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Genes for Alcohol Metabolism and Alcohol Sensitivity
Their Role in the Genetics of Alcohol Dependence

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I. BACKGROUND

As a result of multiple family, adoption, and twin studies over the past 20–30 years, it is generally accepted that there is an inherited component to the risk of alcohol dependence, and that this is due to the additive or interactive effects of multiple genes (1–6). The challenge is to identify these genes, to determine the magnitude of the risk that each variant confers, and to understand the mechanisms by which they exert their effects.

The building blocks for understanding the genetics of alcohol dependence are illustrated in Figure 1. The phenotype of alcohol dependence is determined by contributions from both environmental and genetic sources. In the genetic area, we need to understand how the effects of individual polymorphisms in genes, and the differing risks associated with allelic variants, contribute to the observed heritability.

In searching for genes affecting a complex condition such as alcohol dependence, one can start from physiological/biochemical processes known to affect risk (intermediate phenotypes) and study variations in genes that are likely to be relevant to them; or one can start without preconceptions by conducting a genome-wide search for genes that affect alcohol dependence risk and thereby discover novel and relevant metabolic or neurochemical systems. Each approach has its place, although the second requires a much larger investment in recruiting
Figure 1  Sources of variation in alcohol dependence risk, and routes to understanding of the contributions of individual genes to the overall genetic component of risk. (Top) Partition of phenotypic variance into genetic and environmental sources, usually by twin studies. (Bottom) Ways in which genes affecting the phenotype may be located and characterized. Knowledge of the phenotype and its biochemistry or pathophysiology will often suggest candidate genes. Overall, the fullest picture is obtained when the allelic effects of multiple genes are measured in family or twin studies, so the heritability can be compared against individual genes’ effects.
large numbers of related individuals; and each can be applied in both human and animal studies. Ultimately an integration of knowledge from both will be needed.

Genes that affect alcohol dependence risk may be considered in two classes: those that produce some measurable difference between low-risk and high-risk people in the absence of alcohol, and those whose effects are apparent only after consumption of alcohol. Examples of the first class (Fig. 2, left) may include genes that affect personality characteristics, such as novelty seeking and components of the Eysenck Personality Questionnaire (6); behavior problems such as conduct disorder (7); or addiction to other drugs including nicotine (8). The existence of comorbid psychiatric conditions in many patients with alcohol depen-

![Diagram showing the relationship between genes, proteins, physiology, and behavior.]

Figure 2  Behavior is influenced by genes through proteins and through the metabolic or physiological processes that the enzymes or other proteins control. Genes that contribute to differences in alcohol dependence risk may do so by affecting personality or psychopathology (left), or by affecting alcohol metabolism or susceptibility to alcohol's effects (right). Together, these classes of genes determine genetic risk of alcohol dependence and associated conditions.
dence (9) also suggests that an element of common psychiatric vulnerability—possibly with a genetic component—may exist. However, this class of genes, which are so far uncharacterized, is not considered in this chapter.

The second class (Fig. 2, right) is inherently more time-consuming and expensive to study, because observations made under controlled conditions before and after a standardized dose of alcohol are required. The alcohol-related group of risk factors may be affected by genes whose products bring about the metabolism of ethanol, or determine the degree of intoxication. These phenotypes and genes are the subject of this chapter.

This survey of progress up to the end of 1999 will cover four areas. Two relate phenotype to risk, and two relate genotype to the intermediate phenotypes and thence to dependence risk: (1) alcohol sensitivity and dependence risk; (2) alcohol metabolism and dependence risk; (3) genetic polymorphisms in alcohol sensitivity genes, and their effects on dependence risk; and (4) genetic polymorphisms in alcohol metabolism genes, and their effects on dependence risk.

II. ALCOHOL SENSITIVITY AND DEPENDENCE RISK

The concept of an inverse relationship between sensitivity to the effects of alcohol and the amount of alcohol consumed has intuitive appeal and has been supported by evidence from three areas:

1. The physiological sensitivity to alcohol manifested through facial flushing and other vascular symptoms, mainly seen in Asians but also in some people from other racial groups;
2. Studies of the relationship between psychomotor and subjective alcohol sensitivity, family history of alcohol dependence, and development of dependence in humans; and
3. Selective breeding of animals for alcohol consumption or alcohol sensitivity, and genetic analysis of the resulting inbred strains.

