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REVIEW

Genetic factors influencing alcohol dependence

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Plentiful data from both animal and human studies support the importance of genetic influences in substance abuse and dependence (Bierut *et al.*, 1998; Tsuang *et al.*, 1998; Kendler *et al.*, 2003). This review summarizes the evidence supporting such genetic influences, places them into perspective regarding animal and human studies, discusses the importance of both genes and environment, and highlights some specific genes of interest regarding the vulnerabilities for problems associated with alcohol use disorders. A long history of repetitive heavy use of alcohol exists across generations as well as the high prevalence of alcohol-related problems in Western societies. Moreover, the information offered here addresses the importance of more general issues regarding genetics and gene expression related to alcohol abuse and dependence.
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Abbreviations: ANA, Alko, non-alcohol; AUD, alcohol use disorder; LR, level of response; QTL, quantitative trait locus; SNP, single nucleotide polymorphism

Introduction

Before discussing specific information regarding genetic influences for alcohol use disorders (AUDs), it is important to address the question of whether the genetic variations (polymorphisms) of interest are specific for the drug, or if more generic factors have an impact on repetitive use and associated problems with substances. The answer to both sides of the question is a qualified 'yes'. At least a portion of the genetic influence is likely to reflect a generic predisposition (Rounsaville *et al.*, 1991; Goldman, 1998; Merikangas *et al.*, 1998a; Kendler *et al.*, 2003). As discussed in more detail later, these influences might enhance the risk for problems with a wide range of substances through exaggerated feelings of reward when substances are taken, might operate through genes that contribute to higher levels of impulsivity with associated impaired control of many behaviours, including substance use, or relate to a vulnerability towards some psychiatric conditions that subsequently affect alcohol and drug use disorders such as bipolar disorder or schizophrenia (Mirin *et al.*, 1991; Slutske *et al.*, 1998; Koob and Le Moal, 2001; Schuckit, 2002). It is also probable that some of the genetic loading for a substance-related condition may be relatively specific for an individual substance or class of drugs (Rounsaville *et al.*, 1991; Luthar *et al.*, 1992; Bierut *et al.*, 1998; Merikangas *et al.*, 1998b; Tsuang *et al.*, 1998). This conclusion is supported by reports that substance dependence in close relatives of individuals who themselves have a specific substance use disorder are most likely to demonstrate an enhanced rate of problems on that specific drug (Duncan *et al.*, 1995; Schuckit and Smith, 2000). The more drug-specific vulnerabilities might operate through genes affecting metabolizing enzymes, neurochemical pathways or the intensities of response to a specific drug.

The following sections focus on genetic influences in AUDs, beginning with a review of more clinically oriented investigations and progressing to a more detailed discussion of animal and 'bench-based' work focussing on proteomics, gene expression studies and more detailed intracellular mechanisms. This review ends with a synthesis of human and animal work, along with some thoughts on future directions, including the impact these findings might have on pharmacology.

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Genetic influences in alcohol dependence

Genetic influences

The importance of genetic factors in alcohol dependence has been supported for many years through the same classical questions asked regarding genetic influences for any characteristic. First, there is a fourfold enhanced alcohol-dependence risk in relatives of alcoholics; second, identical

twins of alcohol-dependent subjects carry a higher risk for this disorder than do fraternal twins or full siblings; and third, the adopted children of alcoholics have the same fourfold enhanced risk for this disorder as do offspring raised by their alcohol-dependent parent (Goodwin *et al*., 1974; Cotton, 1979; Prescott and Kendler, 1999). The family and twin studies support the conclusion that the proportion of risk for this disorder explained by genes (that is, heritability) is between 40 and 60% (Prescott and Kendler, 1999; Schuckit *et al*., 2001).

Although it is possible that a small number of genes might directly influence alcohol dependence, it is more likely that the relevant genes influence a range of genetically influenced intermediate characteristics (also called 'endophenotypes') that subsequently affect the risk for heavier drinking and alcohol-related life problems (Goldman, 1998; Gottesman and Gould, 2003). Each of these endophenotypes is likely to reflect the actions of multiple genes and to relate to both genetic and environmental influences (Schuckit *et al*., 2004b; Crabbe *et al*., 2006).

