

Biochemical Markers and Susceptibility to Alcohol Dependence

JB WHITFIELD

Department of Clinical Biochemistry, Royal Prince Alfred Hospital, Camperdown, New South Wales 2050.

There is now strong evidence that alcohol-related problems are in part hereditary and it should therefore be possible to identify genes or gene products which contribute to susceptibility. Since there is heterogeneity in alcoholism, different markers will be abnormal in different patients and may be useful in determining aetiology, prognosis, and treatment. Biochemical characteristics which may be associated with differences in susceptibility include aldehyde dehydrogenase, monoamine oxidase and other aspects of serotonin metabolism, and adenylylase. The current status of these 'trait markers' and the reasons for investing effort in the search for useful markers are reviewed.

There is evidence that genetic and biological factors, as well as behavioural and environmental ones, influence susceptibility to harmful drinking and alcohol dependence. Such factors, existing at the gene level and expressed through variation in the properties of enzymes, receptors or structural proteins, might be useful in the classification of patients and might influence their prognosis and the likelihood of response to behavioural or pharmacological interventions. Identification of such factors through linkage and molecular biology techniques could allow the elucidation of the biochemical mechanisms involved through the so-called 'reverse genetics' (as has been done with muscular dystrophy).

Alcohol-related problems tend to aggregate in families. The most convincing evidence that this is due, at least in part, to genetic factors comes from adoption studies. Goodwin and others¹ showed that drinking problems were about four times as common in adopted-out sons of alcoholics as in adopted-out sons of non-alcoholics. This pattern was not observed in adopted daughters². Conversely, when adopted sons of alcoholics were compared with their siblings or half-siblings raised by the alcoholic parent, the two groups had similar rates of alcohol-related problems and diagnoses of alcoholism³. These adoption studies were based on the extensive records available in Denmark and Sweden, but similar results have also been obtained on adopted men in the USA^{4,5}.

Another important question is whether all of the heritable component of alcoholism is due to the same gene(s), or whether there is heterogeneity. A number of lines of evidence suggest that there are sub-types of alcohol dependence, and it has been reported that patients can be classified into groups differing in age of onset, personality characteristics, biochemical characteristics, and possibly prognosis.

By means of a cross-fostering analysis, using the fact that adoption is a randomising process in which adoptees of various genotypes are placed in varying environments, Cloninger and colleagues^{6,7} searched for genetic and environmental effects, and genotype/environment interactions, in adoptees with alcoholism of varying severity. They postulated that their data could be explained by two types of alcohol dependence, with differing genetic/environmental elements and different courses. Further evidence supporting the existence of heterogeneity in alcoholism comes from a study in St Louis based on analysis of 195 pedigrees of alcoholics⁸.

Cloninger⁹ summarised the characteristics of the proposed types. The first, Type 1 alcoholism is said to be characterised by onset after the age of 25, infrequent spontaneous alcohol-seeking and infrequent aggression after drinking, frequent loss of control and frequent guilt about drinking. Personality traits associated with Type 1 alcoholism include low novelty seeking, high harm avoidance and high reward dependence. Type 1 is found in both sexes and is only moderately heritable. Type

2 alcohol dependence is found only in males, is highly heritable, and shows the opposite pattern for all the above characteristics. The adoption studies cited showed that where a Type 2 genetic background was present, Type 2 alcohol abuse was equally prevalent whether or not there was a Type 2 environmental background, whereas the expression of a Type 1 genetic background in the sons was more likely in the presence of an unfavourable environment.

In summary, the evidence suggests that there are different types of alcoholism, with differing clinical features and differing genetics. Any search for 'markers' of a predisposition to alcoholism must take this heterogeneity into account. Heterogeneity would also explain why no marker of predisposition so far proposed is abnormal in all alcoholics.