Sensitivity to alcohol may be defined in a number of ways. Many psychomotor tests such as reaction time or body sway are altered by alcohol and people vary in the degree of impairment. Subjectively, people can be asked to assess their overall degree of intoxication (although there are difficulties in comparing responses between individuals) or they can assess a number of separate aspects of intoxication such as euphoria or perceived loss of coordination. Physiological responses to alcohol can be assessed from changes in blood pressure or skin temperature, or in superficial blood flow in the skin using Doppler ultrasound. Hormonal or metabolic responses to acute alcohol intoxication, such as the function of the hypothalamic/pituitary/adrenal (HPA) axis, may be measured by tak-
ing blood or, possibly, saliva samples. All of these have been used in past studies of susceptibility to alcohol’s acute effects in humans.

Before going on to consider human intoxication, and the consequences of variation in susceptibility to intoxication, a number of difficulties should be mentioned. One of these is the phenomenon of tolerance; people who consume comparatively large amounts of alcohol become less sensitive to its effects, and this tolerance can be reversed by abstinence. It is therefore a consequence of alcohol use, rather than a cause, but cause and effect can be difficult to resolve. If people who are insensitive to alcohol’s effects are later found to be more likely to develop alcohol dependence, one cannot be certain that the insensitivity leads to dependence unless the subjects are studied before excessive drinking has commenced. Some aspects of causality can be addressed if the study design includes monozygotic twin pairs discordant for the proposed cause—in this case, pairs discordant for excessive alcohol consumption would allow us to decide whether insensitivity to alcohol is caused by alcohol consumption, or is an innate feature of the subjects’ genetic makeup.

Second, large numbers of subjects are needed for a prospective population-based study, because only around 20% of men and 5% of for women will become alcohol dependent by commonly accepted criteria. As always, the size of the study (and the proportion of affected subjects) will determine the power to detect risk factors; there may well be several independent risk factors with small but additive effects. For this reason some investigators have chosen to use a combination of high- and low-risk groups such as family-history-positive and (FHP) family-history-negative (FHN) subjects. Others have studied unselected groups and relied on the natural frequency of alcohol dependence (or other conditions of interest) in the population.

A. Alcohol-Induced Flushing

The alcohol flush reaction in Asians is well characterized. The molecular basis is a mutation in mitochondrial aldehyde dehydrogenase (ALDH2), which leads to low enzymatic activity in both the homozygous and heterozygous states (10) and high acetaldehyde levels during alcohol metabolism. It has been shown in many studies to reduce the risk of alcohol dependence (e.g., 11,12). The reactions to alcohol that occur in Europeans are generally less severe and are probably heterogeneous in their causes (13), but they can decrease alcohol use and seem to have the paradoxical effect of increasing dependence risk (14).

B. Sensitivity to Intoxication: San Diego, 1978 Onward

A series of studies on the effects of alcohol in young adult men with or without a family history of alcoholism was commenced by Schuckit and colleagues in
the 1970s. Initially, groups of around 30 FHP subjects and equal numbers of FHN controls were tested with two different doses of alcohol, or placebo, and a wide range of metabolic, endocrine, psychomotor, and subjective responses were investigated. A number of significant differences between the groups were found, and a discriminant function was constructed (15) that had reasonable success in distinguishing the groups (see Fig. 3). The items that made up the discriminant function were the maximum self-rated ‘terrible feeling,’ the maximum and 210-min plasma cortisol after 1.1 ml/kg of 95% ethanol (0.87 g/kg), and the maximum plasma prolactin after 0.75 ml/kg (0.59 g/kg).

