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ADH AND ALDH GENOTYPES IN RELATION TO ALCOHOL METABOLIC RATE AND SENSITIVITY

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ABSTRACT

Variation in alcohol-metabolising enzymes has been proposed as an explanation for variation in alcohol use and the effects of alcohol, and the elucidation of the effects of ALDH deficiency in Asians has reinforced this view. This paper examines evidence on the relevance of ALDH and ADH variation in people of European descent, in relation to reactions to alcohol which affect alcohol use and to variation in alcohol pharmacokinetics. Genetic variation in ALDH does not explain subjects' self-reported reactions to alcohol. The known genetic variation in ADH2 has a significant effect on blood alcohol concentrations after a standard dose of ethanol but this occurs through an effect on the peak level rather than the rate of metabolism, and the ADH3 polymorphism has no effect. The substantial genetic variation in alcohol pharmacokinetics must be due to other genes, and strategies to locate and identify them are becoming available.

KEY WORDS: Alcohol reactions, blood alcohol concentration, ethanol elimination rate, ADH polymorphism

INTRODUCTION

The search for explanations for individual differences in alcohol use, alcohol dependence and alcohol-related disease has focused on two main areas; the enzymes which carry out alcohol metabolism and the receptors or neurotransmitters which respond to its presence. This paper considers the ways in which genetic variation in alcohol metabolism can affect behaviour and outcomes; in theory and as confirmed or refuted by experimental or epidemiological studies. It concentrates on the alcohol dehydrogenases and aldehyde dehydrogenases which catalyse the first two steps of alcohol metabolism.

Effects of variation in ALDH

Ways in which variation in aldehyde dehydrogenase activity, and hence variation in acetaldehyde concentrations during alcohol metabolism, could affect consumption, dependence and disease are listed in Table 1. In general, inactive ALDH is associated with unpleasant experiences with alcohol and low levels of alcohol use (as shown by the ALDH2 deficiency most commonly found among Asian subjects), but it has been proposed from time to time that slightly increased acetaldehyde concentrations after alcohol could lead to reinforcing effects.

As there are several forms of ALDH, variation in any might be significant. In general it has been assumed that the low-Km, mainly mitochondrial, ALDH2 is mainly responsible for acetaldehyde removal after alcohol but reports of a small number of subjects who have low cytoplasmic ALDH activity and who experience reactions suggests that despite its higher Km this mainly cytoplasmic enzyme, ALDH1, may be important in humans. Other ALDHs have high Km for acetaldehyde and probably do not play a significant role in alcohol metabolism.

Effects of variation in ADH

Variation in alcohol dehydrogenase activity affecting the rate of metabolism of alcohol can also potentially have a number of effects (see Table 2).

Table 1.

Alcohol consumption	Alcohol flush reaction (Wolff 1972, Schwitters et al. 1982). Other effects on experience of intoxication.
Alcohol dependence	Formation of acetaldehyde- neurotransmitter adducts (Collins et al. 1979). Protective effects of alcohol flush reaction (Agarwal & Goedde 1989, Ohmori et al. 1986).
Alcohol-related disease	Immunological response to acetaldehyde-protein adducts (Israel et al. 1988).

Table 2.

Intoxication	Peak and rate of metabolism affect intensity and duration of intoxication.
Alcohol consumption	May be modified by degree of intoxication achieved for each drink taken.
Alcohol dependence	Rate of metabolism determines rate of formation of acetaldehyde - see above.
Alcohol-related disease	Rate of metabolism determines rate of formation of acetaldehyde - see above.

These can be divided into two areas; firstly a more active ADH should reduce the blood alcohol concentration for a given dose of alcohol. In the short term a lower BAC is better because the person will be less impaired by the alcohol. The long-term effects of variation in alcohol metabolism on alcohol use are debatable, because higher BACs could be rewarding and encourage drinking or else lower BACs could encourage one to drink more to achieve a desired level of alcohol effects.

Secondly, faster alcohol metabolism will lead to faster generation of acetaldehyde. Other things being equal, this will result in higher acetaldehyde concentrations during alcohol metabolism so many of the arguments about ALDH can be relevant to ADH variation also. Faster alcohol metabolism also leads to faster generation of NADH and higher concentrations, as reflected in lactate/pyruvate ratios (Mascord et al. 1991), and this may have further effects on carbohydrate and lipid metabolism.

