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META-ANALYSIS OF THE EFFECTS OF ALCOHOL DEHYDROGENASE GENOTYPE ON ALCOHOL DEPENDENCE AND ALCOHOLIC LIVER DISEASE

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Abstract — The purpose of this paper is to assemble and evaluate existing data on the effect of genetic variation in *ADH2* and *ADH3* on the risk of alcohol dependence, and on the risk of alcoholic liver disease. Calculations of odds ratios and their confidence limits, and tests for heterogeneity of the results from the available studies, have been performed. It is clear that possession of the *ADH2-2* allele decreases the risk of alcohol dependence, but it increases the risk of alcoholic liver disease among alcoholics. *ADH3* variation also has significant effects on alcohol dependence, which may be due to linkage to *ADH2*; the *ADH3* effect differs significantly between Asian and European subjects. Therefore *ADH* genotype has substantial effects on risk of alcohol dependence and alcoholic liver disease, but more work is needed on the generalizability of these findings to non-Asian populations, and on possible mechanisms.

INTRODUCTION

The possible relationship between alcohol dehydrogenase (*ADH*) genotype and the risk of alcohol dependence or alcohol-related disease has been a subject for investigation ever since the polymorphic variants of *ADH2* and *ADH3* were recognized. Recently, reliable polymerase chain reaction (PCR)-based methods for *ADH* genotyping have facilitated studies with substantial numbers of subjects. Although the results of these studies indicate a significant role for *ADH* variation in determining the genetic risk for alcoholism, experience with the proposed association between alcoholism and the Taq polymorphism in the dopamine D2 receptor locus suggests a need for caution (see, for example, Gelernter *et al.*, 1993). Association studies are susceptible to effects from linkage disequilibrium and population stratification, and like any other kind of epidemiological study the results may not agree across different authors' reports. Therefore it is desirable to compare all available data, to check whether the results are homogeneous and similar across different populations, and to arrive at the best estimate of the relative risk associated with different alleles of the gene(s) in question.

Over the past 3 years, a number of papers on *ADH* genotype and alcoholism risk have appeared. Most of them report results from Chinese or Japanese subjects, who are more suitable than Europeans for *ADH2* studies, but less suitable for *ADH3* studies, because of the different gene frequencies between these population groups. A smaller number of studies have examined association between *ADH* type and the risk of progression to alcoholic liver disease. Because this information leads to hypotheses about mechanisms, and potentially to the establishment of variation in alcohol metabolism and in metabolite concentrations as a major determinant of alcohol-related disease, it is important to establish the soundness and consistency of these results.

METHODS

Data

A search for all papers containing the terms ('alcohol dehydrogenase' or 'ADH') and ('type' or 'genotype') and ('dependence' or 'alcoholism'), and referring to studies on human subjects, was conducted on the Medline files between 1990 and September 1996. Other papers were added where known. This yielded 17 papers with relevant information. Five (Chao *et al.*, 1994*b*; Nakamura

et al., 1994, 1996; Higuchi *et al.*, 1995, 1996) were excluded, because they reported data which appeared to be repeated in other papers (already included in this analysis) by the same authors. After initial analysis of the results, which showed *ADH2* effects on both dependence and liver disease, but in different directions, two further papers (Ricciardi *et al.*, 1983; Couzigou *et al.*, 1990) were excluded because the groups compared were control (non-alcoholic) subjects and alcohol-dependent subjects with liver disease.

Data were extracted from the remaining ten papers (Thomasson *et al.*, 1991; Gilder *et al.*, 1993; Higuchi, 1994; Chao *et al.*, 1994*a*; Thomasson *et al.*, 1994; Maezawa *et al.*, 1995; Muramatsu *et al.*, 1995; Nakamura *et al.*, 1995; Yamauchi *et al.*, 1995; Tanaka *et al.*, 1996) on *ADH2* and *ADH3* genotype frequencies in the groups classified by the authors as: (1) controls; (2) alcohol dependence without diagnosed liver disease; (3) alcohol dependence and also alcoholic liver disease.

The allele and genotype frequencies were calculated. All subjects had *ADH2-1* or *ADH2-2* alleles only; no subjects were reported as having *ADH2-3*, which is believed to be present only in people of African descent. All data were converted, where necessary, to the actual number of subjects or chromosomes rather than the percentage in each group.

Eight papers contained data on *ADH2* genotype in groups classified as either control or alcohol-dependent. Five contained data on *ADH3* genotype in control and dependent groups. Three of the papers contained data on *ADH2* frequencies in alcohol-dependent subjects free of known alcoholic liver disease and also on subjects with both dependence and alcoholic liver disease, and only one contained data on *ADH3* types in these two groups.