The number of subjects tested was later increased to 453 and these have been followed up at intervals to assess the impact of family history and alcohol sensitivity on the development of alcohol dependence. The success of the follow-up at 8 years was a remarkable 100%, but variation in the testing protocol over the period 1978–1988, death of some subjects, and questions about paternity or the familial alcoholism status in others reduced the number for inclusion in the analysis of results to 335 (16). For the follow-up study, the sensitivity to alcohol (level of response) was defined slightly differently, being based on the prealcohol to 1-hour post 0.75 ml/kg change in subjective scores, body sway, and plasma cortisol. Family history had significant effects on maximum quantity (but not

![Discriminant Function Score](image)

**Figure 3** Discriminant function scores, based on subjective feelings and cortisol and prolactin results, for 30 family-history-positive (FHP) and 30 family-history-negative (FHN) male subjects. Although the groups of FHP and FHN subjects show different means, classification of individual subjects with this discriminant function is only partially achieved. (Reproduced with permission: From Schuckit and Gold, Archives of General Psychiatry 1988; 45:211–316. Copyrighted 1988, American Medical Association.)
frequency) of alcohol use, and on abuse and dependence on alcohol but not on abuse/dependence on cannabinoids or stimulants. This is in accordance with expectation, showing familial transmission of alcohol-specific dependence risk. Data from the full cohort of subjects suggested that both family history and the level of response to alcohol had independent effects on alcohol dependence risk. Restriction of the analysis to subjects with extremely high or low levels of response suggested that the effect of family history was mediated by differences in the level of response.

Studies in these subjects are continuing with the aims of achieving 20-year follow-up, recruitment of children of the original subjects, and progressive inclusion of genotyping for candidate genes in the evolution of the project.

C. Sensitivity to Intoxication: Australia, 1979 Onward

The second large study of sensitivity to intoxication commenced in 1979 with the work of Martin and colleagues in Canberra and Sydney, Australia. They tested pairs of twins with alcohol to determine the relative importance of genetic and nongenetic factors as causes of variation in alcohol pharmacokinetics and in alcohol's effects. Results on alcohol metabolism are discussed below. There were substantial genetic effects on intoxication (17), shown as genetic effects on test performance that were found only in the presence of alcohol. Initial analysis (18) of the relationship between alcohol consumption at the time of testing and susceptibility to intoxication suggested that the direction of causation was from consumption to sensitivity, but long-term follow-up now suggests otherwise (see below).

A total of 412 subjects were included in this Alcohol Challenge Twin Study (ACTS), and many of them also participated in postal surveys of alcohol use over the following decade. In 1990–92 a systematic program of follow-up of the ACTS subjects was initiated with the aim of obtaining blood samples for genotyping. In 1992–93 they were invited to participate in telephone interviews using the SSAGA questionnaire, which provides information on (among other conditions) alcohol dependence. Information on alcohol sensitivity and pharmacokinetics, subsequent alcohol dependence, and genotypes at selected loci could therefore be integrated for 334 of the original 412 subjects.

Examination of the results showed that many of the variables measured after alcohol challenge differed significantly between subjects who did and did not subsequently show alcohol dependence by the DSM-III-R criteria. Among the alcohol sensitivity measures, both body sway (Fig. 4) and self-report intoxication (Fig. 5) were less in the subsequently alcohol-dependent group. These variables were integrated into a composite alcohol sensitivity measure by Heath et al. (19). The difference in sensitivity between groups (alcohol dependence positive and negative) was significant in men but not in women.
Figure 4  Change in body sway by subsequent alcohol dependence status. AD+: alcohol dependent by DSM-III-R criteria, AD—: no alcohol dependence. The four columns in each group represent values for prealcohol and three postalcohol times in the ACTS study where subjects received 0.75 g/kg of ethanol. Sway data have been adjusted for height and weight and increasing (negative) values on the y-axis represent greater body sway, i.e., increased sensitivity to alcohol’s effects. Note the greater sway at all postalcohol times for the male AD— subjects.

