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Molecular Biology of Alcohol Dependence, a Complex Polygenic Disorder

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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.

	Coronary heart disease	Non-insulin-dependent diabetes mellitus (NIDDM)	Osteoporosis	Alcohol-related disease
Complications	Myocardial infarction, heart failure	Nephropathy, retinopathy, vascular disease	Fractures	Cirrhosis, brain damage, cardiomyopathy pancreatitis, trauma
Chronic state	Atherosclerosis	Hyperglycaemia, hyperinsulinaemia	Decreased bone density	Alcohol dependence, alcohol abuse
Risk factors	Lipids, smoking, blood pressure, insulin resistance, age, family history	Obesity, age, impaired glucose tolerance, family history	Sex, age, initial bone density, HRT, family history	Sex, year of birth, alcohol metabolism alcohol sensitivity, conduct disorder, depression, personality
Genes	Genes coding for apolipoproteins or for apoliprotein receptors	MODY genes GCK, HNF1α, HNF4α. Later onset NIDDM, genes unknown	Vitamin D receptor gene; status presently uncertain	*ALDH, ADH.* Possibly dopamine receptors *(DRD2, DRD4)*, monoamine oxidase, $GABA_A$ receptor, *fyn* tyrosine kinase

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, propably 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).

Tab. 2 Whichever approach is taken to discovering "genes for alcoholism", the same assumptions and the same final validation apply.

	Starting point	Intermediate stages	Final test
Traditional genetics	Existence of genetic effects on alcohol dependence in humans	• Identification of metabolic or neurochemical events likely to contribute to variation in risk • Cloning of genes for relevant enzymes and receptors • Identification of polymorphisms in these genes • Initial association studies in patients and controls	Studies showing consistent association between genotype and phenotype in humans from diverse clinical and population sources
"Reverse" genetics (positional cloning)	As above	• Recruitment and characterisation of subjects from large pedigrees, or sibships, with affected subjects • Genotyping of multiple genetic markers across all chromosomes to identify loci showing linkage • Identification of genes at these loci, and polymorphisms in these genes	As above
Animal studies	As above	• Testing and selective breeding of animals for a relevant characteristic • Crossing of pure-bred lines, with testing and genotyping of offspring to identify quantitative trait loci • Identification of genes at these loci, and at equivalent loci in humans, and polymorphisms in these genes	As above

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 repeatibility 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 *ADH2, ADH3,* and up to eight other polymorphic markers on chromosome 4 using blood samples from these twins. *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 quantity and frequency of alcohol consumption, the number of self-reported alcohol-related problems, and DSM-IIIR alcohol dependence. Consumption and problem data have been collected on multiple occasions up to fifteen years apart.

Secondly, we have studied the effects of variation in the dopamine D4 receptor *(DRD4)* on variation in a known risk factor for alcohol dependence, the personality trait of Novelty Seeking. Scores were measured with the Tridimensional Personality Questionnaire, and *DRD4* genotype and genotype for a nearby microsatellite (D11S1984), were determined by PCR and electrophoresis.

Results and Discussion

So far first stage results have been obtained, from the sub-group of subjects who participated in the alcohol challenge study. A series of significant effects of *ADH2* type on measures of alcohol consumption and dependence have been found in men, but only non-significant effects were found in women (Whitfield *et al.,* Alcoholism: Clinical and Experimental Research. In press.). *ADH3* showed weaker associations, but still consistent with the hypothesis that the more active enzyme forms are associated with lower risk. *ADH2* also has significant effects on alcohol pharmacokinetics. Results for other chromosome 4 markers are still being evaluated, with preliminary indications of effects on alcohol metabolism but not alcohol use.

This is the first positive result for *ADH2* and alcohol-dependence in subjects of European descent, and the relative risk associated with *ADH2* type is similar to that found among Asians. The absence of detectable *ADH2* effects in women requires further investigation; only one of the Asian studies considered possible sex differences in *ADH* effects and they also found significant results in men but not women. Because of the low frequency of *ADH2*2* (less than 10% in Europeans), variation at this locus accounts for only about 6% of the variation in alcohol use, even among the men.

These results suggest that *ADH* variation may affect alcohol use through the mechanism suggested by Thomasson *et al.* (11). They proposed that people with the *ADH2*2* or *ADH3*1* alleles would metabolise alcohol faster, produce a higher concentration of acetaldehyde, and be more likely to experience either flushing or undefined aversive effects after drinking alcohol. Against this concept are the lack of evidence for flushing reactions in non-*ALDH*-deficient subjects (even though the *ADH* effects are independent of *ALDH* status (7)), and the similar rates of alcohol metabolism in the post-absorptive phase regardless of *ADH* genotype. However, there does seem to be a greater degree of early alcohol metabolism in *ADH2*12* than *ADH2*11* subjects (12), and this could contribute to differences in the perception of alcohol's euphoric qualities and consequently in alcohol use.

The question of the effects of *ADH3* variation on alcohol dependence is still unresolved. It seems clear that there is no *ADH3* effect on alcohol metabolism *in vivo*, but that there is a highly significant effect on alcohol dependence in Japanese and Chinese subjects. Further studies on *ADH3* and alcohol dependence with larger numbers of European subjects should clarify this issue, and these are in progress.

Our investigations on genes affecting alcohol use or dependence through effects on personality have only recently started. *DRD4* variation was found to have significant effects on Novelty Seeking score, a risk factor for alcohol dependence, in both men and women. However, this effect does not seem to be transmitted through to the alcohol dependence end-point itself, either because the effect is small or because *DRD4* influences some aspect of Novelty Seeking which is not related to alcohol use.

Future progress depends on sophisticated an time-consuming data analysis, which will show whether other loci in the *ADH* region of chromosome 4, or in the *DRD4* region of chromosome 11, contribute to genetic

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.

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