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


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 Reger 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, 1997b). 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 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 context: 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 ADH2 and ADH2 genotype frequencies in alcoholic and control series

<table>
<thead>
<tr>
<th>ADH2 genotype</th>
<th>Penetrance ratios</th>
<th>ADH2 genotype</th>
<th>Penetrance ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1979</td>
<td>1986</td>
<td>1992</td>
</tr>
<tr>
<td>ADH2*1/*1</td>
<td>$f^2_{(1)}$</td>
<td>$f_{(1)N}$</td>
<td>$f_{(2)N}$</td>
</tr>
<tr>
<td>ADH2*1/*2</td>
<td>0.043</td>
<td>0.143</td>
<td>0.25</td>
</tr>
<tr>
<td>ADH2*2/*2</td>
<td>0</td>
<td>0</td>
<td>0.15</td>
</tr>
</tbody>
</table>

$f_i$ denotes the penetrance, i.e. the estimated probability of a history of alcohol dependence, in $ADH2*1/*1$ homozygous Japanese males; $f_i^2$ denotes the corresponding probability in $ADH2*1/*1$ homozygotes who are also $ADH2*1/*1$ homozygotes. For $ADH2$ genotypes, subscripts are used to denote cohort-specific penetrances. The penetrance of the $ADH2*1/*1$ genotype was computed as $a^2 + b^2 - 2a_1b$, where $a_1$ and $b_1$ are the proportions of the controls, and $a_2$ 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.

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**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 $ADH2*1/*1$ 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 (Oser et al., 1999).

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**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

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**AIMS**

The $ADH2$ locus is moderately polymorphic, and the $ADH3$ locus more highly polymorphic, in samples of populations of European ancestry (e.g. Goede 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., 1997a); 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., 1997a). 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., 1985a,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, 43 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., 1997a), 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., 1985a,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., 1997a) 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
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 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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Alcohol dependence</th>
<th>Log-transformed alcohol dependence symptom count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td></td>
<td>$n$ DSM–III–R (%)</td>
<td>$n$ DSM–IV (%)</td>
</tr>
<tr>
<td>ADH2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADH2*1/*1</td>
<td>171</td>
<td>14</td>
</tr>
<tr>
<td>ADH2*1/*2</td>
<td>11</td>
<td>27.3</td>
</tr>
<tr>
<td>ADH2*1/<em>1 v. ADH2</em>1/*2</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

### 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 ($\beta$) and robust standard errors (s.e.) are tabulated

<table>
<thead>
<tr>
<th></th>
<th>Separate analyses</th>
<th>Joint analysis of individual and co-twin predictors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>s.e.</td>
</tr>
<tr>
<td>Individual predictors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not AACTS participant</td>
<td>-0.13</td>
<td>0.06</td>
</tr>
<tr>
<td>ADH2*1/*2 genotype</td>
<td>-0.38</td>
<td>0.17</td>
</tr>
<tr>
<td>High alcohol sensitivity</td>
<td>-0.45</td>
<td>0.10</td>
</tr>
<tr>
<td>Birth cohort 1955–1959</td>
<td>0.11</td>
<td>0.04</td>
</tr>
<tr>
<td>Other Protestant religion</td>
<td>-0.23</td>
<td>0.04</td>
</tr>
<tr>
<td>Tertiary education</td>
<td>-0.10</td>
<td>0.04</td>
</tr>
<tr>
<td>Childhood conduct disorder</td>
<td>0.38</td>
<td>0.04</td>
</tr>
<tr>
<td>History of major depression</td>
<td>0.25</td>
<td>0.05</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.89</td>
<td>0.07</td>
</tr>
<tr>
<td>R$^2$</td>
<td>0.133</td>
<td></td>
</tr>
</tbody>
</table>

| Co-twin predictors    |       |     |       |     |          |                     |
|-----------------------|------------------|-----------------------------------------------|
| MZ co-twin alcohol dependent | 0.42 | 0.06 | 0.33 | 0.06 |
| DZ male co-twin alcohol dependent | 0.10^* | 0.08 | 0.05^* | 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^* | 0.05 | -0.09^* | 0.05 |
| Intercept             | 0.84 | 0.03 |      |      |
| R$^2$                 | 0.098 |      |        |         |

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.
3. AACTS, Australian Alcohol Challenge Twin Study; DZ, dizygotic; MZ, monozygotic.
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 $p_{10} = 1.04$, $P = 0.31$), 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., 1997b), including diagnostic interview studies on large general population samples of twin pairs (Kendler et al., 1992, 1994; True et al., 1996; Heath et al., 1997a; 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. Hämäläinen 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 (Howarth & 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., 1997b), 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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