholism in turn becomes associated with increased risk of alcohol-related medical complications including alcohol-induced asthma (Takao *et al*, 1998) and alcohol-related cancers (oropharyngeal, stomach, colon and lung cancers; Yokoyama *et al*, 1998).

Research by Higuchi and colleagues has also demonstrated the importance of considering jointly the interplay of genetic and environmental factors. Higuchi *et al* (1994) have noted that the proportion of *ALDH2*1/*2* heterozygotes in male alcoholic series has been increasing over time, perhaps because of recent increases in the social pressures of drinking together after work among Japanese men. We may compute from their data that the risk ratio comparing *ALDH2*1/*1* homozygotes with *ALDH2*1/*2* heterozygotes has declined from approximately 25:1 in 1979 to 4:1 in 1992 (see Table 1). Furthermore, while the *ALDH2*1/*1* genotype is associated with increased risk, the overall probability of alcohol dependence associated with that

Table 1 Penetrance ratios for alcoholism in Japanese men, estimated from published data (Higuchi, 1994; Higuchi *et al*, 1994) on *ALDH2* and *ADH2* genotype frequencies in alcoholic and control series

ALDH2 genotype	Penetrance ratios			*ADH2* genotype	Penetrance ratios
	1979	1986	1992		
*ALDH2*1/*1*	$f_{2(79)}$	$f_{2(86)}$	$f_{2(92)}$	*ADH2*1/*1*	f_2*
*ALDH2*1/*2*	$0.043f_{2(79)}$	$0.14f_{2(86)}$	$0.25f_{2(92)}$	*ADH2*1/*2*	$0.23f_2$*
*ALDH2*2/*2*	0	0	0	*ADH2*2/*2*	$0.15f_2$*

f_2 denotes the penetrance, i.e. the estimated probability of a history of alcohol dependence, in *ALDH2*1/*1* homozygous Japanese males; f_2* denotes the corresponding probability in *ALDH2*1/*1* homozygotes who are also *ADH2*1/*1* homozygotes. For *ALDH2* genotypes, subscripts are used to denote cohort-specific penetrances. The penetrance of the *ALDH2*1/*2* genotype was computed as $(a_1b_2/a_2b_1)f_2$, where a_1 and a_2 are the proportions of controls, and b_1 and b_2 the proportions of cases, with **1/*1* and **1/*2* genotypes respectively, with similar computations for the *ADH2*1/*2* and *ADH2*2/*2* genotypes.

genotype (which is shared by almost all those of European and African–American ancestry) is not great.

Higuchi *et al* (1996) presented data from a small general community sample of men (n=230); only 16% of men with the *ALDH2*1/*1* genotypes were estimated to have a history of alcoholism based on their questionnaire responses. The penetrance of the *ALDH2*1/*1* genotype is thus quite modest, suggesting that environmental (or additional genetic) factors must be having important effects. For the same community sample, Higuchi *et al* (1996) also presented data showing significant effects of *ALDH2* genotype on self-reported alcohol consumption levels. From these data we may estimate that the *ALDH2* locus accounts for approximately one-third of the variance in alcohol consumption in that sample. Compared with men homozygous for the low-risk *ALDH2*2/*2* genotype, men homozygous for the high-risk genotype reported monthly alcohol consumption levels that were on average 10-fold higher, the corresponding difference in women being approximately 30-fold. However, the same data also point to the importance of sociocultural influences. Japanese women with the high-risk genotype were drinking at approximately the same level as men with the low-risk genotype, consistent with sociocultural restraints on drinking by Japanese women.

There is no strong tradition of psychiatric epidemiological research on general population samples in Japan. Little is therefore known about how socio-demographic factors, other environmental risk factors, or other history of psychopathology, may moderate *ALDH2* genotype-specific risks of alcohol dependence. Other Asian countries, notably Korea and Taiwan (Helzer *et al*, 1990), have conducted large-scale epidemiological surveys modelled after the US Epidemiologic Catchment Area (ECA; Regier *et al*, 1990) study, but at a time when large-scale collection of genotypic data would not have been feasible. However, a protective effect of the alcohol dehydrogenase *ADH2* locus, albeit one more modest than that associated with the *ALDH2*2* allele, has also been reported in individuals of Asian ancestry; and this *ADH2* locus, and the closely associated *ADH3* locus, are polymorphic in individuals of European and African–American ancestry (Goedde *et al*, 1992; Whitfield *et al*, 1998) as well as in Jewish men in Israel (Neumark *et al*, 1998).

Alcohol dehydrogenase genes (*ADH2*, *ADH3*)

Alcohol dehydrogenase, which is responsible for most of the conversion of alcohol to acetaldehyde, is formed by the combination of three different subunits (alpha, beta, gamma) encoded by closely linked loci on chromosome 4, *ADH1*, *ADH2* and *ADH3*, of which only the two latter are known to be polymorphic. The low-risk *ADH2*2* allele encodes the beta-2 subunit associated with faster metabolism of alcohol (and therefore a faster increase in toxic acetaldehyde levels; Thomasson *et al*, 1991) than the beta-1 subunit associated with the *ADH2*1* allele (Edenberg & Bosron, 1997). The *ADH2*1* allele has been found to be positively associated with increased risk of alcohol dependence in Japanese people, in the work of Higuchi (Higuchi, 1994; see Table 1), as well as in Han Chinese (Thomasson *et al*, 1991; Muramatsu *et al*, 1995; Shen *et al*, 1997) and Atayal Taiwanese (Thomasson *et al*, 1994). In the alcoholic case–control series reported by Higuchi *et al* (1994), in those who were *ALDH2*1/*1* homozygotes, the risk of alcoholism in *ADH2*1/*1* genotypes may be estimated as being approximately four times as great as that for *ADH2*1/*2* heterozygotes, and six to seven times as great as that for *ADH2*2/*2* homozygotes. However, from the small community series (Higuchi *et al*, 1996) the risk of alcoholism in men with both risk-increasing genotypes (*ADH2*1/*1* and *ALDH2*1/*1* homozygotes) is only 29%.