A range of endophenotypes have been described as potential contributors towards the risk of alcoholism. One set of factors appears to be non-specific and reflects a generic predisposition towards dependencies on a range of substances. An example is the constellation of personality characteristics that relate to impulsivity, sensation seeking, neuronal disinhibition and an impaired ability to easily learn from mistakes (Slutske *et al*., 1998; Dick *et al*., 2006). Several other genetically influenced syndromes, primarily schizophrenia and manic depressive disease, are also associated with an enhanced risk for alcohol dependence and additional substance use disorders, perhaps through a vulnerability to impaired functioning with stress, an overlap in the neurochemical systems contributing to all range of syndromes, poor judgement inherent in the psychiatric disorders, or an attempt on the part of the psychiatrically impaired individual to alleviate symptoms of their disorder or side effects of medications (Winokur *et al*., 1996; Caspi *et al*., 2003; D'Souza *et al*., 2006).

Other endophenotypes relate more specifically to alcohol-dependence risk, rather than illicit substance use disorders. These include a low level of response (LR) to alcohol, perhaps reflecting a low sensitivity that is relatively unique to this drug (Erblich and Earleywine, 1999; Schuckit, 2002). A low LR to alcohol can be seen relatively early in life, carries a heritability of 0.4–0.6 (Heath *et al*., 1999; Schuckit *et al*., 2001) and this characteristic predicts later heavy drinking and alcohol dependence but is not associated with repetitive heavy use or problems associated with any other drug (Volavka *et al*., 1996; Schuckit and Smith, 2000; Schuckit *et al*., 2004b). Another example of genetic variations (polymorphisms) that relate specifically to AUDs affects several of the alcohol-metabolizing enzymes, particularly some alcohol and aldehyde dehydrogenases (*ADH* and *ALDH*, respectively) (Li, 2000; Wall *et al*., 2005). The resulting enzymes may produce higher levels of the first breakdown product of alcohol (acetaldehyde), with a subsequent lower alcohol-dependence risk, perhaps through a heightened and, in some cases, aversive response to alcohol. However, these polymorphisms do not appear to have a major impact on dependence syndromes related to other drugs of abuse.

Some specific genes

Most of the work searching for specific human genes that impact on the risk of alcoholism has centred on genetic influences relating to intermediate endophenotypes. This is potentially more efficient than looking for genes influencing more broad substance-dependence phenotypes. Through this process, linkage and association studies have highlighted a wide range of genes that impact on diverse brain systems as having potential relevance to the vulnerability towards AUDs (Schuckit *et al*., 2004a, 2005b; Crabbe *et al*., 2006).

Some genes discussed here are potentially linked to the risk for substance dependence. Thus, this section will serve as a resource for both alcohol and the discussion of genes relevant to illicit drug abuse and dependence. Several studies reported an association of phenotypes of impulsivity, disinhibition and related characteristics with a polymorphism of the GABA A receptor, alpha 2 gene on chromosome 4 (Edenberg *et al*., 2004; Lappalainen *et al*., 2005; Dick *et al*., 2006). On a behavioural level, this gene variation is also related to conduct disorder and the antisocial personality disorder (conditions that incorporate impulsivity and disinhibition) and, not surprisingly, carries an associated predisposition towards dependence on illicit substances as well as on alcohol (Dick *et al*., 2006). Several other polymorphisms potentially associated with disinhibition or related cognitive-based mechanisms include a variation of cholinergic receptor, muscarinic 2 on chromosome 7, and the alcohol dehydrogenase 4 gene on chromosome 4, with the latter potentially carrying its impact via changes in the dopamine reward systems. Another set of genes might operate through polymorphisms in the dopamine receptor D4 gene on chromosome 11 (Franke *et al*., 2000; Li *et al*., 2000) and the dopamine receptor D2 gene on chromosome 2, although the latter might really reflect alterations in the nearby ankyrin repeat and kinase domain containing-1 gene (Gelernter and Kranzler, 1999; Jones *et al*., 2004; Luo *et al*., 2005; Edenberg *et al*., 2006; Dick *et al*., 2007b). Another polymorphism of interest to disinhibition and related phenotypes is in ACN9 homologue on chromosome 7, believed to be involved in gluconeogenesis and the ability of the body to use acetate (Dick *et al*., 2007a). At the same time, a rich literature supports a wide range of genes potentially related to vulnerability to schizophrenia and manic depressive disease (McGough *et al*., 2006), and these disorders carry a heightened risk for both alcohol and drug dependence (Sullivan *et al*., 2003). Finally, it is possible that some individuals experience greater levels of reinforcement or reward from alcohol or other drugs, making them more likely to continue to use their substance or escalate intake (Koob and Le Moal, 2001).