This question of whether the alcohol insensitivity was caused by, or a consequence of, alcohol intake was reexamined by Heath et al (19). The intoxication/dependence association could be due to neurological tolerance to alcohol’s effects (as discussed above) if the subsequently alcohol-dependent subjects had been drinking more heavily at the time of original testing. Therefore, adjustment for the alcohol intake, and an alcohol problem score, at the time of alcohol challenge was incorporated into the analysis; the sensitivity score remained a significant predictor of dependence risk in men. Moreover, there was a substantial genetic correlation (0.72) and negligible environmental correlation (0.04) between alcohol dependence symptom count and alcohol sensitivity score; this means that some genes affect both sensitivity and dependence. Looking at the results in another way, men who were not themselves alcohol dependent but who had a monozygotic (‘identical’) cotwin with alcohol dependence had lower alcohol sensitivity scores than subjects from twin pairs where both twins were unaffected.
**Figure 5** Self-report intoxication by subsequent alcohol dependence status. AD+ : alcohol dependent by DSM-III-R criteria, AD− : no alcohol dependence. The three columns in each group represent three postalcohol times in the ACTS study where subjects received 0.75 g/kg of ethanol. Increasing values on the y-axis represent greater perceived intoxication, i.e., increased sensitivity to alcohol’s effects. Note the greater self-reported intoxication at all postalcohol times for the male AD− subjects.

Despite some limitations discussed in the paper, and the absence of significant effects in women, this twin study and Schuckit’s FHP/FHN study lead to similar conclusions. They support the concept that innate resistance to intoxication increases dependence risk and sensitivity to alcohol’s effects decreases it. It follows that the subjects who are resistant to alcohol’s intoxicating effects are not thereby resistant to its addictive properties; they are more likely to proceed to alcohol dependence.

**D. Other Human Studies of Intoxication**

A number of other studies have attempted to find a link between susceptibility to intoxication by alcohol and risk of alcohol dependence, usually by comparing high-risk and low-risk groups of subjects. Results have been mixed, but a meta-analysis of FHP/FHN studies (20) showed a significant effect. However, it may be relevant that some difference (nonsignificant) between FHP and FHN groups was found to occur with placebo.
A 10-year follow-up study of 43 sons of alcoholics and 28 control subjects was carried out by Volavka et al. (21). The indicator of alcohol sensitivity was changes in the EEG, and subjects with DSM-III-R alcohol dependence (but not alcohol abuse) had significantly less alpha wave changes in response to alcohol than subjects without abuse or dependence. It is unclear whether there is any association between EEG changes and other measures such as body sway or self-rating of intoxication, but the results are consistent with the general hypothesis of a reduced response to alcohol being associated with higher risk of dependence.

Genetic influences on sensitivity to intoxication in humans have also been studied. Genetic (father/son) transmission of sensitivity can be inferred from Schuckit’s studies on sons of alcoholics, and is explicit in a number of twin studies that measured either multiple end-points (17), changes in EEG (22,23), or acute tolerance to alcohol as shown in changes in EEG with time after alcohol (24). All these studies found evidence of significant genetic effects on sensitivity to alcohol.

E. Assessment of Susceptibility to Intoxication

If we accept that sensitivity or resistance to intoxication is an important predictor of alcohol dependence, two technical questions arise. First, which variables should be measured after alcohol consumption to obtain the best prediction? Second, is there any reliable way to obtain this information with a simpler and cheaper method?

In both of the prospective studies discussed above, a large number of variables were measured and only some have been used in the follow-up studies. The twin study of Martin et al. (17) showed that the various measures of intoxication are largely independent and no general intoxication factor can be extracted. Self-report of intoxication after a standard dose of alcohol, and change in body sway, are common to both groups while Schuckit also included plasma cortisol. The amounts of alcohol used in the two studies were similar, either 0.75 g/kg of ethanol or 0.75 ml/kg of 95% ethanol. Because of the alcohol challenge studies needed, the large number of subjects, and the substantial period of follow-up, it seems unlikely that we shall obtain a definitive answer to the question of the best predictor of dependence risk.