Several reports have also stated that the *ADH3*1* allele, which encodes the gamma-1 subunit associated with faster ethanol metabolism than the gamma-2 subunit encoded by *ADH3*2*, is associated with decreased alcohol dependence (e.g. Chen *et al*, 1996; Higuchi *et al*, 1996; Nakamura *et al*, 1996; Shen *et al*, 1997). However, strong linkage disequilibrium (i.e. correlation between the alleles at the two loci) is observed between *ADH2* and *ADH3* (Higuchi, 1994), a factor that has been ignored in most analyses, and may thus cause the evidence for the protective effect of the *ADH3*1* allele to be overstated. In one study which examined this possibility, the protective effect of the *ADH3*1* allele was no longer observed once linkage disequilibrium between the *ADH3*2* allele and the *ADH2*1* allele was controlled for (Osier *et al*, 1999).

AIMS

The *ADH2* locus is moderately polymorphic, and the *ADH3* locus more highly polymorphic, in samples of populations of European ancestry (e.g. Goedde *et al*, 1992). We have published elsewhere papers presenting evidence for an important genetic contribution to alcohol dependence risk in the Australian twin panel (Heath *et al*, 1997*a*); the mediating role of a history of conduct problems (Slutske *et al*, 1997) and genetically determined differences in alcohol sensitivity, as assessed in an alcohol challenge paradigm (Heath *et al*, 1999); and the absence of any major effect of genetically determined differences in blood alcohol metabolism (Grant *et al*, 1999). We have also studied socio-demographic correlates of alcohol dependence risk, including effects of birth cohort, religious involvement and education (Heath *et al*, 1997*a*). A subsample of the Australian twin panel, who participated in an alcohol challenge study, have been genotyped at the *ADH2* and *ADH3* loci, with results generally

consistent with a protective effect of the *ADH2*1/*2* genotype, at least in men (Whitfield *et al*, 1998). Here we attempt an integrated analysis of these data, which illustrates some of the challenges that must be faced in molecular epidemiological research into alcoholism.

METHOD

Sample

Characteristics of the original alcohol challenge sample (Martin *et al*, 1985*a*,*b*) and of the subsamples participating in follow-up assessments (Whitfield *et al*, 1998; Heath *et al*, 1999) have been described in detail elsewhere. Participants in the Australian Alcohol Challenge Twin Study (AACTS), conducted in 1979–1981, were volunteers, recruited chiefly from a volunteer national twin panel, the Australian Twin Register (ATR), which was developed with support from the Australian National Health and Medical Research Council. A total of 206 young adult twin pairs born 1944–1963 successfully completed the original alcohol challenge protocol, including 43 monozygotic (MZ) male, 45 MZ female, 37 dizygotic (DZ) male, 42 DZ female and 39 DZ unlike-sex pairs, i.e. a total of 199 men and 213 women. A mailed questionnaire survey was completed by both members of 133 complete pairs, and 16 singleton twins. An additional 3676 complete twin pairs and 551 single twins born 1893–1964, also recruited from the ATR, participated in the same mailed questionnaire survey, in 1980–1982, allowing characterisation of differences between volunteers for the alcohol challenge study and other same-age registrants with the ATR. Female AACTS participants were disproportionately heavy drinkers, whereas male participants were broadly representative of the ATR (Heath *et al*, 1998, 1999).

In 1992–1994 a telephone interview follow-up survey, which included assessments of history of alcohol dependence and other psychopathology (Heath *et al*, 1997*a*), was conducted with alcohol challenge participants and with most of the pairs who had participated in the questionnaire survey. Follow-up rates for men were 81.4% for the alcohol challenge participants (n=162), and 82.5% for other eligible males from the questionnaire sample (including 1584 men born 1944–1963); for women the rates were 87.4% for alcohol challenge participants (n=187) and 88.3% (2628 women born 1944–1963) respectively.

Assessments

Results of the alcohol challenge study (Martin *et al*, 1985*a*,*b*; Heath & Martin, 1992; Whitfield *et al*, 1998), the 1981 questionnaire survey (Kendler *et al*, 1986) and the 1992–1994 telephone diagnostic interview follow-up assessments (Heath *et al*, 1997*a*) have been reported in detail. For analyses reported here, a summary alcohol sensitivity score was derived for alcohol challenge participants by principal components analysis of subjective intoxication rating and increase in static ataxia in 'eyes open' and 'eyes closed' conditions, at the first post-alcohol assessment point (Heath *et al*, 1999). An assessment of DSM–III–R (American Psychiatric Association, 1987) alcohol dependence was adapted for telephone administration from the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA; Bucholz *et al*, 1994), and from this an approximate algorithm for DSM–IV (American Psychiatric Association, 1994) alcohol dependence could also be derived (no clustering information according to DSM–IV criteria – which did not exist at the time of the survey – was available).