At the same time, other genes appear to relate more specifically to the vulnerability towards AUDs. First, a low LR to alcohol as a risk factor specifically related to heavy drinking and associated problems could theoretically reflect

the results of polymorphisms in any gene that affects any major brain neurochemical system associated with alcohol's effects. Therefore, typical of most complex genetically influenced characteristics, one would expect a wide range of polymorphisms that operate through this mechanism in affecting the alcoholism risk (Schuckit *et al*., 2004b). Several studies in animals and humans support the possible relevance of the l allele of the serotonin transporter on chromosome 17, which produces a transporter with a more rapid reuptake of serotonin. Enhanced transport results in lower levels of this neurotransmitter that might relate to decreased alcohol effects (Rausch *et al*., 1991; Ernouf *et al*., 1993; Hu *et al*., 2005). The l allele has been reported to be associated with a lower LR to alcohol and higher levels of intake in studies carried out in the United States and Germany, with parallel results reported in non-human primates (Barr *et al*., 2003; Hinckers *et al*., 2006). Another polymorphism potentially related to a less-intense cerebellar response to alcohol in rodents, and perhaps humans, is a variation of the GABA A receptor, alpha 6 gene on chromosome 5 (Schuckit *et al*., 1999). This may be associated with a lower LR and higher alcoholism risk, especially when observed in the context of the l allele of the serotonin transporter (Hu *et al*., 2005). Additional genetic variations that potentially relate to the LR to alcohol have included several that may affect the intensity of reaction to alcohol through changes in second messenger systems (for example, adenylyl cyclase) and potassium channels (for example, variations in *KCNMA1*) (Hoffman and Tabakoff, 1996; Davies *et al*., 2003).

Another group of polymorphisms that appear to decrease the risk for repeated heavy drinking and associated problems, although having little effect on the use pattern of other drugs, relate to facial flushing that occurs during drinking. The mechanism operates through genes that alter enzymes having an important function in the metabolism of alcohol, including mutation in the *ALDH**2 gene on chromosome 12, with a resulting enzyme that is incapable of destroying acetaldehyde at the usual levels found in the blood. About 10% of Asian (Japanese, Chinese and Korean) individuals are *ALDH2**2 homozygotes, with a resulting highly intense aversive response to drinking that contributes to a near-zero rate of alcoholism, but does not appear to affect the risk for dependence on other drugs. Heterozygotes for this mutation have a facial flush but lack the intense accompanying symptoms of nausea, diarrhoea and rapid changes in blood pressure associated with alcohol, with the result that fewer individuals with this genotype develop AUDs than the remaining Asian populations. A third variation of these polymorphisms occurs through alterations in several alcohol dehydrogenase forms, especially *ADH1B* and *ADH1C*. The resulting enzymes from *ADH1B**2 and *ADH1C**1 metabolize alcohol a bit more rapidly, potentially increasing the levels of acetaldehyde and contributing to a modest facial flush, along with a more intense (but not necessarily more aversive) response to alcohol that can be observed in most racial and ethnic groups and that is associated with a lower alcoholism risk (Whitfield *et al*., 1998; Li, 2000; Wall *et al*., 2005; Duranceaux *et al*., 2006).

The importance of the environment

The risk for AUDs is complex, with almost all of the intermediate phenotypes and the potential associated polymorphisms explaining only a part of the risk (Dick *et al*., 2001). The possible exception is the strong aversive reaction to alcohol associated with a homozygote status for the *ALDH2**2 alleles in Asians in genes where the protection from alcoholism is almost 100% (Li, 2000). For most characteristics, an optimal understanding of how the phenotype or a specific polymorphism affects the AUD vulnerability requires an evaluation of the environment in which the genes operate. These epigenetic phenomena associated with culture, attitudes, stresses and so on are likely to explain 40% or more of the variance of risk for heavier drinking and associated problems. Understanding more about how phenotype or gene by environment relationships operate may offer clues to early identification of individuals with high alcoholism risk and highlight potentially important approaches to prevention. For example, in both cross-sectional and prospective studies, a low LR to alcohol as an endophenotype that can be documented relatively early in life has been shown to enhance the risk for heavier drinking and problems through association with heavier-drinking peers, alterations in the expectations of the likely effects of drinking and the enhanced probability of using alcohol to cope with stresses (Schuckit *et al*., 2005a; Schuckit and Smith, 2006). The combination of LR and the additional domains explained over 50% of the variance for heavy drinking and associated outcomes in some models. These comments highlight the importance of emphasizing that this section focusses on only one main aspect of alcohol and drug-dependence risk, and additional work is needed to evaluate the role of environmental contributors as well.