Biological Markers

A number of biochemical or physiological measures differ between alcohol-dependent subjects and controls, and some can be used clinically to distinguish patients whose problems are related to alcohol consumption. These measurements have been called 'state markers' because they distinguish between people on the basis of a state created by current or previous alcohol intake^{10,12}. However, there have also been reports of differences between alcohol-dependent subjects and controls which do not seem to be related to the subject's state, but persist even through months or years of abstinence. These are postulated to be markers of the genetic 'trait' which leads to alcohol dependence.

One of the major difficulties in establishing the role of a risk factor in a disease is determining that the risk factor, or trait marker, precedes the disease. Usually, patients present for treatment after the pathological process has been under way for months or years and any differences between them and controls might be secondary to the disease. In the case of alcoholics, any difference from the general population could be due to a primary genetic characteristic causing or predisposing to the disease, or a genetic characteristic linked to the causative gene by proximity on the chromosome, or it could be secondary to the development of alcohol dependence or to other effects of alcohol. Changes secondary to the development of dependence could conceivably persist through many months or years of abstinence and only prospective studies can finally decide the issue.

Alcohol Metabolism

One way in which people who become dependent on alcohol might differ from others is in the metabolism of alcohol itself. There have been various claims about this, centring on either alcohol dehydrogenase (ADH) and the rate of metabolism of ethanol or aldehyde dehydrogenase (ALDH)

and the circulating concentrations of acetaldehyde during alcohol metabolism. In general, the concept was that the rate of conversion of alcohol to acetaldehyde by ADH and the rate of conversion of acetaldehyde to acetate by ALDH together control the circulating concentrations of acetaldehyde during alcohol metabolism. The acetaldehyde concentration would in turn determine the rate of production of morphine-like compounds from reactions between acetaldehyde and endogenous compounds, leading in time to addiction (see for example¹³).

Although many alcoholics have an increased rate of alcohol metabolism, this reverts to normal with abstinence. Study of alcohol metabolism in relatives of alcoholics has shown normal alcohol disappearance rates^{14,15}. Although one study¹⁶ reported a difference in blood acetaldehyde levels, with sons of alcoholics having higher acetaldehyde levels during alcohol metabolism than control subjects, doubts about technical aspects of the acetaldehyde measurements¹⁷ have led to this theory failing to gain general acceptance.

Both ADH and ALDH are polymorphic enzymes, with genetic variation between and within populations¹⁸. The various ADH enzyme sub-units do show quite wide variation in kinetic properties *in vitro* but there is no association between ADH polymorphism and the incidence of alcoholism¹⁹ and the effect of ADH type on alcohol metabolism *in vivo* has not yet been investigated in sufficient numbers of subjects.

It has recently been reported²⁰ that some people — maybe around 20% of the population — can increase their rate of alcohol metabolism within a short period of exposure to alcohol. One interesting feature of this phenomenon is that subjects who showed such an adaptive increase did not seem to have any family history of alcoholism, whereas the other subjects had about the expected rate of alcoholism in relatives. The significance and mechanism of any such difference has still to be established.

Genetic variation in ALDH can have an effect on the incidence of alcoholism, but it is mediated through an aversive effect rather than being a predisposing factor. ALDH exists in both cytoplasmic and mitochondrial forms. Genetic absence of ALDH leads to the so-called alcohol flush reaction, which usually leads those affected to avoid alcohol. This phenomenon has been best studied in Japan, where about half the population is affected by a deficiency of mitochondrial ALDH^{21,22}, but the deficiency gene also occurs elsewhere in Asia and in South America. The rate of ALDH deficiency in Japan has been compared between alcoholic and non-alcoholic subjects, and was found to be 2% and 41% respectively²³. Recently a small number of European subjects who flush in response to alcohol have been found to have unusual forms of cytoplasmic ALDH²⁴, but the prevalence and significance of this has not been determined.