Assessments of religious affiliation and of social attitudes (Martin *et al*, 1986), used as indicators of possible population stratification effects, were included in the 1981 questionnaire survey. The *ADH2* and *ADH3* genotypes for 369 participants in the alcohol challenge study (176 men, 193 women) were determined, either at the time of interview follow-up, or at a separate follow-up of AACTS participants (Whitfield *et al*, 1998), using DNA extracted from white blood cells, polymerase chain reaction (PCR) and restriction digestion, followed by electrophoresis of the PCR products (von Wartburg *et al*, 1988; Xu *et al*, 1988). No *ADH2*2/*2* homozygotes were observed in the sample.

Analyses

Alcohol dependence was analysed both as a binary variable and as a quantitative symptom count. For the latter purpose, the number of DSM–III–R alcohol dependence symptoms (e.g. tolerance, withdrawal and withdrawal relief) reported by a respondent was log transformed ($\ln(x+1)$) prior to analysis. As a summary measure of high alcohol sensitivity, a dummy variable was created to indicate whether a participant in the alcohol challenge study had an alcohol sensitivity principal component score that fell in the upper quartile of the sensitivity distribution. Multiple regression and multiple logistic regression were used to identify predictors of dependence symptom count or diagnosis. Since there was significant evidence for linkage disequilibrium between *ADH2* and *ADH3* (Whitfield *et al*, 1998), tests for *ADH3* effects always included *ADH2* as a covariate. Robust variance estimators were used to derive estimates of 95% confidence intervals or standard errors of parameter estimates, using STATA software (StataCorp, 1999) to adjust for the non-independence of observations on twin pairs, which would otherwise inflate estimates of statistical significance.

Where statistical tests were clearly non-significant even without adjustment for non-independence, unadjusted test statistics are reported. In some analyses, we included variables that were assessed for the entire sample (e.g. socio-demographic measures and history of psychopathology) and variables assessed only on the subsample of participants in the alcohol challenge sample (*ADH2*, *ADH3* genotypes and the summary measure of alcohol sensitivity). A dummy variable was created indicating whether a respondent had participated in the alcohol challenge study, values for non-participants being set to zero. This allowed us to estimate effects both of variables that had been assessed for the entire sample, and of those that could only be assessed in challenge study participants.

RESULTS

ADH2, *ADH3* and alcohol dependence risk

Table 2 summarises the joint association in the Australian alcohol challenge sample of *ADH2* and *ADH3* genotypes with a history of alcohol dependence (DSM–III–R and approximated DSM–IV) at follow-up in 1992–1994. Associations with alcohol dependence symptom count measures are also shown. Once we adjusted for the non-independence of observations on twin pairs, there was no overall association between history of alcohol dependence assessed as a binary variable and *ADH2* genotype in either men or women, though there was a trend for reduced risk of DSM–III–R alcohol dependence in male

Table 2 Associations between alcohol dehydrogenase genotypes (*ADH2*, *ADH3*) and history of alcohol dependence (DSM–III–R or DSM–IV), and DSM–III–R alcohol dependence symptom count in the Australian Twin Register

Genotype		Alcohol dependence						Log-transformed alcohol dependence symptom count			
		Women			Men			Women		Men	
		n	DSM–III–R (%)	DSM–IV (%)	*n*	DSM–III–R (%)	DSM–IV (%)	$\bar{X}$	95% CI	$\bar{X}$	95% CI
ADH2											
*ADH2*1/*1*		171	14	12.3	140	25.7	19.3	0.53	0.42–0.64	0.88	0.77–0.99
*ADH2*1/*2*		11	27.3	18.2	19	5.3	10.5	0.6	0.01–1.19	0.47	0.18–0.76
*ADH2*1/*1 v. ADHD2*1/*2*			NS	NS		NS	NS	NS		$P<0.01$	
ADH3											
ADH2 genotype	*ADH3 genotype*										
**1/*2*	*Any*[1]	11	27.3	18.2	19	5.3	10.5	0.60	0.04–1.16	0.47	0.18–0.76
**1/*1*	**1/*1*	43	20.9	18.6	40	27.5	15.0	0.68	0.45–0.91	0.78	0.58–0.98
**1/*1*	**1/*2*	101	9.9	8.9	75	21.3	20.0	0.47	0.34–0.60	0.91	0.77–1.05
**1/*1*	**2/*2*	26	19.2	15.4	24	33.3	25.0	0.51	0.18–0.84	0.94	0.68–1.19
Linear trend		NS		NS		NS	NS	NS		$P<0.01$	

1. Because *ADH2*1/*2* heterozygotes were rare, we have collapsed across *ADH3* genotypes for this group.

*ADH2*1/*2* heterozygotes, consistent with prediction (OR=0.16, 95% CI 0.02–1.23). There was, however, a significantly reduced alcohol dependence (DSM–III–R) symptom count for heterozygous men. When information on *ADH3* genotype was included, no overall trend for an association of this genotype with alcohol dependence or symptom count was observed, except in the case of alcohol dependence symptom count in men. This effect was entirely explained by the protective effect of the *ADH2*1/*2* genotype, with no significant evidence for an additional effect of the *ADH3* genotype (unadjusted $F_{2,154}=0.26$, $P=0.77$).

