Molecular Biology of Alcohol Dependence, a Complex Polygenic Disorder

John B. Whitfield1, Brian N. Nightingale1, Martin E. O’Brien1, Andrew C. Heath2, Andrew J. Birley3 and Nicholas G. Martin3

1 Department of Clinical Biochemistry, Royal Prince Alfred Hospital, Sydney, Australia
2 Department of Psychiatry, Washington University, St. Louis USA
3 The Queensland Institute of Medical Research, Brisbane Australia

Alcohol dependence, and the medical conditions which arise from prolonged excessive alcohol use, have no single cause. Like other complex diseases, they result from a combination of social, personal and genetic contributions; but within any society genetic variation has a substantial influence on individual risk. The genes presently known to affect alcohol dependence produce variation in alcohol metabolism; other genes which affect personality or susceptibility to intoxication are likely to be significant but so far reproducible evidence is scanty. Designs which include related subjects have advantages for the study of complex diseases, because any association effects can be placed in the context of overall heritability and because linkage analysis can also be included. Examples of our studies of alcohol metabolism, consumption and dependence are presented.

Introduction

Excessive alcohol consumption, alcohol dependence, and alcohol-related diseases form an overlapping group of conditions affected by social attitudes, availability and price of alcohol, and genetic factors which cause variation in susceptibility between different individuals. This mix of social, environmental and genetic factors is seen in a number of common and clinically significant conditions. Such complex diseases follow a pattern of underlying contributing factors, many of which are unknown, leading to a definable and frequently long-term pathology, and ultimately to complications or clinical end-points producing death or severe impairment (Tab. 1). At each of these stages, genetic variation may modify risk. The challenge is to characterise the genes (and, of course, the non-genetic risk factors) and estimate their relative importance.

Identification of the genes which contribute to risk of excessive alcohol consumption and dependence, or to the organ damage which results in some patients, would establish which biochemical or cellular systems are involved in the pathogenesis of these conditions and might improve the quality of the advice which can be offered to individual patients. Such benefits of genetic epidemiology are of course applicable to, and the aim of research on, many diseases.

Tab. 1 Examples of complex diseases in which genetic factors are thought to play a significant role. In each case the genes must exert their effects through known or unknown risk factors. Frequently there is a definable chronic stage which proceeds to one or several clinical end-points, which in turn may be subject to separate genetic or environmental influences.

<table>
<thead>
<tr>
<th>Complications</th>
<th>Coronary heart disease</th>
<th>Non-insulin-dependent diabetes mellitus (NIDDM)</th>
<th>Osteoporosis</th>
<th>Alcohol-related disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic state</td>
<td>Atherosclerosis</td>
<td>Nephropathy, retinopathy, vascular disease</td>
<td>Fractures</td>
<td>Cirrhosis, brain damage, cardiomyopathy, pancreatitis, trauma</td>
</tr>
<tr>
<td>Risk factors</td>
<td>Lipids, smoking, blood pressure, insulin resistance, age, family history</td>
<td>Hyperglycaemia, hyperinsulinaemia Obesity, age, impaired glucose tolerance, family history</td>
<td>Decreased bone density</td>
<td>Alcohol dependence, alcohol abuse</td>
</tr>
<tr>
<td>Genes</td>
<td>Genes coding for apolipoproteins or for apolipoprotein receptors</td>
<td>MODY genes GCK, HNF1α, HNF4α. Later onset NIDDM, genes unknown</td>
<td>Vitamin D receptor gene; status presently uncertain</td>
<td>Sex, year of birth, alcohol metabolism, alcohol sensitivity, conduct disorder, depression, personality</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ALDH, ADH, possibly dopamine receptors (DRD2, DRD4), monoamine oxidase, GABAA receptor, fyn tyrosine kinase</td>
</tr>
</tbody>
</table>
Genetic Factors in Alcoholism

The existence of genetic factors for alcohol dependence was suggested by the fact that it clusters in families, and has been confirmed by a substantial number of adoption and twin studies (1–3, and summarised in 4). With the increasing use of alcohol by women in many countries, investigators have sought to determine whether the causes of variation in alcohol consumption or susceptibility to dependence are the same in women as in men. Adoption studies suggested that one form of alcoholism is strongly heritable and confined to men, while another has a greater environmental component and can occur in either sex. Meta-analysis of published twin studies, and a recent twin study including a majority of women (4), suggest that the causes of alcohol dependence are substantially similar in men and women.

The next question to arise from such a finding is, what specific genes are causing the heritable variation in susceptibility? So far, studies in Asian subjects have revealed that genetic variation in alcohol metabolism (ADH and ALDH genes) can affect alcohol dependence and alcoholic cirrhosis (5–7); while studies on a dopamine receptor gene in multiple populations have yielded conflicting results (8). The sequence of publications in both these areas underlines the importance of multiple studies on independent populations in establishing the role of a polymorphism in causing alcohol dependence or any other complex disease.