GENETIC VARIATION IN PHARMACOKINETICS AND SENSITIVITY: EXPERIMENTAL STUDIES

Sensitivity to alcohol

Taking sensitivity first, many groups have studied the effects of ALDH deficiency in Asian subjects on the physiological response to alcohol and on alcohol use and dependence. We have tested its effect on the impairment in psychomotor skills after alcohol. This is important because there are social pressures on some ALDH2-deficient subjects to drink, and such people may be at particular risk if they drive after drinking. These results will be presented elsewhere.

Of course, not only Asians but also some Europeans react badly to alcohol. We have found that around 5% report an unpleasant reaction to small amounts of alcohol; this could be due to many causes but a small number of Europeans have been reported as flushing after alcohol and having ALDH1 deficiency. We have also studied ALDH1 activity, and ALDH2 genotypes, and their effects on reactions to alcohol in subjects of European descent.

Alcohol pharmacokinetics

Many studies have been done on alcohol pharmacokinetics and on alcohol's acute effects. A smaller number have considered the range of variation within a population, the constancy of an individual's placing in the scale of sensitivity, and the causes of variation. The major source of information on genetic effects, and the precursor to our current studies, is the Alcohol Challenge Twin Study (ACTS) conducted in Sydney in 1979-81 (Martin et al. 1985a,b). In that study, alcohol was consumed by 412 subjects (206 twin pairs) and the blood and breath alcohol curves, and performance on a number of psychomotor tests, were monitored over the following three hours. Eighty of the subjects repeated the study on a second occasion. From these data, the repeatability and heritability of alcohol pharmacokinetic variables were calculated; the two variables considered here are the peak blood alcohol concentration (BAC) and the rate of decline in the BAC after the peak. The heritabilities and repeatabilities of these two measures are shown in Fig. 1, and it can be seen that essentially all the repeatable variation in both peak and rate is due to genetic factors.
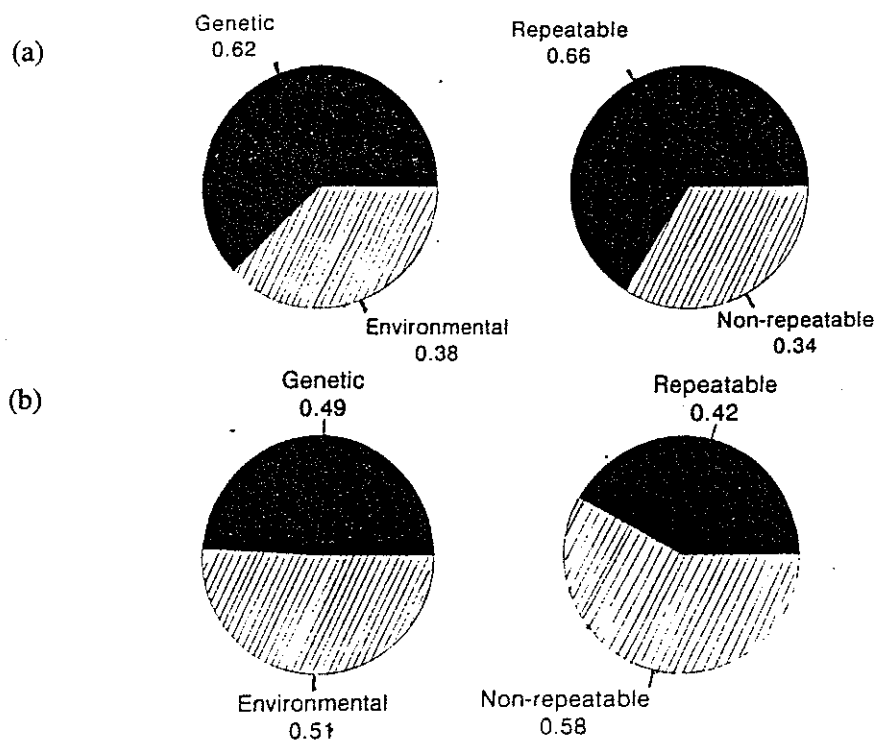

Fig. 1. Proportions of variance in (a) peak BAC and (b) rate of decrease of BAC accounted for by repeatable and genetic factors. (From data of Martin et al, 1985.)