Predictors of alcohol dependence symptoms

Since we found no evidence for associations between *ADH2* genotype and alcohol dependence symptoms in women, subsequent analyses focused only on data from the Australian male twins. Table 3 summarises major predictors of alcohol dependence symptom count in these male twins. These data are based on all male twins born 1944–1963, with missing data on those who did not participate in the alcohol challenge study handled as described in the Method section. Columns 2 and 3 of Table 3 summarise regression coefficients from

Table 3 Results of separate and joint analyses predicting male respondents' log-transformed alcohol dependence symptom counts from own ('individual predictor') and co-twin characteristics. Unstandardised regression coefficient (β) and robust standard errors (s.e.) are tabulated

	Separate analyses		Joint analysis of individual and co-twin predictors	
	β	s.e.	β	s.e.
Individual predictors				
Not AACTS participant[1]	−0.13	0.06	−0.11	0.06
*ADH2*1/*2* genotype	−0.38	0.17	−0.31[NS]	0.16
High alcohol sensitivity[2]	−0.45	0.10	−0.36	0.09
Birth cohort 1955–1959	0.11	0.04	0.10	0.04
Other Protestant religion	−0.23	0.04	−0.2	0.04
Tertiary education	−0.10	0.04	−0.10	0.03
Childhood conduct disorder	0.38	0.04	0.35	0.04
History of major depression	0.25	0.05	0.22	0.04
Intercept	0.89	0.07	0.91	0.06
	$R^2=0.133$		–	
Co-twin predictors				
MZ co-twin alcohol dependent	0.42	0.06	0.33	0.06
DZ male co-twin alcohol dependent	0.10[NS]	0.08	0.05[NS]	0.08
DZ female co-twin alcohol dependent	0.43	0.10	0.38	0.10
MZ male co-twin unaffected	−0.24	0.05	−0.20	0.04
DZ male co-twin unaffected	−0.08[NS]	0.05	−0.09[NS]	0.05
Intercept	0.84	0.03	–	–
	$R^2=0.098$		$R^2=0.198$	

1. Respondent did not participate in the alcohol challenge study, has unknown status on *ADH2*1/*2* genotype and alcohol sensitivity variables.
2. Respondent scored in the highest quartile on the distribution of alcohol sensitivity scores.
AACTS, Australian Alcohol Challenge Twin Study; DZ, dizygotic; MZ, monozygotic.

Table 4 Sociocultural correlates of *ADH2*1/*2* genotype in the Australian Twin Register

	Genotype		OR	95% CI
	*ADH2*1/*1* (%)	*ADH2*1/*2* (%)		
Religious affiliation				
Church of England (CoE)	35.2	54.6	2.33^{NS}	0.93–5.84
Both parents CoE	30.9	52.2	3.44^{NS}	0.91–13.00
Single parent CoE	25.9	26.1	2.21^{NS}	0.50–9.72
Neither parent CoE	43.2	21.7	1.00	–
Social attitudes, 1981 survey[1] (% agreement with)				
Men and women:				
Computer music	W: 12.4 M: 18.2	50 30.8	3.09	1.02–9.38
Women only:				
Working mothers	21.5	75	10.93	2.25–52.97
Pyjama parties	27.5	85.7	16.29	1.72–154.23
Men only:				
Socialism	40.2	81.8	6.89	1.41–33.51

1. Using a standard methodology in social attitude research, respondents were asked to indicate whether they agreed with a series of one-word and two-word 'probe' items designed to assess high or low conservatism. See Martin *et al* (1986) for further details.

separate analyses which predicted the respondent's alcohol dependence symptom count from (a) characteristics of the respondent only, or (b) the self-report history of alcohol dependence of the respondent's co-twin, plus zygosity status of the twin pairs. Columns 4 and 5 list regression coefficients estimated under (c), a joint analysis including both sets of predictor variables.

In the first analysis, significant protective effects of the *ADH2*1/*2* genotype, as well as of high alcohol sensitivity assessed in the alcohol challenge paradigm, were observed even when socio-demographic effects on alcohol dependence symptom count were controlled for. The risk-decreasing effects of the *ADH2*1/*2* genotype, and of high alcohol sensitivity, were individually similar in magnitude to the risk-increasing effect of a childhood history of conduct disorder. There were more modest risk-increasing effects associated with a history of major depression, and with being born in the years 1955–1959, while 'other Protestant' religious affiliation and tertiary education were each associated with decreased symptom count. Participants in the alcohol challenge study reported marginally more alcohol dependence symptoms than non-participants, although this was not a strong effect.

The second analysis confirmed our previously reported evidence for genetic effects on risk, a history of alcohol dependence in an MZ co-twin being associated with a significantly elevated symptom count, compared with a positive history in a DZ co-twin, and absence of history of alcohol dependence in an MZ co-twin being associated with a significant reduction in alcohol dependence symptom count. Finally, in the joint analysis, the association between *ADH2*1/*2* genotype and reduced alcohol dependence symptom count was no longer significant, once standard errors were adjusted for the non-independence of observations on twin pairs. None the less, the estimated risk-increasing effects associated with having an alcohol-dependent MZ twin brother, or an alcohol-dependent DZ twin sister or of having a history of childhood conduct disorder, and the risk-decreasing effects of possessing an *ADH2*1/*2* genotype, or of showing high alcohol sensitivity, were all comparable in magnitude.

Anglican genes or population stratification effects?