Four of the papers contained Tables or data which allowed the effects of *ADH2* genotype on alcohol dependence to be contrasted across aldehyde dehydrogenase (*ALDH2*) groups. Two contained sufficient information to allow this to be done for *ADH3* and *ALDH2*.

Statistical methods

Tests for homogeneity of odds ratio (OR) across studies, calculation of the OR for each study and the associated 95% confidence intervals (CI), and (where there was no evidence of heterogeneity across studies) the OR and 95% CI for the combined data, were performed using StatXact-Turbo (Cytel Software Corporation, Cambridge, MA, USA).

Procedure

Firstly, ORs were calculated from the *ADH2* and *ADH3* allele frequencies in each group (control, dependence only, or dependence with alcoholic liver disease). This gives an estimate of the allele effect on the assumption of additive effects; that is, the difference between the 11 and 12 genotypes is the same as that between the 12 and 22 genotypes. This gives greater statistical power because of greater numbers, but will not distinguish between additive and dominant genetic effects.

Subsequently the ORs were compared across genotype to test whether heterozygotes were intermediate in risk between the homozygote groups. This was done by calculating ORs for 11 vs 12, 12 vs 22 and 11 vs 22 groups, using the number of subjects with each genotype.

Comparison of *ADH2* and *ADH3* effects between the *ALDH2-11* and *ALDH2-12* groups was achieved by extracting and summing the numbers of control and alcoholic subjects, by *ALDH2* and either *ADH2* or *ADH3* genotypes. The resulting summary tables were tested for homogeneity of odds ratios, in order to determine whether *ALDH2* type modifies the effects of *ADH* types.

RESULTS

Risk of alcohol dependence by ADH2

The effects of *ADH2-1* vs *ADH2-2* type on the odds ratio for dependence are shown in Fig. 1. The positive values indicate a greater risk of alcohol dependence associated with *ADH2-1* than with *ADH2-2*.

There was an apparent difference between the results of Gilder *et al.* (1993), on European subjects, and the rest, on Asian subjects. However, the test for homogeneity (across all eight reports) showed that the ORs were not significantly different (Breslow–Day statistic 6.733, $P = 0.457$). The estimated common OR was 2.78, 95% CI = 2.42–3.21, $P < 0.0001$. Therefore

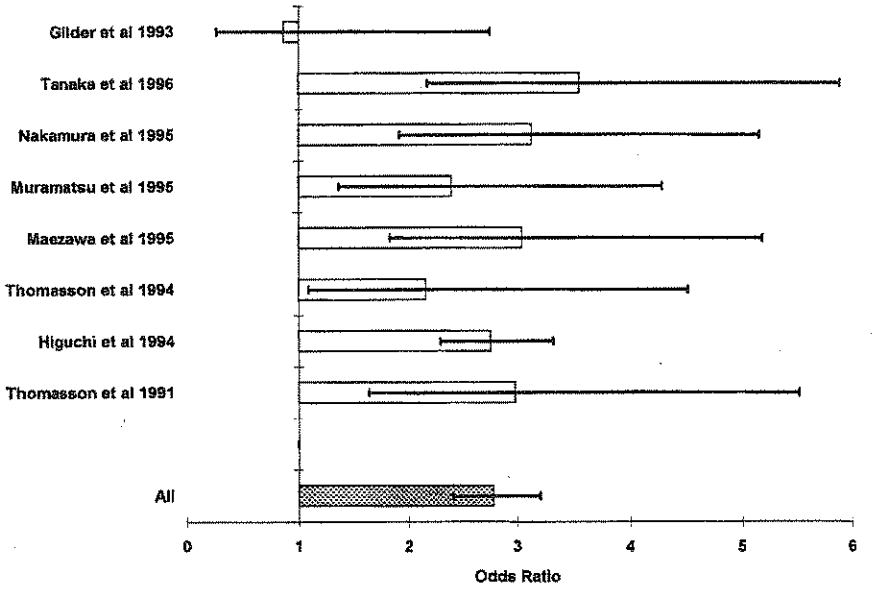

Fig. 1. *ADH2* and alcohol dependence, from allele frequencies.
The odds ratios (shown by the horizontal columns) and their 95% confidence intervals (shown by the error bars) were calculated for each paper individually, and for all of them.