Comparison of within-pair similarities between monozygotic and dizygotic twin pairs does not, of course, determine which genes are producing the genetic effect. In order to determine whether genetic effects on alcohol use and metabolism are mediated by variation in alcohol or aldehyde dehydrogenases, we need to know what variation exists in these enzymes, to have practical methods of typing them, and to match the genotypes with observations (short- or long-term) on the subjects.

In theory, and based on the in vitro properties of the various ADHs, subjects with ADH2-2 or (possibly) ADH2-3 should metabolise alcohol faster than subjects with ADH2-1, and subjects with ADH3-1 faster than those with ADH3-2. The in vitro characteristics of the ADH variants have been

known for some years (Bosron & Li 1986).

We now have the ability to type for these common variants of ALDH and ADH using DNA obtained from white blood cells, in order to test these hypotheses. Because extensive information had already been gathered on the ACTS subjects, they were re-contacted in 1990-93 and asked to provide a blood sample for further studies on ADH and ALDH variation and its effect on responses to alcohol. Eighty-five per cent of the original subjects were successfully followed up, and DNA from their blood samples has been used for ALDH2, ADH2 and ADH3 genotyping.

RESULTS

Effects of ALDH2 genotype and ALDH1 activity on alcohol sensitivity

As mentioned above, a significant number of these twin subjects, who are of European rather than Asian descent, reported that they have unpleasant reactions to small amounts of alcohol. We have previously reported (Whitfield & Martin 1993) that these reactions are associated with lower levels of habitual alcohol consumption. Further investigation of the cause of these reactions is in progress, but only one subject with the ALDH2-1,2 genotype has been found and so far no other variants of ALDH2 have been discovered. Investigation of ALDH1 activity of red blood cells has shown no association between the reported reactions and the enzyme activity. It therefore seems that the basis for European alcohol reactions is not an inborn error of metabolism in the same way as the Asian alcohol flush reaction; but in view of its significant effects further work on its cause is needed.

Effects of ADH genotype on alcohol metabolism in the ACTS subjects

The hypothesis that genetic variation in ADH2 and ADH3 can account for genetic variation in alcohol metabolism has been tested by comparison of mean values for the peak and rate between ADH2-11 and ADH2-12 subjects (no subjects with ADH2-3, and no homozygotes for ADH2-2 were encountered), and between ADH3-11, 12 and 22 subjects. Separate analyses were conducted for male and female subjects.

The results are shown in Fig. 2. Female subjects had higher peak BACs but the same rate of decrease, and ADH2-12 subjects had significantly lower peak values than ADH2-11 subjects, but similar rates of decrease on the unadjusted figures. ADH3 genotype had no effects on either peak BAC or rate of decrease.
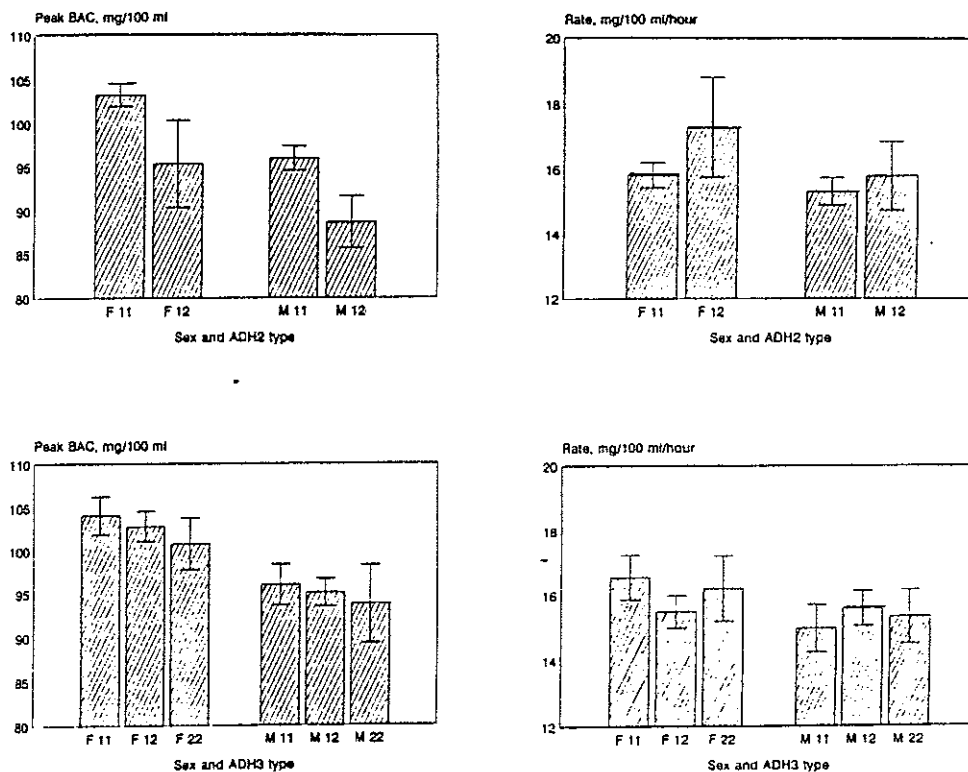