Monoamine Oxidase (MAO)

Another probable path through which genetic variation could lead to alcohol use and dependence is neurochemical. It has been shown, for example, that rats bred for alcohol preference have low concentrations of serotonin and increased serotonin receptors in their brains and the preference can be overcome by serotonin uptake inhibitors²⁵. This, together with other evidence of altered serotonin metabolism obtained in humans, directs attention to this neurotransmitter system. Other reports^{26,27} show changes in second messenger systems within cells, raising the possibility that the response to alcohol may be less in some people so that they consume more to achieve the desired effect and in time induce dependence.

There have been a considerable number of reports of association between platelet monoamine oxidase activity and psychiatric illness, including alcohol dependence²⁸⁻³⁷, or personality traits thought likely to lead to behaviour regarded as

deviant³⁸⁻⁴¹. Factors affecting pMAO in the general population include sex, possibly race⁴², and genetic factors of an unknown nature. Conditions affecting platelet formation, age or survival can also alter the MAO activity of the platelets prepared by the usual procedures. These factors, together with the alterations reported in various psychiatric and neurological disorders, were reviewed by Sandler, Reveley and Clover⁴³. It is also important to note that the conditions of platelet harvesting have been reported to affect measured pMAO activity^{44,45}.

Monoamine oxidases have a fairly broad specificity but the two types, MAO A and MAO B, have different substrate preferences and inhibitor sensitivities. The form found in platelets is MAO B⁴⁶ with greatest activity on the substrates benzylamine, phenylethylamine and dimethylaminobenzylamine. The assay is usually carried out with radiolabelled substrate and the product is separated after incubation by solvent extraction or on an ion-exchange column.

Although a variety of methods and substrates have been used for MAO measurement nearly all the published papers agree that alcoholics have, as a group, lower platelet MAO values than controls. There is in most cases a considerable overlap between the groups.

Table 1. Reports on platelet MAO levels in alcoholics.

Reference	Substrate	Low in alcoholics
Major and Murphy 1978 ²⁸		Yes
Sullivan 1978 ²⁹	Tryptamine, 70 μ M	Yes
Sullivan 1979 ³⁰	Tryptamine, 70 μ M	Yes
Fowler 1981 ³¹	Tyramine, 50 μ M	Yes
	Tryptamine, 50 μ M	
	β -phenylethylamine, 50 μ M	
Alexopoulos 1983 ³²		Yes
Giller and Hall 1983 ³³		No (8 subjects)
Giller 1984 ³⁴	Tyramine, 10-500 μ M	Yes
von Knorring 1985 ³⁵	β -phenylethylamine	Yes (Type 2)
	Tryptamine	
Faraj 1987 ³⁶	Tyramine, 40-700 μ M	Yes
Pandey 1988 ³⁷	Tyramine, 250 μ M	Yes
von Knorring 1987 ³⁸	β -phenylethylamine	Yes (with drug abuse)
Tabakoff 1988 ⁴⁷	Phenylethylamine, 1.2 and 12 μ M	No (except in presence of ethanol)

One paper, however, reports that the basal platelet MAO in alcoholics is not significantly different from controls but the degree of inhibition by ethanol is greater⁴⁷. This alcohol inhibition effect is contrary to some other reports (e.g. reference 28) and further investigation of this finding is required in order to determine the optimum protocol for platelet MAO measurement. (The optimum reaction conditions will be those which give the greatest differentiation between patients and controls, not necessarily those which maximise the enzyme activity.) Inhibition of human platelet MAO activity by ethanol had previously been reported by the same group⁴⁸ and was of a competitive nature. Therefore the degree of inhibition will depend on the substrate concentration as well as the inhibitor concentration and low concentrations of substrate may be needed to demonstrate inhibition. This may explain other workers' negative results for ethanol inhibition but it does not, of course, explain why so many studies have found low pMAO activity in the absence of ethanol in alcoholics.

The consensus is that the platelet MAO difference is not secondary to the level or duration of alcohol intake, but represents a genetically determined risk factor. However, no substantial prospective studies have yet been published to confirm this view. Schuckit et al⁴⁹ compared 15 young men with alcoholic first-degree relatives (who would be expected to have an increased risk for future development of alcohol-

lism) with 15 control subjects of comparable age and drinking history without family history of alcoholism. The mean pMAO activity in the high-risk group was 10% below that of the controls but the difference was not statistically significant, possibly because of the small number of subjects.