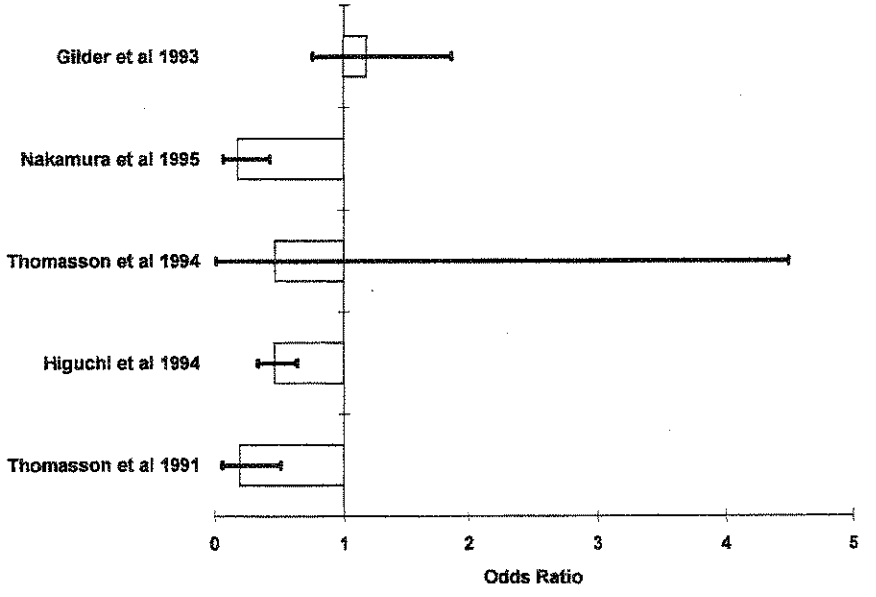

Fig. 2. *ADH3* and alcohol dependence, from allele frequencies.
The odds ratios (shown by the horizontal columns) and their 95% confidence intervals (shown by the error bars) were calculated for each paper individually.

each *ADH2-1* allele seems to be associated with an almost threefold increase in dependence risk, compared to an *ADH2-2* allele.

Risk of alcohol dependence by ADH3

Results for the effect of *ADH3* type on dependence are shown in Fig. 2. For *ADH3* variation, studies on European subjects made it easier to test the hypothesis of association, because the *ADH3-1* and *ADH3-2* gene frequencies are approximately equal. Nevertheless, the study using European subjects (Gilder *et al.*, 1993) showed negative results, whereas most of the studies on Asians showed a significant effect of *ADH3* genotype. The test for heterogeneity (taking all five reports) was highly significant: Breslow–Day statistic 24.1, $P = 0.0001$. By including only the Asian groups, no heterogeneity of results across studies was found: Breslow–Day statistic 6.085, $P = 0.108$. For the four Asian studies, the common OR was 0.38, 95% CI 0.29–0.51, $P < 0.0001$.

Risk of alcoholic liver disease by ADH2

Figure 3 shows the combined data for *ADH2* and alcoholic liver disease. Note that the comparison is with alcoholics without known liver disease, rather than with non-alcoholic controls, because only subjects with alcohol dependence are at risk of alcoholic liver disease. Although there are only three reports available, the results are compatible with each other (test for homogeneity of odds ratios, $P = 0.355$) and the estimate of the common odds ratio is 0.565, 95% CI 0.385–0.825, $P = 0.0024$.

Risk of alcoholic liver disease by ADH3

For *ADH3*, there is only one relevant report (Chao *et al.*, 1994*a*); the OR is 1.01, 95% CI 0.46–2.31. It seems that *ADH3* type has little or no effect on alcoholic liver disease, but an effect up to a twofold difference in risk would still be compatible with the data so far.

Relative effect of one or two copies of ADH2-2 or ADH3-2

Figure 4 summarizes the relative risk for subjects with 11 genotype compared to either 12 or 22 genotypes. The figure shows firstly the effect of *ADH2* genotype on alcohol dependence risk, secondly the effect of *ADH3* genotype on alcohol dependence risk (Asian subjects only), and thirdly the effects of *ADH2* genotype on alcoholic liver disease.

Comparisons were also made between 12 heterozygotes and 22 homozygotes. For alcohol dependence, *ADH2-12* heterozygotes were at significantly greater risk than *ADH2-22* homozygotes (common OR 1.60, 95% CI 1.30–1.98, $P < 0.0001$), but *ADH3-12* heterozygotes and *ADH3-22* homozygotes had essentially the same risk (common OR 1.00, 95% CI 0.27–3.16). For alcoholic liver disease, the risk was less in the *ADH2-12* heterozygotes than in the *ADH2-22* homozygotes (common OR 0.73) but not significantly so (95% CI 0.40–1.33).

