We have tested for effects of alcohol dehydrogenase (ADH) genotypes on self-reported alcohol consumption and symptoms of alcohol dependence, recorded on three occasions up to 15 years apart, in 377 male and female subjects of European descent. ADH2 genotype had significant effects on both consumption and dependence in the men, but not in the women. The effects of ADH3 genotype were considerably less than those of ADH2, but significant results could be demonstrated when the combined genotypes were considered. The direction of the effects on alcohol consumption and dependence risk were consistent with reports on Asian subjects, and with the in vitro properties of ADH isoenzymes. As with previous studies on the relationship between ADH type and alcohol use, population stratification cannot be excluded as a contributing factor in these results.

Key Words: Alcohol Dehydrogenase, Alcoholism, Association, Female, Male.

Multiple reports indicate that alcohol dehydrogenase (ADH)2 and ADH3 genotypes influence the risk of alcohol dependence among Japanese or Chinese subjects, independent of aldehyde dehydrogenase (ALDH) genotype. Meta-analysis of these reports shows that the relative risk for alcohol dependence increases by 3-fold for each copy of ADH2*1 and a similar amount for each copy of ADH3*2—but, on present evidence, in Asians only. The alleles associated with lower risk (ADH2*2 and ADH3*1) code for proteins with greater in vitro enzymatic activity. This is consistent with the hypothesis that faster conversion of ethanol to acetaldehyde deters subject from alcohol consumption, although it has not yet been demonstrated that acetaldehyde concentrations are higher nor that ethanol metabolism is faster in vivo in subjects with these forms of ADH. From the in vitro data, the effect of ADH2 type should be greater than that of ADH3 so that the order of rates of alcohol metabolism would be ADH2*22 > 12 > 11 and within each ADH2 group ADH3*11 > 12 > 22. On this hypothesis, the risk of alcohol dependence, or the quantity of alcohol used and the number of problems encountered, would trend in the opposite direction.

However, studies on ADH2 genotypes in other, non-Asian populations have produced negative results. This might be because of the low prevalence of ADH2*2 in non-Asian groups, or because of some factor that modifies the effects of genotype and that differs between populations. For ADH3, there are indications that the significant effects found in Asian populations do not extend to European ones. Therefore, it is desirable to assess the effects of ADH genotype in diverse non-Asian groups in order to determine whether these effects are universal.

We have data on lifetime prevalence of alcohol dependence, together with measures of alcohol consumption and alcohol-related problems, in a group of subjects of European descent studied on multiple occasions over the past 19 years. We have therefore tested for association between ADH2 or ADH3 genotype and these alcohol-related variables, and the expected trend across ADH2/ADH3 haplotypes.

Because we have tested for association between genotype and phenotype, a positive result could be produced by population stratification. This can occur if two or more ancestral populations are represented in the sample, and one genotype is overrepresented in a subgroup that happens (for unrelated reasons) to drink either more or less than other subjects in the sample. However, this problem may also have occurred in the studies that have shown ADH effects on alcohol dependence in China or Japan.

Our study group differs from those in most previous reports, which have been based on recruitment of patients diagnosed as having alcohol dependence rather than assessment of quantity and frequency of alcohol use or the number of alcohol-related problems encountered. Also, in previous reports, the majority of subjects have been men and possible sex differences in the effects of ADH variation have not been examined.

Subjects and Methods

Four hundred twelve adult male and female twin subjects (206 pairs) were recruited in Sydney and Canberra in 1979 to 1981 for a study of genetic and environmental influences on alcohol metabolism and susceptibility to intoxication. At that time, their ages were between 18 and 34.
with a mean of 23 years; there were 199 men and 213 women. Recruitment of subjects was from a register of twins, from advertising, and from word of mouth. The number of potential subjects who declined to take part once the nature of the study was explained is not recorded, and there was a small number (~5%) of subjects who agreed to participate, but were unable to drink or retain the alcohol dose (0.75 g/kg). Information is only available on subjects who were able to complete the experimental protocol. At that time (Initial Study), all subjects answered a questionnaire that included items on the usual number of drinks taken per week, and on the frequency and quantity of alcohol use (variables IC-1 and IC-2 in Tables 3 and 4, and Fig. 1). This questionnaire was completed before the subjects drank any alcohol.