Alternative approaches may offer a way of assessing susceptibility to intoxication without experimental administration of alcohol. Schuckit has developed a brief questionnaire (Self-Rating of the Effects of alcohol, SRE) designed to determine the number of drinks required to attain intoxication in the ‘real-life’ situation (25). This covers the subjects’ experiences when they first began to use alcohol, during their period of heaviest alcohol use, and currently. The questionnaire results have been compared retrospectively with data collected at the time of alcohol testing (15 years previously) in 94 of the men in the FHP/FHN studies,
and with alcohol dependence diagnosis in 551 subjects interviewed as part of the COGA study (26). There were highly significant associations between SRE scores and subjective "high" results after alcohol, and between SRE scores and alcohol dependence diagnosis, but the sensitivity and specificity of SRE in predicting response or dependence for any individual were only moderate. Nevertheless this questionnaire is likely to be useful in tracing the pathways from genotype to sensitivity to dependence in large studies where alcohol challenge is impractical, and in identifying subgroups of subjects in whom alcohol insensitivity is, or is not, an important factor in the development of dependence.

F. Animal Breeding: Selection for Consumption and Sensitivity

In addition to these human studies, many strains of rats and mice have been developed by selection for extremes of alcohol sensitivity or alcohol preference. It was found that various strains of rats that differed in their alcohol preference differed, in the opposite direction, in their sensitivity to alcohol's effects (27). More recent studies have used inbred animals, initially produced as model organisms in which to investigate neurobiological mechanisms of dependence. After 25–30 generations of selection and inbreeding, animals with predictably high or low preference or sensitivity can be produced (28,29).

Initial studies concentrated on differences in neurotransmitter systems between the contrasting strains, while more recent work has identified quantitative trait loci (QTLs) through linkage studies in crosses between the strains. The QTLs are discussed below, but it is relevant to note that strains selected on preference tend to differ in sensitivity, and vice versa, reinforcing the concept that sensitivity to alcohol's effects reduces intake and the probability of dependence. Sensitivity to alcohol withdrawal in mice has also been analyzed (30).

III. ALCOHOL METABOLISM AND DEPENDENCE RISK

Some of the practical issues with prospective studies of alcohol metabolism or alcohol pharmacokinetics are similar to those mentioned above for alcohol sensitivity. Metabolic tolerance, in which the rate of alcohol metabolism is increased as a reversible response to regular alcohol use, can confound any association between blood alcohol results and risk of dependence. Study of subjects before they adopt hazardous drinking habits is necessary. Again as discussed above, substantial numbers of subjects are required to give the study adequate power; and the costs associated with initial testing of subjects with alcohol and of maintaining contact for follow-up are a consideration.
Probably because of these reasons, the Alcohol Challenge Twin Study and related work seem to be the only source of published information about alcohol pharmacokinetics and prospective dependence risk in humans. In the original ACTS (31), data were collected on both blood and breath alcohol concentrations at times between 40 and 210 min after the subjects’ consumption of alcohol. From these data it was possible to calculate the peak concentration and the rate of decrease in the postabsorptive phase, or to work with the individual readings. Both the peak and the rate showed significant genetic effects. Studies of the relationships between either peak or rate and reported usual alcohol consumption at the time of testing showed that both peak and rate were significantly and positively correlated with consumption, even at quite low levels of alcohol use. These associations seemed to depend on genes that affected both alcohol consumption and the alcohol pharmacokinetics (32).

Extension of this work became possible when alcohol dependence diagnoses were derived from the SSAGA interview results for the ACTS subjects. Initial

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**Figure 6**  Blood alcohol concentrations in men and women after 0.75 g/kg of ethanol. AD+ (continuous lines): subsequently alcohol dependent by DSM-III-R criteria, AD− (dashed lines): no alcohol dependence. Error bars show standard errors.
division of subjects into dependence-positive (AD+) and -negative (AD−) groups showed a difference in blood alcohol values between these groups for both men and women (Fig. 6). Differences between groups were also found for breath alcohol readings. Correction for usual alcohol consumption at the time of alcohol challenge did not abolish the difference between the AD+ and AD− groups, which appears to be based on a relationship between the early alcohol metabolism and risk of future dependence. Further analysis of these data is in progress, with the aims of correcting the results for gene sharing between members of twin pairs and clarifying the relationships between alcohol metabolism in vivo, alcohol dehydrogenase genotypes, and alcohol dependence risk. So far, it seems that ADH2 and ADH3 polymorphisms do not fully explain the link between alcohol metabolism and dependence.