Does ALDH2 type modify the ADH2 or ADH3 effects?

The calculated odds ratios for *ADH2*1* vs *ADH2*2* alleles were 2.74 (95% CI 2.25–3.34) for the *ALDH2*11* subjects and 3.88 (95% CI 2.69–5.58) for the *ALDH2*12* subjects. These odds ratios were not significantly different ($P = 0.106$). Similarly, the effects of *ADH3* on dependence, and of *ADH2* on liver disease, did not differ significantly between the *ALDH2*11* and *ALDH2*12* groups (homogeneity P 0.643 and 1.000, respectively).

DISCUSSION

ADH2 type and alcohol dependence

ADH type undoubtedly has an effect on alcohol dependence in Asians, as shown by aggregation of the data from multiple independent studies. The *ADH2-1* allele is associated with a greater risk. Several reports have shown that this is not conditional upon *ALDH2* status (Higuchi, 1994; Maezawa *et al.*, 1995; Nakamura *et al.*, 1995), and this is confirmed by the analysis of *ADH2* odds ratios by *ALDH2* type, shown above.

There may also be an effect of *ADH2* in Europeans, but the small number of *ADH2-2* alleles encountered in European subjects (whether control or alcoholic) makes this uncertain. At least 90% of most European populations are *ADH2-11*, with <10% heterozygotes and a very small proportion of *ADH2-2* homozygotes. If we postulate that the frequency of the *ADH2-11*

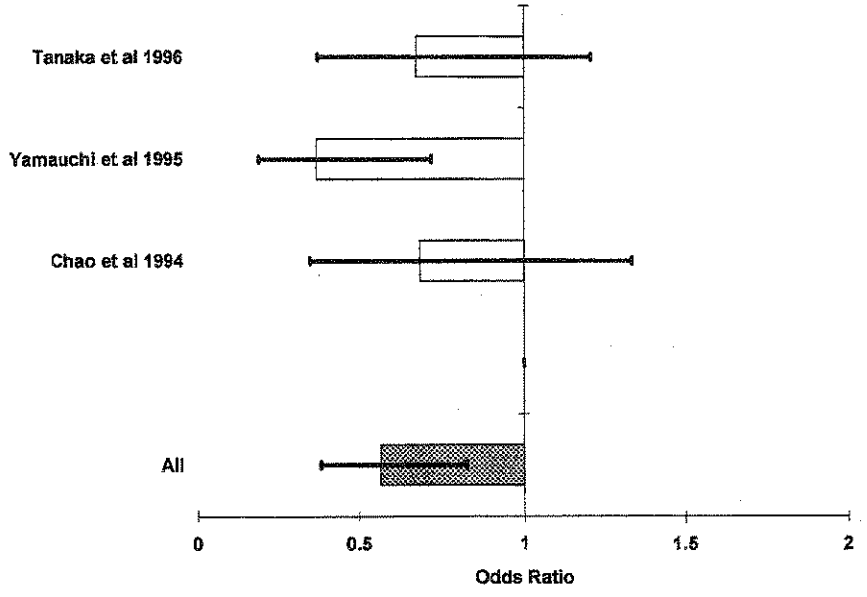

Fig. 3. *ADH2* and alcohol liver disease, from allele frequencies.
The odds ratios (shown by the horizontal columns) and their 95% confidence intervals (shown by the error bars) were calculated for each paper individually, and for all of them.