More information is available about subjects who have been drinking heavily and have then become abstinent. There may be changes in pMAO activity after cessation of drinking; Major et al⁵⁰ reported on eight subjects who were admitted to an alcohol rehabilitation unit and had serial blood samples taken starting within 72 hours of their last drinks. An increase of about 60% over baseline values had occurred at six days with a subsequent fall to a plateau about 15% above baseline by 15-20 days. Alexopoulos et al⁵¹ compared 14 men who had ceased drinking about 48 hours before a blood sampling with 34 men who had been abstinent for six weeks; both groups had significantly lower pMAO than controls but the values were about a third higher in the immediate post-drinking period than they were at six weeks.

On a longer-term perspective, Brown⁵² reported that six alcoholics who had been abstinent for less than six months had significantly lower pMAO activity than eight alcoholics who had been abstinent for 1-22 years. Sullivan et al⁵³ found that alcoholics had significantly lower pMAO than controls after 3, 6 and 12 months of abstinence. Faraj et al⁵⁶ found that both hospitalised chronic alcoholics (within 24 hours of admission) and abstinent alcoholics (2-10 years abstinence) had lower pMAO than controls.

Therefore one may reasonably collect samples for pMAO during active drinking or within 2-3 days of cessation, or at a later stage (say three weeks after cessation of drinking); but results may be unrepresentative in the intervening period.

MAO and Alcoholism in Relatives

The low platelet MAO activities of alcoholics are of even greater interest when considered in the light of possible differences between Type 1 and Type 2 alcoholics. The relevant papers are those which distinguish between the postulated types of alcoholism or which include information on the relatives of the alcoholic subjects studied.

Major and Murphy²⁸ studied three groups of subjects; alcoholics and controls from the US military forces, and civilian controls, finding significant differences between each of these groups. The highest mean value was found for the civilian controls and the lowest for the military alcoholics. The military control group, with an intermediate mean value, contained about 20% of men who had features of alcoholism. When the family histories of the military alcoholics and the military controls were evaluated, they found that low-MAO subjects in both these groups were significantly more likely to have first degree relatives who were alcoholics, or who had 'psychosocial disturbances' (psychiatric hospitalisation, felony conviction, actual or attempted suicide) than the high-MAO subjects. (No information was available on the families of the civilian controls.) Conversely, subjects in either military group with alcoholic first-degree relatives had significantly lower pMAO than those without.

Similar results were found by Sullivan et al⁵⁰; after finding a significantly lower mean pMAO in alcoholics than controls, they contrasted the first-degree relatives of alcoholics in the lowest and highest deciles of pMAO activity. Relatives of low-pMAO alcoholics had lower pMAOs themselves, and there was also a significantly higher incidence of psychiatric hospitalisation, suicide attempts, and alcoholism than in relatives of high-MAO alcoholics. Relatives of low-MAO probands with a history of psychiatric illness had significantly lower pMAO than their relatives without such a history, but for the relatives of the high-MAO alcoholic probands there was no such difference.

Lower pMAO, and a significantly increased risk of alcoholism, in relatives of low-MAO alcoholic probands was also

found by Alexopoulos et al⁵². All these results point to an association between low pMAO and familial alcoholism.

Explicit use of the Type 1/Type 2 categorisation developed from the adoption studies referred to above also gave results consistent with this idea. For example, when a group of alcoholics were classified into Type 1 and Type 2 according to the criteria developed from the adoption studies, it was found⁵⁵ that platelet MAO in male Type 1 alcoholics, and all female alcoholics, did not differ significantly from controls, but male Type 2 alcoholics had significantly lower values than either Type 1 or controls. In another study³⁸, low-MAO alcoholics were more likely to be younger and have an earlier onset of problems, and they used illicit drugs more frequently, than high-MAO alcoholics, and they were also more likely to have two or more alcoholic family members. Here, however, it was found that not only male but also female alcoholics had lower pMAO than same-sex controls. These authors concluded that 'characteristics of the low MAO group are very similar to the ones described for Type 2 alcoholics' and suggested that 'platelet MAO may be a useful diagnostic marker for subtyping alcoholic patients'.