Comparison of the separation between AD+ and AD− subjects, by calculating Z-scores to put the variables on a common scale (Fig. 7), suggests that the

![Graph showing differences in blood and breath alcohol values, body sway, and self-report intoxication between AD+ and AD− subjects. The graph compares male and female subjects.](image)

**Figure 7** Summary of differences in blood and breath alcohol values, body sway, and self-report intoxication after alcohol challenge, between subjects with and without subsequent alcohol dependence. All variables have been transformed to Z-scores by dividing the mean difference between AD+ and AD− groups by the standard deviations.
association between alcohol dependence and blood or breath alcohol concentrations is at least as strong as those between alcohol dependence and body sway or self-report intoxication. Moreover, the blood and breath alcohol values are associated with subsequent dependence risk in women as well as men. There is little relationship between blood alcohol results and sensitivity to alcohol effects (17), and in particular the sensitivity to alcohol is not associated with ADH type (19), so there appear to be two distinct groups of genes involved in the pathways from alcohol metabolism, and from intoxication, to the common end-point of dependence risk.

IV. POLYMORPHISMS IN ALCOHOL SENSITIVITY GENES

Phenotypes have been defined that lead to increased alcohol dependence risk, and these phenotypes show significant heritability. Therefore, the next step is to determine what genes may affect these phenotypes, and to test whether they also show significant effects on alcohol dependence. At the same time, genes found by other routes to influence alcohol dependence can be tested for effects on the postulated intermediate phenotypes of alcohol sensitivity and alcohol metabolism.

Animal work has been quite productive in defining the neurochemical differences between animal lines that differ in their sensitivity to alcohol’s effects. Multiple receptor systems, including serotonin, dopamine, GABA, and opioid, have been shown to differ between the contrasting inbred lines in one or more of the animal models (33). This is a helpful initial step in defining candidate genes. Crossing of the inbred lines and use of genetic linkage markers to detect quantitative trait loci (QTLs) has located one highly significant locus in the P/ NP rats, which includes the neuropeptide Y (NPY) gene (34,35). QTLs have also been located in mice (36) and ways of moving forward from QTL location have been discussed (37).

Testing of genetically engineered mice (knockout or overexpressing for the gene of interest) for alcohol’s effects has shown a number of instances where genes affect alcohol preference and/or sensitivity to its effects. Such studies confirm and extend the results from the more pharmacologically oriented studies on the inbred rats and show which genes and gene products deserve further study.

Some of the results of these animal studies are summarized in Table 1. The existence of a reciprocal relationship between alcohol sensitivity and alcohol preference has been shown in several cases. The genes involved are mainly neurotransmitters and their receptors, or protein kinases that may modify receptors and their binding properties. The modification of receptors by phosphorylation may be a mechanism of acute tolerance to alcohol, and such tolerance may well be a relevant aspect of alcohol sensitivity. The role of insulin-like growth factor 1
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Table 1  Genes Shown to Affect Alcohol Sensitivity in Studies of Recombinant Mice

<table>
<thead>
<tr>
<th>Gene</th>
<th>Knockout</th>
<th>Overexpressed</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine receptor D2</td>
<td>Intake –</td>
<td></td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Sensitivity –</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine receptor D4</td>
<td>Sensitivity +</td>
<td></td>
<td>39</td>
</tr>
<tr>
<td>FYN tyrosine kinase</td>
<td>Sensitivity +</td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Insulin-like growth factor 1</td>
<td>Sensitivity:</td>
<td></td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Sleep time –</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tolerance –</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF binding protein 1</td>
<td></td>
<td></td>
<td>41</td>
</tr>
<tr>
<td>Neuropeptide Y</td>
<td>Intake +</td>
<td></td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Sensitivity –</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intake –</td>
<td></td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Sensitivity +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein kinase C epsilon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotonin receptor 5HT1B</td>
<td>Intake +</td>
<td></td>
<td>44</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>45</td>
</tr>
<tr>
<td>Serotonin receptor 5HT3</td>
<td>Intake –</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Sensitivity +</td>
<td></td>
<td>47</td>
</tr>
</tbody>
</table>

(IGF-1), however, is unexpected and so far unexplained; it illustrates the potential of gene studies to provide a starting point for further physiological studies.