Fig. 2. Effects of ADH2 (top row) and ADH3 (bottom row) genotypes on peak BAC (left) and rate of decrease in BAC (right) after a standard dose of alcohol (0.75 g/kg). Results for men and women are shown separately.

It has previously been reported that such factors as body fat content and habitual alcohol intake, as well as sex, influence blood alcohol concentrations in these subjects (Martin et al. 1985a); and also that there is a correlation between the peak BAC and the rate of decrease (Whitfield & Martin 1994). These factors were therefore introduced as covariates in further analysis of variance for peak and rate.

Introduction of these covariates increased the statistical significance of the effect of ADH2 type on peak BAC (to $p < 0.001$), but comparison of Fig. 3, which shows the proportion of variance in peak and rate accounted for by the factors and covariates, with Fig. 1 shows that there is still a high proportion of the total genetic variance not accounted for. Inclusion of all factors and covariates, as listed in Fig. 3, produced a significant effect of ADH2 type on rate of alcohol metabolism ($p = 0.018$) but again there is evidence that other genes are contributing to the variation between subjects. ADH3 type did not become significant.
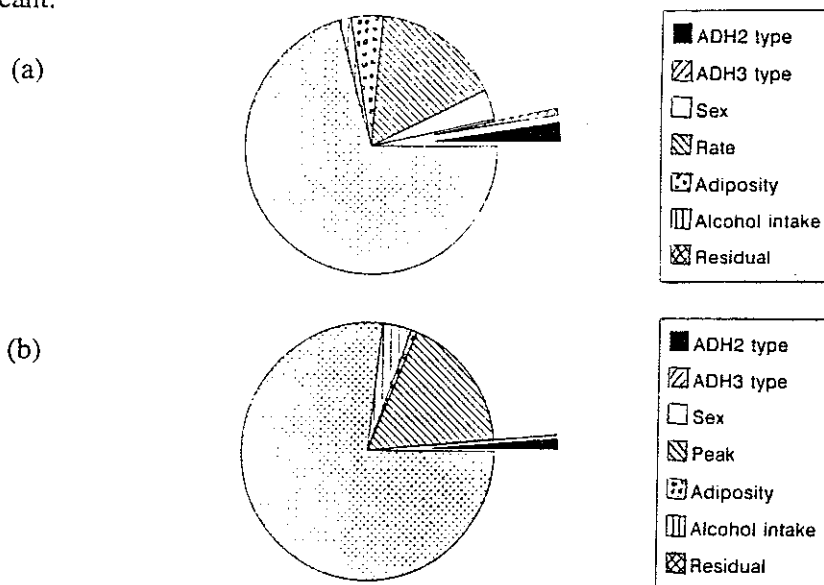
(a)

(b)

Fig. 3. Proportions of variance in (a) peak BAC and (b) rate of decrease of BAC accounted for by ADH genotypes, covariates, and undetermined factors.