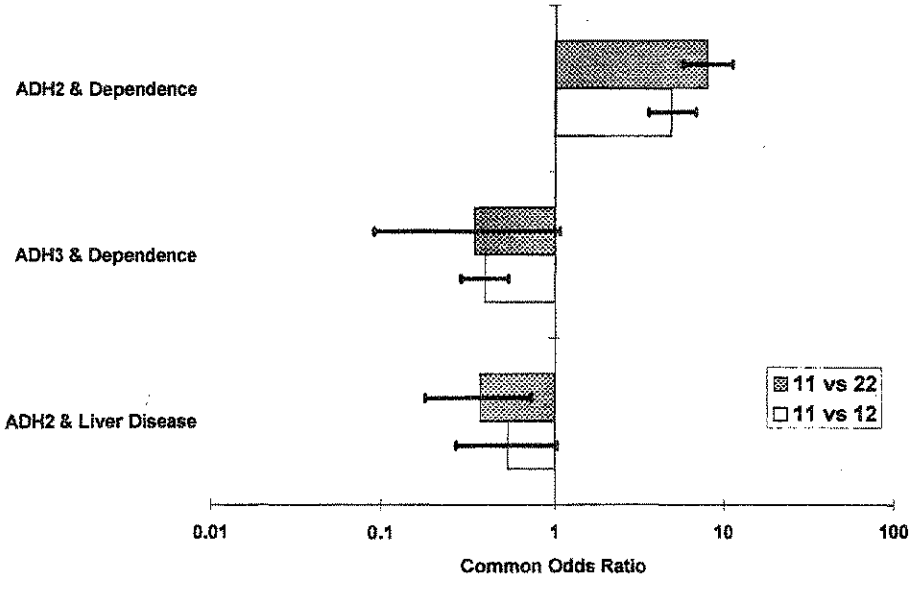

Fig. 4. Calculated odds ratios for homozygotes and heterozygotes for *ADH2* and *ADH3*.
Comparisons were made of the 12 heterozygotes (open columns) and the 22 homozygotes (shaded columns) against the 11 homozygotes. Error bars show the 95% confidence intervals. (Note the logarithmic scale.)

genotype is 90% in controls and 95% in alcoholics, it would require 863 controls and 863 alcoholics to have a 90% chance of detecting a difference at the $P < 0.01$ level (see Table A3 in Fleiss, 1981). However, the failure to show an *ADH* effect on dependence in European studies is not due to the absence of the inactive forms of *ALDH* among this population.

Examination of the results for each *ADH2* genotype showed that the heterozygotes were intermediate in risk between the two homozygous groups. The OR estimates suggest that the *ADH2-12* heterozygotes are rather closer to the *ADH2-22* group than to the *ADH2-11* group, but in both comparisons the difference was highly significant.

ADH3 type and alcohol dependence

In Asians, *ADH3-1* is associated with lower risk. However, comparison across genotypes shows that the difference is essentially between *ADH3-11* subjects, on the one hand, and homozygotes or heterozygotes with an *ADH3-2* gene, on the other. Comparison of the *ADH3-12* and *ADH3-22* groups shows no significant difference. The case of *ADH3* in Europeans is different; there is significant heterogeneity of the ORs for *ADH3*, which is removed when only the reports from Asia are considered. This suggests that the *ADH3* effect may be due to the known association of *ADH3-2* with *ADH2-1* in Asians, but further data are needed from other populations.

Alcoholic liver disease

ADH2 type has a significant effect on the probability of liver disease in alcohol-dependent subjects, and this appears to increase across genotype in the order 11 < 12 < 22. This is the opposite order to the dependence risk, and these opposing effects mean that studies which compare only control (non-dependent) subjects against alcoholics with cirrhosis may give falsely negative results. For this reason, two such studies were omitted from the analysis of published results.

GENERAL CONCLUSIONS AND COMMENTS

The effect of variation at the *ADH2* or *ADH3* loci is substantial. Calculation of the relative risks between *ADH2-11*, *ADH3-22* subjects, on the one hand, and *ADH2-22*, *ADH3-11* subjects on the other, implies an ~20-fold difference in the probability of alcohol dependence among Asian subjects. To put this in some perspective, data from a nationally representative population sample in the USA (Light *et al.*, 1996) showed that having a second- or third-degree relative; a first-degree relative; and both first- and second- or third-degree relatives with alcohol dependence was associated with 50%, twofold, and fourfold increases in DSM-III-R alcohol dependence risk, respectively. In the same study, being female rather than male decreased risk by ~50%. Apart from *ALDH* deficiency, *ADH* type is the most potent known influence on alcoholism.

From the information available so far, we cannot be sure whether these *ADH* effects are general, or restricted to Asian subjects. Indeed, we cannot exclude variation at another, closely linked, locus as the true cause. The *ADH2* polymorphism is widespread (Goedde *et al.*, 1992) and further information on *ADH* type and alcohol use in non-Asian subjects is likely to appear soon.

There is also a need for information relevant to the mechanisms of the *ADH* effect. The assumption that these effects on dependence risk or risk of alcoholic liver disease are mediated by differences in ethanol metabolism, and possibly by consequential differences in acetaldehyde concentration, is not strongly supported by experimental evidence from the limited number of studies *in vivo* (Yamamoto *et al.*, 1993; Whitfield, 1994). Further consideration will need to be given to possible mechanisms of these *ADH* effects.

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