However, the fact that a gene produces a protein that is involved in the chain of events leading to intoxication does not prove that the equivalent gene in humans is responsible for the heritability of susceptibility to intoxication or dependence. The gene may not be polymorphic in humans, or the variants may be rare. With this reservation in mind, genes found to be relevant in experimental animals provide prime candidates for human linkage or association studies. Some results from such studies are starting to appear. However, we do not yet know whether variation in the syntenic genes affects alcohol sensitivity in humans. Studies on dopamine receptor genes (probably not related to alcohol sensitivity) have given contradictory results, possibly because of variation between and within populations and possibly because the effects are small and the power of some studies has been insufficient. These association studies illustrate some of the difficulties that must be overcome.

Although large-scale studies with linkage techniques and microsatellite markers have yielded QTLs in rats and mice, this has not yet been a route to discovery of alcohol sensitivity genes in humans. Investigation of the serotonin transporter gene (5-HTTLPR) appears to have arisen from the probable involve-
ment of serotonin systems in impulsive or violent behavior, and from the existence of a deletion/insertion variant that affects gene transcription and transporter expression. Turker et al. (48) reported an association between the short form of the 5-HTTLPR and high ethanol tolerance in young adults, but the association was significant only in this subgroup of subjects. Schuckit et al. (49) found an association between serotonin transporter genotype and response to alcohol, and development of alcoholism, in a small number of subjects from their long-term study but Edenberg et al. (50) were unable to find any linkage or association between this polymorphism and alcohol dependence.

V. POLYMORPHISMS IN ALCOHOL METABOLISM GENES

Animal breeding and human genome-scan methods have not been applied to discovery of alcohol metabolism genes per se, and animals selected for alcohol preference or sensitivity have not been found to have major differences in their alcohol metabolism. Nevertheless, enzyme and protein techniques revealed a number of polymorphisms in human alcohol-metabolizing enzymes and the molecular basis of these has now been clarified.

The impact of variation in \textit{ALDH2} is restricted to subjects from Northeast Asia and their descendants. No common polymorphisms in \textit{ALDH2} outside Asia have been found despite searches (51–53), and the frequency and significance of any variation in \textit{ALDH1} (54) is uncertain. A polymorphism in \textit{ALDH2} in rats bred for alcohol preference or nonpreference (P/NP) offers a cautionary example as it causes a significant amino acid substitution and was found at higher frequency in the nonpreferring line (55), but it was later shown to be unrelated to alcohol preference (56). However, it is possible that a recently described promoter polymorphism in human \textit{ALDH2} (57,58) affects expression and hence enzyme activity.

\textit{ADH2} variation also has significant effects on alcohol dependence risk, both within Asia (59,60) and beyond (61,62). This is independent of \textit{ALDH2} status, as the risk varies by \textit{ADH2} genotype even among subjects with the active form of \textit{ALDH2}. Associations between \textit{ADH3} genotype and alcohol dependence risk have also been reported, but it has recently been shown that this effect is due to linkage disequilibrium with \textit{ADH2} at least in Asian populations (60,63). Variation in other \textit{ADH}s is unlikely to affect alcohol metabolism, although a recently reported \textit{ADH4} polymorphism (64) may affect expression of this comparatively low-affinity isoenzyme.

The effects of \textit{ADH} variation on dependence risk are usually ascribed to faster alcohol metabolism and higher acetaldehyde concentrations in subjects with the more active forms of the enzymes. \textit{ADH2} genotype has strong effects on alcohol dehydrogenase activity in vitro (65), but the in vivo effects are not
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as predicted (66,67). It is possible that other mechanisms for the ADH2 effect, such as variation in competitive inhibition of the metabolism of other ADH substrates by ethanol, must be sought.