In a Follow-up Study in 1990 to 1992, we attempted to contact, and obtain blood samples for genotyping from, all subjects who had completed the Initial Study, 10 to 12 years previously. Those who were located and agreed to participate were asked to complete another questionnaire that included items on alcohol consumption (variables FC-1, FC-2, and FC-3) and on their lifetime experience of symptoms of alcohol dependence (variable FD-1). Three hundred thirty-four subjects (158 male, 176 female) completed this questionnaire and, at that time, their average age was 34 years.

In 1992 to 1996, 336 of the subjects (159 male, 177 female; mean age: 37 years) also participated in a larger study of the genetics of alcohol dependence and comorbid conditions. This involved a structured interview (Semi-Structured Assessment for the Genetics of Alcoholism; SSAGA) that included items on quantity and frequency of alcohol use (variable SC-1) and the number of dependence symptoms (variable SD-1). This interview allows diagnosis of alcohol dependence according to the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-III-R).

Therefore, the consumption and dependence information are covered by nine variables (those listed in Tables 3 and 4) and the DSM-III-R diagnosis of alcohol dependence. All items except the dependence diagnosis had large numbers of possible values and were treated as continuous variables. Because of the skewness of their distributions, all except the weekly drinks category at follow-up were transformed to \( \log_{10}(x + 1) \) before use.

Estimates of consumption were significantly correlated (\( R \approx 0.23 \) to 0.83) both within and across occasions, as were estimates of dependence score; and consumption and dependence showed a considerable overlap. This is evidence for the reliability of the assessment of phenotype. The large proportion of men meeting DSM-III-R criteria for alcohol dependence is a frequent finding in community studies, but many such subjects do not seek treatment and are therefore omitted from studies that rely on clinical recruitment.

Blood was obtained for genotyping, either at the time of the Follow-up Study or after the SSAGA interview, from 369 subjects (176 male, 193 female). Genotypes for \( ADH2 \) and \( ADH3 \) were determined on DNA extracted from white blood cells, using the polymerase chain reaction and restriction digestion, followed by electrophoresis of the polymerase chain reaction products. \( ADH2 \) genotype was established for all 369 samples and \( ADH3 \) for 367. Genotypes for a further eight subjects were inferred from the genotypes of their monozygotic co-twins.

Data on ancestry were provided by 203 of the subjects as part of another project. They gave information on each of their four grandparents, and because of the large number of countries involved, the categories were combined into “Northern European” (including the UK, Eire, Germany, the Netherlands, Scandinavia, and also for this purpose Australia and New Zealand), “Southern European” (including Italy, Greece, Spain, Portugal, France, Turkey, and Lebanon), and Asian (from which there were only three reported grandparents). For the four categories of grandparent, the proportions reported as having a Southern European ancestry were 7.9%, 9.3%, 6.9%, and 8.0%. For further analysis, we classified individual subjects as having either 100% Northern European ancestry (\( n = 157 \)) versus <100% (\( n = 24 \)).

Statistical analysis was performed using BMDP Dynamic (BMDP Statistical Software, Inc., Los Angeles, CA) for analysis of variance and tests for trend across groups, or StatXact Turbo (Cytel Software Corporation, Cambridge, MA) for exact tests on contingency tables. Because the subjects were twins and therefore not genetically independent, tests for effects of genotype on the dependent variables were performed both with the subjects treated as independent observations of genotype, and after halving the number of degrees of freedom to allow for the fact that the subjects were related within twin pairs. This is a conservative approach; in either case, the estimates of the mean value for each genotype, and the estimate of the difference between them, are unbiased.

**RESULTS**

**Genotype Frequencies**

The frequencies of the observed \( ADH2 \) and \( ADH3 \) genotypes are shown in Table 1, separately for men and
ADH GENOTYPE EFFECTS

ADH2*2, ADH2, ADH3*22, ADH3*11

ADH GENOTYPE EFFECTS

1465

ADH3'11 subjects (odds ratio: 5.43; 95% CI: 1.63–18.06) than in ADH3'22 subjects. Significantly higher in ADH3'11 subjects (odds ratio: 2.33; 95% CI: 1.28–4.25) and in women (no significant sex difference). Subjects with genotype: male, p = 0.032 (one-tailed) or p = 0.046 (two-tailed); female, p = 0.95 (Fisher’s exact test). No subjects were homozygous for the quantitative variables.

