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Commentary

Need for power in genetic explorations of alcoholism in India

Alcoholism continues to be implicated in both medical presentations and psychosocial health outcomes in India as much as in other countries[1]. The proportion of beds occupied in psychiatry facilities by persons with alcoholism in India continues to remain high[2]. Knowing the aetiopathology of compulsive drinking is a major driver of research. One needs to distinguish proximate and ultimate causal factors in public health research. Easy availability continues to be seen as a key contributor to excessive consumption of alcoholism, and public health measures continue to be taken in this regard, to this day. Socio-cultural factors continue to be implicated[3] in its development, at least as explanatory models.

The genetic model of alcoholism presupposes genetic alterations as arguably ultimate factors that increase vulnerability to this condition. Related studies include those studying family history[4] to establish familiality along with adoption studies to confirm biological causality, those looking at endophenotypes (alcohol metabolizing enzymes, morphometry of the brain and evoked potential-based studies in the first-degree relatives)[5,6] to determine pathogenetic processes as well as potential genetic markers and finally direct studies of altered genes and their expression in select cohorts such as the COGA (Collaborative Studies on the Genetics of Alcoholism) studies[7]. A few studies have been published from India on exploration of candidate genes in the area of alcoholism[8,9]. The study by Malhotra *et al*[10] in this issue explores multiple candidate genes for this condition amongst a North Indian population.

Before seeking to understand the nature of potential candidate genes, the genetic hypothesis of alcoholism needs to be revisited for an adequate perspective. In a condition that can be seemingly socio-culturally mediated, it is necessary to remember that path analytical studies of alcoholism have been predominantly carried out in certain developed countries and the genetic contribution is seen to be just over 50 per cent[11]. Absence of such studies from India where cultural factors may be seen as having an impact on its development remains a limitation. Further, such studies need special populations (twins registers, adoption registers) and are labour/resource intensive.

The key aspects that are of relevance to the study by Malhotra *et al*[10] are the role of candidate genes, selection of markers samples and study design. The role of candidate genes depends on the current perspectives on the nature of altered biology underlying alcoholism. Focus areas include those biochemical pathways involved in metabolism of alcoholism such as alcohol dehydrogenase and aldehyde dehydrogenase (ADH3 and ALDH2)[11] and those related to the biochemistry of the neural substrates that respond to alcohol (GABA, opioid, dopamine receptors)[11]. Alternatively, effect of alcohol on altering gene expression (epigenetics) which further contributes a modified vulnerability has been gaining importance[12].

Altered metabolism of alcohol leading to different concentrations of alcohol (and/or its metabolites) for varying time duration, with correspondingly increased or reduced sensitivity to behavioural effects of alcohol, has been a long-standing view[11]. Those which are slow to metabolize alcohol may enhance its undesirable behavioural effects, even at lower intake, and may potentially create an aversion to continued use. Thus, ADH and ALDH have remained under major focus for the last few decades. There are various forms of these enzymes that metabolize alcohol and various genetic underpinnings of these in alcoholism have been described[13]. A few Indian studies have provided evidence for variation in the components of these enzymes and their impact on alcoholism[5,9]. The study by Malhotra and colleagues[10] has also looked at ADH1B. However, no significant association was

found in this study. The findings are different from those reported from other countries[13]. Such genetic analysis can be more comprehensible if the enzymes are simultaneously assayed.