The AACTS sample provides a striking illustration of the potential dangers of ignoring population stratification effects when a traditional case–control methodology is used to incorporate collection of genetic information in epidemiological research. Table 4 summarises the associations between religious affiliation – Church of England (CoE) versus 'Other' – and *ADH2* genotype. Although the associations fall just short of conventional significance levels, approximately one-third of those who were *ADH2*1/*1* homozygotes, but 55% of those who were *ADH2*1/*2* heterozygotes, reported a religious affiliation of CoE. The heterozygotes were also more likely to report that both biological parents had a CoE religious affiliation (52.2% *v.* 30.9%). Religious affiliation is an important correlate of a number of other sociocultural variables. It was therefore not surprising to observe several correlations between *ADH2* genotype and self-reported social attitudes (also shown in Table 4). It seems plausible that these represent associations introduced by population stratification effects, and rather less likely that a gene influencing alcohol metabolism has an indirect effect on social attitudes. Further analysis revealed, however, that religious affiliation was not associated with alcohol dependence symptom count in these data (unadjusted $F_{1,107}=1.04$, $P=0.31$), so that this aspect of the population stratification effect (i.e. the association between *ADH2*1/*2* genotype and CoE religious affiliation) could not account for the observed association with alcohol dependence symptom count.

CONCLUSION

In populations of predominantly European ancestry, twin and adoption studies (reviewed by McGue, 1994; Heath *et al*, 1997*b*), including diagnostic interview studies on large general population samples of twin pairs (Kendler *et al*, 1992, 1994; True *et al*, 1996; Heath *et al*, 1997*a*; Prescott & Kendler, 1999), have produced consistent evidence of a strong genetic contribution to differences in alcohol dependence risk, perhaps accounting for as much as 50–60% of the variance in risk. It is to be anticipated that current gene-mapping studies will ultimately be successful in identifying important but as yet unknown genetic risk factors for alcohol dependence. Because such gene-mapping studies typically use high-density pedigrees containing many affected family members, their findings cannot easily be generalised to the general population – a problem well illustrated in the field of breast cancer research, where a long delay occurred between identification of the risk-increasing *BRCA1* gene, and characterisation of its effects in the

general population (Couch, 1998; Newman *et al*, 1998). This problem may be even more pronounced in the case of alcoholism, where a high proportion of cases in the community remain untreated (e.g. Regier *et al*, 1990; True *et al*, 1996).

It is in principle both easy and cheap to incorporate DNA collection (by blood samples or buccal scraping) in epidemiological surveys of general population samples, or in case–control series, allowing typing of candidate genes that are believed to be risk or protective factors. However, because there may be pronounced gene frequency differences among individuals of different ancestries, there is a very real danger that studies using a traditional case–control methodology, which ignores possible population stratification effects, will generate innumerable false-positive findings. This danger is especially grave for addictive behaviours such as smoking or alcohol misuse, for which there are pronounced sociocultural variations in prevalence rates. Even in the case of functional polymorphisms, population stratification effects may be important confounders, as was illustrated by the association between the protective *ADH2*2* allele and an Anglican religious affiliation, and various associated social attitudes, in the Australian twin panel.

One solution to the problem of population stratification lies in targeting research efforts at population isolates such as those in Finland (e.g. Hastbacka *et al*, 1992) or Japan, which show much greater genetic homogeneity than societies such as the USA or Australia. Even within such societies, however, regional variations in patterns of substance use may be correlated with gene frequency differences, leading to the possibility of false-positive findings (Kittle *et al*, 1998). Furthermore, much lower rates of illicit drug problems in such societies may limit the generalisability of findings to US and other Western populations.

Use of within-family comparisons offers a much surer way to control for population stratification effects. This can be achieved most powerfully by the genotyping of both biological parents as well as the proband, in an approach popularised by Spielman and Ewens as the transmission disequilibrium test (TDT; Spielman *et al*, 1993; Ewens & Spielman, 1995). In comparisons limited to heterozygous parents, the frequencies with which a candidate allele versus other alleles are transmitted to an affected offspring are compared by McNemar's test or similar matched pairs techniques. While only heterozygous parents will be informative for a TDT analysis, using dummy variables to compare disease–candidate allele associations where the candidate allele has been transmitted from heterozygous versus homozygous parents can provide a check for whether stratification effects seem to be inflating the association observed in standard case–control comparisons.

A potential concern is the availability of parents for such investigations, considering the mortality associated with alcoholism and its associated behaviours (especially smoking: Hurt *et al*, 1996). However, this is less of an issue once it is recognised that epidemiological surveys of the origins of alcohol dependence need to focus on the period of adolescence and early adulthood. In the US National Comorbidity Survey, the median age of onset of alcohol dependence symptoms was approximately 20 years (Nelson *et al*, 1998), and the much larger US National Longitudinal Alcohol Epidemiologic Survey (Grant, 1997) likewise found very high life-time rates of alcohol dependence in the youngest age cohort. Where DNA from one or both parents is unavailable, comparison of unaffected and affected full siblings permits an equivalent test, comparing allele counts or genotype frequencies in discordant sib pairs (Spielman & Ewens, 1998). Using discordant sib pairs is much less powerful than using case–parent trios; however, families with at least two affected and one unaffected siblings are particularly informative, and for high prevalence traits can compare favourably with the standard trios (Hovarth & Laird, 1998). Unfortunately, given the small sample sizes and quasi-random sampling used in the AACTS, we had insufficient power to use such methods to confirm the association that we observed between *ADH2* and reduced alcohol dependence symptom count in men.