In view of reported differences in natural history and prognosis between the types, MAO values may also have prognostic implications.

Serotonin and 5-HIAA

So far the low platelet MAO seen in some alcoholics has been considered only as an empirical observation, but it must be linked (either in the genetic or, more probably, in the causal sense) with some feature of alcoholism susceptibility. It might be supposed that the changes in platelet MAO reflect the situation in the CNS, where one of the physiological functions of MAO is to convert the neurotransmitter serotonin (5-HT) to the inactive metabolite 5-HIAA. As mentioned above, animals bred for alcohol preference have low levels of brain serotonin. However, the literature is confusing and it is difficult both to formulate a unifying hypothesis, and to find ethically acceptable ways of testing it.

In humans (although only small numbers of subjects have been studied and there are obvious limitations on the studies which can be done) there is some evidence suggesting that CSF 5-HIAA levels are low in alcoholics, suicide attempters, and impulsively violent criminals (see reference 54). It has been proposed that impulsive and suicidal behaviour and alcoholism may be associated with a deficiency of serotonin; alcoholics in whom this was present would start to abuse alcohol at an early age, develop antisocial personality disorder, and manifest impulsive violent behaviour towards themselves and others⁵⁴. However, there is no well-documented relationship between pMAO and CSF monoamine metabolites in alcoholics; and in normal subjects, at least, pMAO is not correlated with CSF homovanillic acid, MOPEG or 5-HIAA.

Another difference in serotonin metabolism between alcoholics and controls has been observed by measuring serotonin uptake by platelets *in vitro*^{55,56}. Firstly, alcoholics (whether currently drinking, recently abstinent, or abstinent for 1-11 years) had a significantly lower K_m for serotonin uptake than controls and there was a trend towards a lower V_{max} . Secondly, the low K_m was confirmed in a different group of 'dependent alcoholics' but serotonin affinity was normal in 'alcohol abusers with no sign of alcohol dependence', while the V_{max} was significantly below control levels in both the dependent and non-dependent alcohol abusers. The difference in K_m is particularly interesting, both because it suggests a qualitative rather than a quantitative change and because it appears to be a marker of current or past dependence rather than of the amount of alcohol used. Another report⁵⁷ agreed with a low K_m for serotonin uptake in alcoholics but found that it rose to normal levels after a week's abstinence; this may indicate that the low K_m is a temporary

phenomenon or the rise into the normal range may be a transient one, similar to the changes in platelet MAO which occur after drinking ceases.

Adenylate Cyclase

Another possible biological marker of susceptibility to alcoholism is adenylate cyclase. This is an enzyme system which is stimulated by cell surface receptors to convert ATP to cAMP and three papers have reported differences in aspects of this system between alcoholics and control subjects.

In the first²⁷, intracellular levels of cAMP were measured in white blood cells and it was found that both the basal levels and the post-stimulation levels were much lower in actively drinking alcoholics than in normal controls or patients with liver disease.

The second paper⁵⁶ reported measurement of the basal and stimulated enzyme activity in platelets; the stimulated enzyme activity was highest in control subjects, lower in recently abstinent alcoholics, and lowest of all in 12-48 month abstinent alcoholics. (Median values for these three groups appeared to be 105, 75 and 45 pmole cAMP/min/mg of protein, respectively.)

Another group of reports on human subjects^{59,60} suggest a low platelet adenylate cyclase during withdrawal from alcohol, reverting to normal with abstinence, but a continuing reduced sensitivity of the enzyme system to alcohol, demonstrated in both platelets and white blood cells and present in subjects who had been abstinent for 1-10 years.