Ancestry Effects

Because of the difference in ADH2*2 allele frequency between subjects of Northern and Southern European ancestry, it became important to determine whether there was a difference in the quantitative alcohol consumption or problem variables between these groups. Only ADH2*11 subjects were used in this analysis, to avoid confounding any true effects of ADH2*2 with the ancestry information. For each of the eight variables, the mean was greater in the all-Northern-European grandparents group (n = 151) than in the other group (n = 18). However, the difference was only statistically significant (p = 0.044) for one of the variables (FC-1).
Table 3. Mean Values for Alcohol Consumption or Dependence Measures by ADH2 Genotype

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of weekly drinks in 1979-1981 (FC-1)</td>
<td>0.942 0.896 0.17 1.180 0.677 0.001</td>
<td>0.724 0.736 0.01 1.191 0.925 0.000</td>
</tr>
<tr>
<td>Quantity x frequency measure in 1979-1981 (FC-2)</td>
<td>1.317 1.078 3.61 1.176 0.059 0.020</td>
<td>1.113 0.982 0.06 1.192 0.407 0.004</td>
</tr>
<tr>
<td>Consumption in past week at follow-up (FC-1)</td>
<td>0.966 0.731 4.91 1.152 0.028 0.031</td>
<td>0.641 0.761 0.06 1.173 0.409 0.004</td>
</tr>
<tr>
<td>Quantity x frequency measure at follow-up (FC-2)</td>
<td>0.904 0.622 9.71 1.144 0.002 0.063</td>
<td>0.693 0.645 0.15 1.160 0.700 0.001</td>
</tr>
<tr>
<td>Weekly drinks category at follow-up (FC-3)</td>
<td>3.47 2.16 10.57 1.156 0.001 0.064</td>
<td>2.51 2.30 0.27 1.175 0.602 0.002</td>
</tr>
<tr>
<td>Quantity x frequency measure in SSAGA (SC-1)</td>
<td>0.896 0.560 11.21 1.158 0.001 0.066</td>
<td>0.824 0.405 3.65 1.175 0.058 0.020</td>
</tr>
<tr>
<td>No. of symptoms positive at follow-up (FD-1)</td>
<td>0.457 0.296 4.63 1.156 0.033 0.029</td>
<td>0.328 0.333 0.00 1.175 0.952 0.000</td>
</tr>
<tr>
<td>No. of symptoms positive at SSAGA (SD-1)</td>
<td>0.606 0.421 6.46 1.150 0.012 0.041</td>
<td>0.427 0.425 0.00 1.167 0.965 0.000</td>
</tr>
</tbody>
</table>

Consumption and dependence variables are indicated by C or D, and the occasions by I (initial study), F (follow-up study), or S (SSAGA study). All variables except FC-3 are log-transformed.

Note: for male subjects, 3 of 8 comparisons significant at \( p < 0.01 \), 3 of 8 significant at \( p < 0.05 \), 1 significant at \( p < 0.10 \), and 1 NS. This is still the case if \( p \) values are calculated from the same \( F \) statistics but with half the degrees of freedom. \( p \) values < 0.05 are shown in bold font.

Table 4. Mean Values for Alcohol Consumption or Dependence Measures by ADH3 Genotype

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADH3*11</td>
<td>ADH3*12 F df p</td>
</tr>
<tr>
<td>Sum of weekly drinks in 1979-1981 (FC-1)</td>
<td>0.959 0.927 0.399 0.08 2.178 0.924</td>
<td>0.720 0.714 0.796 0.51 2.189 0.599</td>
</tr>
<tr>
<td>Quantity x frequency measure in 1979-1981 (FC-2)</td>
<td>1.236 1.290 1.409 0.86 2.174 0.423</td>
<td>1.091 1.094 1.204 0.59 2.190 0.557</td>
</tr>
<tr>
<td>Consumption in past week at follow-up (FC-1)</td>
<td>0.924 0.903 1.110 1.99 2.151 0.141</td>
<td>0.775 0.592 0.617 3.15 2.171 0.045</td>
</tr>
<tr>
<td>Quantity x frequency measure at follow-up (FC-2)</td>
<td>0.803 0.877 0.962 0.55 2.143 0.579</td>
<td>0.844 0.680 0.666 3.65 2.158 0.028</td>
</tr>
<tr>
<td>Weekly drinks category at follow-up (FC-3)</td>
<td>3.267 3.202 3.792 1.16 2.155 0.315</td>
<td>2.857 2.340 2.407 2.91 2.173 0.057</td>
</tr>
<tr>
<td>Quantity x frequency measure in SSAGA (SC-1)</td>
<td>0.755 0.805 0.942 1.76 2.156 0.175</td>
<td>0.697 0.542 0.563 3.12 2.173 0.047</td>
</tr>
<tr>
<td>No. of symptoms positive at follow-up (FD-1)</td>
<td>0.376 0.433 0.570 3.16 2.155 0.045</td>
<td>0.281 0.294 0.355 0.69 2.173 0.503</td>
</tr>
<tr>
<td>No. of symptoms positive at SSAGA (SD-1)</td>
<td>0.587 0.573 0.608 0.14 2.148 0.869</td>
<td>0.486 0.396 0.460 1.46 2.165 0.235</td>
</tr>
</tbody>
</table>