The growing understanding of the role of genetic factors in the aetiology of alcohol dependence has important clinical implications. There is no simple 'one gene–one disease' relationship, but rather a multiplicity of genetic factors (including, in Asians, *ALDH2* and *ADH2* as well as possibly *ADH3* variants) that contribute, in conjunction with environmental risk factors, to differences in alcohol dependence risk. Already, for alcoholic patients of Asian origin, a case could be made for determining *ALDH2* genotype, since *ALDH2*1/*2* heterozygotes who develop problems with alcohol appear at heightened risk of medical complications. With the growth of transracial adoptions, an increasing number of children of Asian ancestry are being reared by parents of European ancestry, whose physicians are less likely to be aware of such relationships between genotype, alcohol exposure and risk of medical complications of alcohol dependence.

The Australian data that we have used to illustrate our discussion are of course not without limitations. The Australian twin panel was developed as a volunteer panel through appeals in the media, and while a broad range of socio-economic levels and educational backgrounds are represented in the sample (Heath *et al*, 1997*b*), it cannot be considered to be truly representative of the population of Australia. Genotyping at the *ADH2* and *ADH3* loci has as yet been completed only for participants in the alcohol challenge study and some of their relatives, so that failure to detect significant protective effects of the *ADH2* locus in women may be a consequence of low power. Furthermore, we know that women who volunteered to participate in the alcohol challenge study were disproportionately heavy drinkers (Heath *et al*, 1998), and thus we might expect to find that *ADH2*1/*2* heterozygotes were underrepresented among these volunteers; there is indeed some evidence to this effect, a higher frequency of the heterozygotes being observed in men than in women (Whitfield *et al*, 1998). Notwithstanding these limitations, the Australian data provide a useful example both of the possibilities of studying jointly the effects of genetic and environmental risk factors, and of the potential confounding factors that may arise.

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REFERENCES

American Psychiatric Association (1987) *Diagnostic and Statistical Manual of Mental Disorders* (3rd edn, revised) (DSM–III–R). Washington, DC: APA.

___ 1994 *Diagnostic and Statistical Manual of Mental Disorders* (4th edn) (DSM–IV). Washington, DC: APA.

Bucholz, K. K., Cloninger, C. R., Dinwiddie, S. H., *et al* (1994) A new, semi-structured psychiatric interview for use in genetic linkage studies: a report of the reliability of the SSAGA. *Journal of Studies on Alcohol*, **55**, 149–158.

Chen, W. J., Loh, E. W., Hsu, Y. P., *et al* (1996) Alcohol-metabolising genes and alcoholism among Taiwanese Han men: independent effect of ADH2, ADH3 and ALDH2. *British Journal of Psychiatry*, **168**, 762–767.

Couch, F. J. (1998) BRAI testing – advances and retreats. *Journal of the American Medical Association*, **279**, 955.

Edenberg, H. J. & Bosron, W. F. (1997) Alcohol dehydrogenases. In *Comprehensive Toxicology* (ed. F. P. Guengerich), pp. 119–131. New York: Pergamon Press.

Ewens, W. J. & Spielman, R. S. (1995) The transmission/disequilibrium test: history, subdivision and admixture. *American Journal of Human Genetics*, **57**, 455–464.

Goedde, H. W., Agarwal, D. P., Fritze, G., *et al* (1992) Distribution of ADH2 and ALDH2 genotypes in different populations. *Human Genetics*, **88**, 344–346.

Grant, B. F. (1997) Prevalence and correlates of alcohol use and DSM–IV alcohol dependence in the United States: results of the National Longitudinal Alcohol Epidemiologic Survey. *Journal of Studies on Alcohol*, **58**, 464–473.

Grant, J. D., Heath, A. C., Madden, P. A. F., *et al* (1999) An assessment of the relationship between alcohol metabolism and alcoholism risk in Europeans. *Behavior Genetics*, **29**, 291–302.

Hastbacka, J., de la Chapelle, A., Kaitila, I., *et al* (1992) Linkage disequilibrium mapping in isolated founder populations: diastrophic dysplasia in Finland. *Nature Genetics*, **2**, 204–211.

Heath, A. C. & Martin, N. G. (1992) Genetic differences in psychomotor performance decrement after alcohol: a multivariate analysis. *Journal of Studies on Alcoholism*, **53**, 262–271.

___ , Bucholz, K. K., Madden, P. A. F., *et al* (1997*a*) Genetic and environmental contributions to alcohol dependence risk in a national twin sample: consistency of findings in women and men. *Psychological Medicine*, **27**, 1381–1396.

___ , Slutske, W. S. & Madden, P. A. F. (1997*b*) Gender differences in the genetic contribution to alcoholism risk and to alcohol consumption patterns. In *Gender and Alcohol: Individual and Social Perspectives* (eds R. W. Wilsnack & S. C. Wilsnack), pp. 114–149. Rutgers, NJ: Rutgers University Press.

___ , Madden, P. A. F. & Martin, N. G. (1998) Assessing the effects of cooperation bias and attrition in behavioral genetic research using data-weighting. *Behavior Genetics*, **28**, 415–427.