In the case of ALDH all reports agree on its influence in Asians, where the inactive ALDH2*2 form is quite common, and on its lack of significance (because of absence of ALDH2*2) for other populations. For ADH, the importance of ADH2 and ADH3 variation took longer to emerge and again was firmly established by studies in Japan and China. There are still questions about whether ADH3 variation has effects on alcohol use or dependence in non-Asian populations.

The literature on the dopamine D2 receptor (DRD2) polymorphism is even more conflicting; an initial highly publicised report has been followed by a stream of mainly negative papers (see 8). Such a series of events means that each positive report needs to be replicated, probably several times, before acceptance and this greatly increases the resources which have to be employed. Meta-analysis techniques can be applied to multiple reports on diverse populations to determine whether the reports are consistent and jointly significant, and to estimate the magnitude of the relative risk by genotype (7).

Strategies for Characterising Genes Contributing to a Complex Disorder

Several approaches are possible and under active investigation. All start from the position that genetic effects on alcohol dependence exist, and all have the common aim of defining mutations or polymorphisms in humans which cause this genetic variation. The routes between these two points diverge (Tab. 2).

<table>
<thead>
<tr>
<th>Tab. 2</th>
<th>Whichever approach is taken to discovering “genes for alcoholism”, the same assumptions and the same final validation apply.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting point</td>
<td>Intermediate stages</td>
</tr>
<tr>
<td>Traditional genetics</td>
<td>Existence of genetic effects on alcohol dependence in humans</td>
</tr>
<tr>
<td>&quot;Reverse&quot; genetics (positional cloning)</td>
<td>As above</td>
</tr>
<tr>
<td>Animal studies</td>
<td>As above</td>
</tr>
</tbody>
</table>

| Strategies for Characterising Genes Contributing to a Complex Disorder |

Several approaches are possible and under active investigation. All start from the position that genetic effects on alcohol dependence exist, and all have the common aim of defining mutations or polymorphisms in humans which cause this genetic variation. The routes between these two points diverge (Tab. 2).
A large study in the USA is attempting to identify "alcoholism genes" by testing markers across the entire genome for linkage with alcohol dependence, using families selected for multiple affected members. Some promising loci have been reported (9), but no further details are available. Several groups have bred rats or mice for sensitivity or resistance to intoxication, or for voluntary alcohol consumption, and these lines are being used for detection of quantitative trait loci (QTLs) (10). Multiple association studies on candidate genes have been conducted, with variable results as alluded to above.

Our approach has been to test for association or linkage between candidate genes and loci, and alcohol dependence or its risk factors, in twin subjects who have already been extensively studied on multiple occasions over the past twenty years. This allows integration of repeatability and heritability information with the effects of individual alleles or loci, and places the effects of each genotype in the appropriate context.

Subjects and Methods

The subjects of our studies are adult male and female twins of European descent, recruited through the Australian National Health and Medical Research Council (NHMRC) Twin Registry for a study of genetic and environmental effects on alcohol consumption, alcohol dependence and common co-morbid conditions, and biological markers of alcohol use. Blood samples have been obtained from approximately 3300 subjects, and data from an alcohol challenge study is also available for around 400 of them.

As a first step in identifying the components of the genetic variation, which is substantial and highly significant in this group, we have typed \(\text{ADH2, ADH3,}\) and up to eight other polymorphic markers on chromosome 4 using blood samples from these twins. \(\text{ADH2 and ADH3 were typed using polymerase chain reaction (PCR) followed by restriction digestion and electrophoresis, while microsatellite markers were typed by PCR with fluorescent labelled primers and gel electrophoresis.}\) These data are being analysed using the three main approaches presently available: association of phenotype with genotype at a candidate locus, linkage disequilibrium mapping, and linkage using sib-pairs. The phenotype variables are measures of alcohol's euphoric qualities and consequently in the absorptive phase regardless of ADH genotype. However, there does seem to be a greater degree of early alcohol metabolism but not alcohol use.

Future progress depends on sophisticated and time-consuming data analysis, which will show whether other loci in the \(\text{ADH}\) region on chromosome 4, or in the \(\text{DRD4}\) region of chromosome 11, contribute to genetic type on measures of alcohol consumption and depend-
variation in alcohol use and dependence. Extensive typing at other loci will be required to test for involvement of other candidate genes such as dopamine or γ-amino butyric acid (GABA) receptors.

**Conclusion**

There is no doubt that alcohol dependence is an example of a complex syndrome with a strong genetic component. This concept should help to reduce lingering prejudices against patients with this condition, and make biological approaches to it more widely accepted. Like any other complex genetic condition, large numbers of well-characterised subjects and a great deal of laboratory work and data analysis will be required to define the genes involved. The comparatively brief history of these efforts shows that certain principles must be kept in mind, such as replication of results by independent groups, generalisability to diverse populations, and the desirability of a plausible mechanism for any genotype effects found.

**Acknowledgements**

Collection of data from the twins described in this paper was supported in part by grants from the National Health and Medical Research Council (Australia) and the National Institute of Alcohol Abuse and Alcoholism (USA).

**References**


Received 20 April 1998; accepted 28 May 1998