Consumption and dependence variables are indicated by C or D, and the occasions by I (initial study), F (follow-up study), or S (SSAGA study). All variables except FC-3 are log-transformed.

Nevertheless, this result fuels the suspicion that the relationship between \( ADH2 \) genotype and drinking habits or problems that we have observed is confounded by ethnic stratification. The best way to avoid this confounding is to rely only on within-family comparisons. Unfortunately, in our sample, we only have five pairs of same-sex dizygotic twins, where one twin was \( ADH2*11 \) and the other was \( ADH2*12 \). We compared the eight alcohol consumption and problem variables within each of these five pairs, on the hypothesis that the \( ADH2*11 \) twin would have the higher values. Of the 40 possible comparisons, 29 showed a higher result for the \( ADH2*11 \) twin, 5 showed a lower result, and there were 6 ties. In all five pairs, there was a majority (ranging from 4-2 to 7-0) of higher results in the \( ADH2*11 \) subject. Although the number of pairs is small, the probability that in all five \( ADH2 \) discordant pairs the \( ADH2*11 \) genotype would be the heavier drinking twin is only 1 in 32 (\( p = 0.031 \)).

DISCUSSION

\( ADH, Alcohol Consumption, and Alcohol Dependence \)

\( ADH \) genotype effects have been reported consistently in studies on Japanese or Chinese subjects, and are assumed to be mediated by differences in alcohol metabolism. The aim of this study was to test for effects of \( ADH \) genotype on alcohol consumption, alcohol-related problems, and alcohol dependence in men and women of European descent.

We have found that \( ADH2 \) genotype does influence alcohol dependence, consumption, and problems in the men;
but, variation in this factor has nonsignificant effects in women of the same background. ADH3, which has effects on alcohol dependence that are almost as great as those of ADH2 in Asians, had mainly nonsignificant effects (or effects contrary to expectation) in both men and women (Table 4), but there is some evidence for ADH3 effects in the male ADH2*11 subjects (Fig. 1). These results extend our knowledge of genetic factors influencing alcohol use and dependence to a different population group (Europeans), and to alcohol use in the general population.

The consistent direction of these effects across several occasions of study spread over 15 years reinforces our general conclusions, although the nonsignificant results from the initial phase suggest that genetic factors may be overcome by other influences in younger men. The effects of ADH2 genotype at initial study in 1979 to 1981, when the average age of the subjects was 23, are less than those seen later when the subjects were 12 to 15 years older. It has previously been shown that stability of alcohol consumption is greater after age 25,24 and social or other non-ADH-related influences may predominate among the young.

Despite the comparatively small number of ADH2*12 subjects, evidence of effects on both dependence and consumption was found. Because the frequency of ADH2*2 is <10% among Europeans,25 and because genotyping techniques capable of handling large numbers of samples have only recently become available, previous studies that produced negative results12-15 may have done so because of insufficient power. Negative results may also have arisen from comparing subjects with alcoholic liver disease with controls who are not alcohol-dependent, because ADH2 variation has the opposite effects on dependence and alcoholic liver disease.18

For ADH3, where the gene frequencies of ADH3*1 and 2 are nearly equal in Europeans and the power to detect differences between genotypes is greater, one previous study showed negative results15 in contrast to multiple positive reports on Asians.1,3,4,7

**Male-Female Differences in ADH2 Effects**

There are a number of possible reasons why significant effects were found in men, but not in women. First, some important aspect of alcohol metabolism could differ between men and women and nullify the effects of ADH2. Although the rate of decrease in blood alcohol concentration after a test dose was the same in men and women in this group of subjects,16 there have been a number of claims that sex hormones affect alcohol metabolism or ADH activity,26 or are associated with differences in post-alcohol acetaldehyde concentrations.28 In mice,29 some but not all inbred strains show differences in hepatic ADH activity between males and females.