___ , ___ , Bucholz, K. K., *et al* (1999) Genetic differences in alcohol sensitivity and the inheritance of alcoholism risk. *Psychological Medicine*, **29**, 1069–1081.

Helzer, J. E., Canino, G. J., Yeh, E. K., *et al* (1990) Alcoholism – North America and Asia: a comparison of population surveys with the Diagnostic Interview Schedule. *Archives of General Psychiatry*, **47**, 313–319.

Higuchi, S. (1994) Polymorphisms of ethanol metabolizing enzyme genes and alcoholism. *Alcohol and Alcoholism. Supplement*, **2**, 29–34.

___ , Matsushita, S., Imazeki, H., *et al* (1994) Aldehyde dehydrogenase genotypes in Japanese alcoholics. *Lancet*, **343**, 741–742.

A. C. HEATH, DPhil, P. A. F. MADDEN, PhD, K. K. BUCHOLZ, PhD, L. J. BIERUT, MD, Missouri Alcoholism Research Center at Washington University, and Departments of Psychiatry, Psychology and Genetics, Washington University School of Medicine, St Louis, Missouri, USA; J. B. WHITFIELD, PhD, Department of Clinical Biochemistry, Royal Prince Alfred Hospital, Sydney, Australia; S. H. DINWIDDIE, MD, Department of Psychiatry, North Chicago Medical School, Chicago, Illinois, USA; W. S. SLUTSKE, PhD, Missouri Alcoholism Research Center at the University of Missouri, and Department of Psychology, University of Missouri, Columbia, Missouri, USA; D. B. STATHAM, MA, NICHOLAS G. MARTIN, PhD, Department of Epidemiology and Population Health, Queensland Institute of Medical Research, Brisbane, Australia

Correspondence: Dr Andrew C. Heath, 40 North Kingshighway, Suite 1, St Louis, MO 63108, USA. Tel: +1 314 286 2204; fax: +1 314 286 2213; e-mail: andrew@matlock.wustl.edu

___ , ___ , Muramatsu, T., *et al* (1996) Alcohol and aldehyde dehydrogenase genotypes and drinking behavior in Japanese. *Alcoholism: Clinical and Experimental Research*, **20**, 493–497.

Hovarth, S. & Laird, N. M. (1998) A discordant-sibship test for disequilibrium and linkage: no need for parental data. *American Journal of Human Genetics*, **63**, 1886–1897.

Hurt, R. D., Offord, K. P., Croghan, I. T., *et al* (1996) Mortality following inpatient addictions treatment. Role of tobacco use in a community-based cohort. *Journal of the American Medical Association*, **275**, 1097–1103.

Kendler, K. S., Heath, A. C., Martin, N. G., *et al* (1986) Symptoms of anxiety and depression in a volunteer twin population: the etiologic role of genetic and environmental factors. *Archives of General Psychiatry*, **43**, 213–221.

___ , ___ , Neale, M. C., *et al* (1992) A population-based twin study of alcoholism in women. *Journal of the American Medical Association*, **268**, 1877–1882.

___ , Neale, M. C., Heath, A. C., *et al* (1994) A twin-family study of alcoholism in women. *American Journal of Psychiatry*, **151**, 707–715.

Kessler, R. C., Nelson, C. B., McGonalge, K. A., *et al* (1996) The epidemiology of co-occurring addictive and mental disorders: implications for prevention and service utilization. *American Journal of Orthopsychiatry*, **66**, 17–31.

Kittle, R., Perola, M., Peltonen, L., *et al* (1998) Geographic patterns of autosomal dinucleotide repeat variation and linkage disequilibrium in Finnish provinces. *American Journal of Human Genetics*, **63**, A42.

Long, J. C., Knowler, W. C., Hanson, R. L., *et al* (1998) Evidence for genetic linkage to alcohol dependence on chromosomes 4 and 11 from an autosome-wide scan in an American Indian population. *American Journal of Medical Genetics*, **81**, 216–221.

Martin, N. G., Perl, J., Oakeshott, J. G., *et al* (1985*a*) A twin study of ethanol metabolism. *Behavior Genetics*, **15**, 93–109.

___ , Oakeshott, J. G., Gibson, J. B., *et al* (1985*b*) A twin study of psychomotor and physiological responses to an acute dose of alcohol. *Behavior Genetics*, **15**, 305–347.

___ , Eaves, L. J., Heath, A. C., *et al* (1986) Transmission of social attitudes. *Proceedings of the National Academy of Sciences of the USA*, **83**, 4364–4368.

McGue, M. (1994) Genes, environment and the etiology of alcoholism. In *The Development of Alcohol Problems: Exploring the Biopsychosocial Matrix of Risk* (eds R. Zucker, G. Boyd & J. Howard). Rockville, MD: US Department of Health and Human Services.

Muramatsu, T., Zu-Cheng, W., Y-Ru, F., *et al* (1995) Alcohol and aldehyde dehydrogenase genotypes and drinking behavior of Chinese living in Shanghai. *Human Genetics*, **96**, 151–154.

Nakamura, K., Iwahashi, K., Matsuo, Y., *et al* (1996) Characteristics of Japanese alcoholics with the atypical aldehyde dehydrogenase 2*2. I. A comparison of the genotypes of ALDH2, ADH2, ADH3, and cytochrome P-4502EI between alcoholics and nonalcoholics. *Alcoholism: Clinical and Experimental Research*, **20**, 52–55.