It was shown above that variation in blood and breath alcohol levels after a test dose was associated with variation in subsequent alcohol dependence. Since ADH2 genotype determines the in vitro enzyme activity and affinity for substrate and coenzyme, and affects dependence risk, we need to investigate whether ADH2 variation accounts for all the association between alcohol pharmacokinetics and alcohol dependence. Preliminary results suggest that ADH2 type does not offer a full explanation, and other genes that affect alcohol metabolism (31) need to be sought and tested for their effects on dependence risk.

VI. CONCLUSIONS

It seems that some, but not all, of the genetic effects on alcohol dependence risk are mediated through genes that affect alcohol metabolism or sensitivity to intoxication. Animal studies suggest that around a half-dozen genes are involved in alcohol sensitivity. Presumably other genes will be located that will account for genetic effects on other pathways to alcohol dependence, including the influence of personality variation and susceptibility to addiction to other drugs including nicotine. Not all genes whose products are involved in alcohol metabolism or intoxication will prove to be polymorphic in humans.

Both candidate gene and genome-scan approaches have been widely and successfully used in the investigation of inborn errors of metabolism and other single-locus diseases. The so-called “reverse genetics” has been successful with conditions such as cystic fibrosis, muscular dystrophy, or Huntington disease where the biochemical defect was not understood until the gene was discovered. However, this approach may be less successful for complex diseases where the additive effects of many genes are important, because the power of linkage techniques to detect small effects is limited. For alcoholism, as for other multifactorial conditions such as diabetes or heart disease, an understanding of risk factors and the investigation of candidate genes suggested by this understanding will continue to be important.

If and when genes affecting alcohol dependence risk in humans are identified, what are the likely consequences? The number of loci that would have to be genotyped to obtain a reasonable estimate of dependence risk for an individual will depend on the number of relevant polymorphic genes, the frequencies of the alleles, and the risk associated with each allele. There may be many such genes but this presents little technical difficulty; the problems lie in establishing data for the conversion of genotypic information to risk assessment and in developing useful risk-based counseling approaches. However, even those genes and their
products that are not polymorphic in humans will be potential targets for therapeu-
tic agents; and in this respect animal studies offer a useful complement to
purely human ones because the details of the relevant polymorphisms will differ
across species.

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REFERENCES

1. DW Goodwin, F Schulsinger, L Hermansen, SB Guze, G Winokur. Alcohol prob-
lems in adoptees reared apart from alcoholic biological parents. Arch Gen Psychiatry
2. DW Goodwin, F Schulsinger, N Moller, L Hermansen, G Winokur, SB Guze. Drink-
ing problems in adopted and nonadopted sons of alcoholics. Arch Gen Psychiatry
3. M Bohman, S Sigvardsson, CR Cloninger. Maternal inheritance of alcohol abuse: cross-
4. Z Hrubec, GS Omenn. Evidence of genetic predisposition to alcoholic cirrhosis and
psychosis: twin concordances for alcoholism and its biological points by zygosity
5. KS Kendler, CA Prescott, MC Neale, NL Pedersen. Temperance board registration
for alcohol abuse in a national sample of Swedish male twins born in 1902–49.
6. AC Heath, KK Bucholz, PA Madden, SH Dinwiddie, WS Slutske, LJ Bierut, DJ
Statham, MP Dunne, JB Whitfield, NG Martin. Genetic and environmental contribu-
tions to alcohol dependence risk in a national twin sample: consistency of findings
7. WS Slutske, AC Heath, SH Dinwiddie, PA Madden, KK Bucholz, MP Dunne, DJ
Statham, NG Martin. Common genetic risk factors for conduct disorder and alcohol
8. WR True, H Xian, JF Scherrer, PAF Madden, KK Bucholz, AC Heath, SA Eisen,


47. SR Engel, AM Allan. 5-HT3 receptor over-expression enhances ethanol sensitivity in mice. Psychopharmacology (Berl) 144:411–415, 1999.
56. LG Carr, J Kirchner, L Magnes, L Lumeng, TK Li. Rat mitochondrial aldehyde