The interpretation of the adenylate cyclase results is still an open question; methods differ, and it is obviously important to distinguish between actively drinking subjects, recent withdrawal from alcohol, and long-term abstinence. The suggestion that alcohol normally stimulates this enzyme system, but is less stimulatory in alcoholics, is interesting because it could be part of a pattern of reduced response leading to greater intake of alcohol, but more work is needed.

There is no information on whether there is a difference between Type 1 and Type 2 alcoholism, but it appears that there is no correlation between MAO inhibition by alcohol and adenyl cyclase stimulation⁴⁷. Therefore these two phenomena seem to be related to different aspects of alcoholism.

Endocrine Markers of Predisposition to Alcohol Dependence

A number of endocrine responses controlled through the hypothalamus and pituitary have been shown to differ between alcoholics and controls. While these may be secondary effects, there is some evidence from relatives of alcoholics of pre-existing differences.

The TSH response to TRH is said to be low in alcoholics, and other abnormalities in thyroid hormone status have been reported, but these are probably secondary to alcohol intake⁶¹. Reports on relatives are mixed; of three studies reported, one⁶² found that three out of ten sons of alcoholics had a reduced TSH response to TRH, while a second⁶³ found an increased response. A third came down on the side of a reduced response but the group studied were selected for having 'a family or personal history of depression or alcoholism', a rather heterogeneous group⁶⁴.

Prolactin release in response to ethanol is reported to be less in normal men with a family history of alcoholism than in family history negative controls^{65,66} but, although this is an interesting aspect of overall response to alcohol, the difference between the two groups was small. A trend towards a diminished response to alcohol in sons of alcoholics has also been found for subjective response to a test dose of ethanol, and for changes in cognitive and motor performance after drinking⁶⁷ and it has been suggested that such a decreased response could lead to a greater alcohol intake.

Endocrine responses to stimuli are time-consuming to measure in large numbers of subjects and the literature does

not so far offer much prospect of their use in classifying subjects into different groups.

Markers of Current Alcohol Intake

There is an extensive literature on biological markers of alcohol intake (see, for example, references 10-12). These measurements can detect 50% or more of subjects taking harmful amounts of alcohol and can be used to monitor progress of intervention programmes. In addition, the level of such markers (which is related to the amount of alcohol consumed) may be an indirect measure of the degree of dependence on alcohol and of the likely response to treatment.

Screening Instruments and Intervention Programmes

As discussed above, there are both scientific and clinical reasons for attempting to identify markers of predisposition to alcohol dependence. But such biochemical markers, whether they are concerned with fundamental mechanisms of drinking behaviour or reflect peripheral effects such as liver damage, must be capable of guiding therapy if they are to be of clinical use and there must be evidence of the efficacy of treatments for drinking problems. Until the past decade such evidence has been hard to find. However, over this time evidence has accumulated of the effectiveness of intervention, particularly if it is undertaken at an early stage before dependence has become severe and permanent physical and neuropsychiatric damage has occurred.

Kristenson and colleagues⁶⁸ identified a cohort of middle-aged men who had drinking problems. After excluding those with an established dependence he allocated them randomly to treatment, which consisted of a session of counselling and periodic feedback of laboratory results, or to a control group. Over the next five years there was a 61% reduction in the number of days of hospitalisation in those receiving counselling compared with the control group; mortality was halved. Among hospital patients Chick et al⁶⁹ and Elvy et al⁷⁰ reported fewer alcohol-related problems and better psychological health in previously untreated problem drinkers who received counselling than control groups. In a multicentre study in general practice in the UK, Wallace et al⁷¹ found that 47% of men identified by screening as having an excessive alcohol intake had reduced this to the target level one year after counselling whereas only 25% of the control group had modified their intake in this way.

There is now sufficient evidence of benefit from intervention to justify a research effort to identify markers of susceptibility to alcohol problems, to conduct trials of their use in early intervention programmes, and to attempt to distinguish subgroups with differing responses to treatment.

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