What are the substrates in the brain for alcohol that mediate its effects and more importantly underlie the transition to dependent use of the substance? Are these specific to alcohol or do these tap into any generic pathways that can induce and perpetuate a recurrent cycle of behaviours? The evidence at present appears to be for (*i*) gamma aminobutyric acid (GABA) receptors, (*ii*) opioid receptors, and (*iii*) dopamine receptors and the enzymes involved in the synthesis of the neurotransmitters and receptors[11]. The genes of interest in the study[10] include *COMT* (catechol-o-methyl transferase), *GABRA1* and *GABRA2*, *5'HTTLPR* (5-HT transporter long promoter region), *CHRM2* (cholinergic receptor muscarinic) and *ALDH2* and *ADH1B*. The authors defend the selection of genes of interest in their study by describing key biochemistry literature linked to this condition. Studies focussed on endophenotypes in alcoholism from India include evoked potentials related studies, particularly the P300[14]. Amongst others, pathways related to dopamine have been linked to the physiology of P300[15]. Thus, a focus on genes such as *COMT* seems justified although the absence of focus on dopamine receptor D2 (*DRD2*-) and *DRD4*-related genes in this study (while keeping serotonin and cholinergic mechanisms under purview) may be considered significant. It is necessary to keep in mind that the power of study with finite subjects weakens by adding genes for exploration. Further, the main underlying intent here appears to be to demonstrate feasibility of carrying out such studies. Accordingly, one can anticipate more comprehensive studies from this group in the coming years.

The strength of this study lies in the use of multiple genes for exploration in the same sample. It would be useful to compare the cases here with that in a study looking *DRD2* polymorphisms from south India[8]. A population-based sample has inherent advantages in the study of genetic polymorphisms. Arguably, a well-characterized clinical sample permits clinically meaningful conclusions. While this sample is carefully selected, the sample size may have stopped the authors from reporting specific associations between clinical characteristics and allele frequencies. They have thus limited their analysis to the presence and absence of alcohol dependence. However, multiple genes being explored for association in a sample of 200 individuals, with use of just one marker per gene, can significantly affect the power to detect (and particularly, exclude) associations and the confidence in the results. It is reasonable to suggest that an exploration of the genes marked out as significant in this study, be attempted across many different centres in the country.

The issue of markers for use in candidate studies is a complex one. There are many single nucleotide polymorphism (SNP)-based markers available for each gene; and several have been reported in association studies of alcoholism with markers for *GABRA1* and 2 genes[16]. While the rs980791 used in this study as a marker of *GABRA1* gene has shown robust association with alcoholism in the COGA studies[16], certain other markers have also shown an equally strong association[16]. However, some of the markers of the same gene have not shown an association with alcoholism. The marker rs279871 used in this study for *GABRA2* has been found to be strongly associated with alcoholism in the presence of drug dependence, but not with pure alcoholism alone, in the COGA study[17]. The sample in the current study was selected for absence of dependence on any other substance (other than nicotine). Apart from reduced power for studies evaluating a single marker for each gene, the choice of markers also becomes a key determinant of the study's outcomes. While this study provides proof of technical feasibility in carrying out association studies across several markers for candidate genes in alcoholism from India, one needs to consider 3-5 markers per gene or haplotype-based studies to explore alcoholism across centres. The reported variations in terms of possible protective effect against alcoholism for *GABRA1* and *2* genes in the Indian context need to be explored in larger samples with multiple genetic markers so that the study remains powered enough to rule out a significant association. Till then, the nature of conclusions arrived at in this study remains uncertain.

The results of a genetic association study depend on the nature of the controls. An appreciable effort to recruit non-confounding controls is evident in the methodology here. In this study, the controls underwent a complete Semi-structural Assessment for Genetics of Alcoholism-II (SSAGA)-based interview along with the evaluation of family history. Such methods significantly improve the reliability of the results. On the other hand, case-control design does not adequately make up for the key advantages inherent to availability of ethnicity-based prevalence data for the genotypes of interest. Results from large databases such as the

proposed Genome Asia 100K[18] could potentially help to arrive at more meaningful conclusions, while using clinical genomic data.

If key genotypic differences in India for a phenotypically universal condition are proposed, further questions include the nature of phenomenological and endophenotypic differences that exist in the condition of interest, in our country. Unfortunately, careful phenotypic and endophenotypic studies that demand biochemical or genetic explanations different from those internationally reported have not yet been reported from India. If more such studies become available, one can explore genotypic differences more confidently.

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