Second, an effect might be missed because of the lower mean and narrower range of alcohol consumption in women than men, or because of insufficient ADH2*12 women in our study. As seen in Table 1, the proportion of ADH2*12 subjects was significantly greater among the men, either because ADH2*12 women were underrepresented or because ADH2*12 men were overrepresented. Women who are genetically disposed toward low consumption might have chosen not to participate in the initial alcohol challenge procedure, which determined admission to the study group. The smaller number of ADH2*12 women would have reduced our power to detect ADH2 genotype effects on drinking in women.

Most previous reports do not compare the effects of ADH in men and women. Of the nine cited studies1-9 from China and Japan, five appear to have studied only men and three have pooled results from men and women without comment on the justification for this procedure. The one study that did consider women's results separately9 reported that the women drank <5% of the average male amount, and "there were no significant differences between the drinking patterns of women with any of the ADH2 or ALDH2 genotypes." The difference in alcohol intake between men and women was less extreme in our study; at each of the three times studied, the geometric mean of alcohol intake was approximately twice as great in the men as in the women.

Study of larger numbers of women, without the selection procedure imposed on our subjects by the alcohol challenge study, should decide between the negative results being due to lack of power to detect effects or true sex differences.

**Combined Effects of ADH2 and ADH3 Genotypes**

The expectation from previous reports was that ADH2 type would have a stronger effect than ADH3 type, and that those most at risk would be the 11 homozygotes for ADH2 and the 22 homozygotes for ADH3. Oriental ADH2*11 subjects have approximately eight times the risk of alcohol dependence of ADH2*22, whereas ADH3*11 subjects have around half the risk of ADH3*22.3

ADH3 effects were much smaller than ADH2 effects in this study (as anticipated), but when the subjects were grouped by ADH2 and ADH3 genotype in the order which is predicted (from previous reports) to show increasing alcohol consumption or dependence, a significant trend in the expected direction was present for nearly all the measures. This is also the decreasing order of in vitro enzyme activity.

**Possible Effects of Population Stratification**

There was suggestive evidence both that ADH2*2 is more common in people of partial Southern European descent than in people of entirely Northern European descent (see also Ref. 25), and that alcohol consumption and problems were lower in the Southern group. It is therefore possible that population stratification is producing the associations between ADH genotype and phenotype. Unfortunately, we do not have complete ancestry data for all our
subjects and, even if it was available, it would not necessarily give a definitive answer.

The way to avoid such problems is to use only within-family comparisons. Having genotype information from parents allows use of the transmission disequilibrium test or, in this case, we make use of siblings discordant for ADH2 genotype. Intrapair comparisons from the five dizygotic twin pairs discordant for ADH2 type do not support population stratification as the sole explanation of our results. Rather, they point to a genuine effect of the ADH2*2 allele as aversive to drinking and protective against alcohol-related problems.

However, it is clear that a much larger sample of ADH2 discordant sibling pairs, with full drinking histories, is needed to settle this issue. We are currently assembling such a sample. It is perhaps worth noting that previous studies on association between alcohol metabolizing enzymes and drinking behavior in Asian populations are not necessarily free of the population stratification problem.

Conclusions

These results support the importance of some aspect of alcohol metabolism in influencing consumption and dependence, presumably by affecting the nature of the “alcohol experience.” They are consistent with the hypothesis that the rate of generation of acetaldehyde from ethanol may be a significant determinant of alcohol use, because the more active isoenzymes of ADH are the ones associated with lowest alcohol consumption and the least risk of alcohol dependence. Nevertheless, further consideration of the mechanism of this effect is required because ADH genotype has only small effects on the rate of ethanol metabolism measured in vivo, and because increased postacetaldehyde acetaldehyde concentrations in non-ALDH-deficient subjects carrying ADH2*2 or ADH3*1 have not been demonstrated. Studies using family structures are required to rule out effects of population stratification.

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