Nelson, C. B., Heath, A. C., Kessler, R. C., *et al* (1998) Temporal progression of alcohol dependence symptoms in the US household population: results from the National Comorbidity Survey. *Journal of Consulting Clinical Psychology*, **66**, 474–483.

Neumark, Y. D., Friedlander, Y., Thomasson, H. R., *et al* (1998) Association of the ADH2*2 allele with reduced ethanol consumption in Jewish men in Israel: a pilot study. *Journal of Studies on Alcoholism*, **59**, 133–139.

Newman, B., Mu, H., Butler, L. M., *et al* (1998) Frequency of breast cancer attributable to *BRCA1* in a population-based series of American women. *Journal of the American Medical Association*, **279**, 915–921.

Osier, M., Pakstis, A. J., Kidd, J. R., *et al* (1999) Linkage disequilibrium as the ADH2 and ADH3 loci and risk of alcoholism. *American Journal of Human Genetics*, **64**, 1147–1157.

Prescott, C. A. & Kendler, K. S. (1999) Genetic and environmental contributions to alcohol abuse and dependence in a population-based sample of male twins. *American Journal of Psychiatry*, **156**, 34–40.

Regier, D. A., Farmer, M. E., Rae, D. S., *et al* (1990) Comorbidity of mental disorders with alcohol and other drug abuse: results from the Epidemiologic Catchment Area (ECA) study. *Journal of the American Medical Association*, **264**, 2511–2518.

Reich, T., Edenberg, H. J., Goate, A., *et al* (1998) Genome-wide search for genes affecting the risk for alcohol dependence. *American Journal of Medical Genetics*, **81**, 207–215.

Shen, Y. C., Fan, J. H., Edenberg, H. J., *et al* (1997) Polymorphism of ADH and ALDH genes among four ethnic groups in China and effects upon the risk for alcoholism. *Alcoholism: Clinical and Experimental Research*, **21**, 1272–1277.

Slutske, W. S., Heath, A. C., Dinwiddie, S. H., *et al* (1997) Common genetic risk factors for conduct disorder and alcohol dependence. *Journal of Abnormal Psychology*, **107**, 363–374.

Spielman, R. S. & Ewens, W. J. (1998) A sibship test for linkage in the presence of association: the sib transmission/disequilibrium test. *American Journal of Human Genetics*, **62**, 450–458.

___ , McGinnis, R. E. & Ewens, W. J. (1993) Transmission test for linkage disequilibrium: the insulin

gene region and insulin-dependent diabetes mellitus (IDDM). *American Journal of Human Genetics*, **52**, 506–516.

StataCorp (1999) *Stata Statistical Software: Release 6.0.* College Station, TX: Stata Corporation.

Takao, A., Shimoda, T., Kohno, S., *et al* (1998) Correlation between alcohol-induced asthma and acetaldehyde dehydrogenase-2 genotype. *Journal of Allergy and Clinical Immunology*, **101**, 576–580.

Tanaka, F., Shiratori, Y., Yokosuka, O., *et al* (1996) High incidence of ADH2*1/ALDH2*1 genes among Japanese alcohol dependents and patients with alcoholic liver disease. *Hepatology*, **23**, 234–239.

Thomasson, H. R., Edenberg, H. J., Crabb, D. W., *et al* (1991) Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. *American Journal of Human Genetics*, **48**, 677–681.

___, Crabb, D. W., Chen, C. C., *et al* (1994) Low frequency of the ADH2*2 allele among Atayal natives of Taiwan with alcohol use disorders. *Alcoholism: Clinical and Experimental Research*, **18**, 640–643.

True, W. R., Heath, A. C., Romeis, J. C., *et al* (1996) Models of treatment-seeking for alcoholism: the role of genes and environment. *Alcoholism: Clinical and Experimental Research*, **20**, 1577–1581.

von Wartburg, J. P., Gennari, K., Muellener, D., *et al* (1988) Determination of the genotype of human class I alcohol dehydrogenase. In *Biomedical and Social Aspects of Alcohol and Alcoholism* (eds K. Kuriyama, A. Takada & H. Ishii), pp. 35–38. New York: Elsevier Science.

Wall, T. L., Peterson, C. M., Peterson, K. P., *et al* (1997) Alcohol metabolism in Asian-American men with genetic polymorphisms of aldehyde dehydrogenase. *Annals of Internal Medicine*, **27**, 376–379.

Whitfield, J. B., Nightingale, B. M., Bucholz, K. K., *et al* (1998) ADH genotypes and alcohol use and dependence in Europeans. *Alcoholism: Clinical and Experimental Research*, **22**, 1463–1469.

Xu, Y., Carr, L. G., Bosron, W. F., *et al* (1988) Genotyping of human alcohol dehydrogenases at the ADH2 and ADHS loci following DNA sequence amplification. *Genomics*, **2**, 209–214.

Yamamoto, K., Ueno, Y., Mizoi, Y., *et al* (1993) Genetic polymorphism of alcohol and aldehyde dehydrogenase and the effects on alcohol metabolism. *Arukoru Kenkyo-To Yakubutsu Ison (Japanese Journal of Alcohol Studies and Drug Dependence)*, **28**, 13–25.

Yokoyama, A., Muramatsu, T., Ohmori, T., *et al* (1998) Alcohol-related cancers and aldehyde dehydrogenase-2 in Japanese alcoholics. *Carcinogenesis*, **19**, 1383–1387.