DISCUSSION

Alcohol sensitivity

Sensitivity to alcohol has two components; some people report that they always or sometimes have unpleasant reactions to small amounts of alcohol, and this reduces their alcohol use. The other component is the degree of intoxication (subjective or objective) after alcohol; this may influence alcohol use (Schuckit 1993) or may be influenced by it, and it shows significant genetic variation. The importance of ALDH or ADH variation in determining alcohol sensitivity seems to be small in European subjects and work on variation in neurotransmitter metabolism and receptors is more likely to define the causes.

ADH in vitro/in vivo comparisons

From the in vitro properties of the ADH2 and ADH3 variants it would be expected that subjects with different genotypes would show considerable differences in rate of alcohol metabolism. For example, ADH2-2 is approximately forty times as active, per molecule, as ADH2-1. Different ADH3 enzymes show a two- to three-fold difference in activity. Despite these differences, only minimal differences in the rate of alcohol metabolism in vivo can be found. We are forced to conclude that either there are differences in the enzyme protein concentrations to counteract the differences in activity (which would imply that ADH expression is regulated to produce the same level of activity regardless of genotype), or genes which control the intracellular metabolic environment have the major influence on rates of alcohol metabolism.

Future directions

Although a significant influence of ADH2 type on peak BAC has been demonstrated, there are clearly further genes involved; and the genes affecting the rate of alcohol metabolism are still to be defined. There are a number of ways to proceed, based to a greater or lesser degree on systematic searches. For example, it has been reported that a restriction fragment length polymorphism detectable with a beta-ADH probe affects the probability of alcoholic liver disease in at-risk drinkers (Sherman et al. 1993) and presumably it does so by influencing some aspect of alcohol metabolism. Testing of this polymorphism, and any others discovered in ADH genes, may reveal the 'missing' genes; this approach relies on finding polymorphisms within candidate genes and testing them for *association* with phenotypic variation. However, there is an alternative strategy, which relies on *linkage* rather than association to locate genes affecting a quantitative characteristic, and preliminary results suggest it may be applicable to variation in alcohol metabolism.

Linkage methods are applicable to pedigrees or pairs of relatives. For most genetic diseases, where the phenotype of each member of the pedigree can be determined from the family history and/or cause of death, large pedigrees with affected members in multiple generations have generally been used. For quantitative characteristics such as alcohol metabolism or susceptibility to intoxication, which must be measured in an experimental setting, this is not feasible. Pairs of siblings, and in particular DZ twins, offer a promising alternative.

For any pair of full siblings (except monozygotic twins) some genes will be shared because of inheritance from the same parent, while others will differ. Use of polymorphic markers, either in large numbers evenly spaced throughout the genome or close to a candidate gene, will identify sib pairs as either concordant or discordant for each marker locus and also for any other loci close to it. If such a linked locus is influencing a quantitative characteristic, then concordant pairs will be more similar for that characteristic than discordant pairs, and this can be tested by an F-ratio test on the within-pair mean squared differences.

Although this sib-linkage approach requires large numbers of subjects and highly polymorphic markers, there is evidence from the twin results that ADH3-concordant DZ pairs are more similar for their peak blood alcohols than ADH3-discordant pairs. Further analysis of the data already collected, and testing with other markers and larger numbers of subjects, should reveal whether some presently unknown locus in or near the ADH gene cluster plays a major role in determining blood alcohol concentrations after drinking.

CONCLUSIONS

The alcohol flush reaction caused by ALDH2 deficiency in many Asian subjects has been very influential in focusing attention on genetic factors in alcohol sensitivity and use. However, aversive reactions to alcohol in other populations do not seem to have a similar cause and may not be very heritable.

The degree of intoxication experienced after a test dose of alcohol is another aspect of alcohol sensitivity. It varies greatly and is significantly influenced by genetic factors, but we do not know what they are. The interaction between alcohol sensitivity (in this sense) and alcohol use requires further work.

Alcohol pharmacokinetics vary for genetic reasons but the prime candidates, the known polymorphisms in ADH2 and ADH3, have less effect than in-vitro studies would suggest. Other genes influencing alcohol pharmacokinetics must be sought, and studies on pairs of siblings offer a way forward for both this and the alcohol sensitivity variation.

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