Whole genome associations in human studies

The goal of association genome scanning is to identify markers for genetic variants that contribute to vulnerability to complex disorders such as alcohol dependence. This approach is then used with unrelated alcohol-dependent versus control individuals sampled from the Collaborative Study on the Genetics of Alcoholism. Positive single nucleotide polymorphism (SNP) clusters point to potential genes of interest whose products are implicated in cellular signalling, gene regulation, development, and cell adhesion. The results are consistent with linkage and association results for alcohol and other addictive phenotypes. These data support polygenic contributions to vulnerability to alcohol dependence. These SNPs provide new tools to aid the understanding, prevention and treatment of alcohol abuse and dependence (Johnson *et al*., 2006).

Alcohol dependence is a leading cause of morbidity and mortality in Native Americans. One study mapped susceptibility loci for diagnostic and statistical manual-III-R alcohol dependence and two narrower alcohol related phenotypes in Mission Indian families. The alcohol use severity phenotype mapped to chromosomes 4 and 12, whereas the withdrawal phenotype mapped to 6, 15 and 16. Evidence for linkage to chromosomes 4, 15 and 16 have been reported previously for alcohol-related phenotypes, but there are no reports for

chromosomes 6 and 12. A combined linkage and association analysis demonstrated that alcohol dehydrogenase 1B gene polymorphisms are at least partially responsible for the linkage result on chromosome 4 in this population. These results demonstrate the potential importance of several chromosomal regions in alcoholism and identify new regions of the genome that may be unique to either the restricted phenotypes evaluated or this population of Mission Indians (Ehlers *et al*., 2004).

In summary, the initial sections of this review focussed on evidence supporting the importance of genetic influences in AUDs and the key role of endophenotypes in the search for genes affecting the vulnerability towards substance dependence. Examples of some gene variations hypothesized to relate to overall vulnerabilities towards drug dependence, as well as risk factors associated with alcohol abuse, have been presented. The emphasis on linkage and association studies in humans, although important, cannot adequately address other important issues related to genetic influences in the psychopharmacology of substance abuse. These include gene expression studies, investigations looking into the genetic influences on the actions of the substances of abuse on specific regions of the brain or types of neurons, or the mechanics of specific intracellular mechanisms. Such studies, primarily as carried out in animal models, tissue cultures and isolated cells, are reviewed in the remaining sections of this review.

Gene expression profiling in human post-mortem brain

Microarray technology

Microarray technology has changed the way in which genes are studied. Traditionally, single (or only a few) transcripts (mRNAs) were studied at a time. In contrast, many investigators currently use expression profiling to define global 'transcriptomes' from various tissues. Such 'omic' approaches have proven to be valuable tools in the study of genetically complex diseases such as cancer, neurodegeneration and drug abuse because they allow large numbers of elements (for example, RNA transcripts) to be examined simultaneously in an unbiased fashion.

Expression profiling requires the use of high-quality RNA that can be extracted from a variety of tissues and cells including post-mortem human brain. A number of studies have focussed on the identification of quality control guidelines that should be considered when performing expression profiling of human post-mortem brain (Tomita *et al*., 2004; Jackson *et al*., 2005; Atz *et al*., 2007). For example, post-mortem interval, agonal state and pH are all important variables that affect transcript quality. Clearly, high-quality RNA can be obtained, as expression profiling of post-mortem human brain has been successfully applied to a number of neurological conditions, including Rett syndrome (Colantuoni *et al*., 2001), Alzheimer's disease (Loring *et al*., 2001; Blalock *et al*., 2004) and multiple sclerosis (Lock *et al*., 2002; Dutta *et al*., 2006). In addition, psychiatric disorders such as schizophrenia (Mirnics *et al*., 2000, 2001; Pongrac *et al*., 2004), major depression (Evans *et al*., 2004), bipolar disorder (Iwamoto *et al*., 2004b) and autism (Purcell *et al*., 2001) have been studied successfully. The results of these studies indicate that the levels of numerous genes are altered and identification of such changes using the 'single target' approach would be inefficient.

Global gene expression and alcohol abuse and dependence

Long-term alcohol abuse produces persistent adaptations in the brain that can result in tolerance, physical dependence, craving and other behavioural changes. These changes in brain function are likely a consequence of altered gene and protein expression that likely underlie the cellular adaptations to chronic alcohol abuse (Nestler, 2000; Anni and Israel, 2002). As stated above, microarray technology has greatly improved our ability to study complex genetic disorders by allowing entire transcriptomes to be defined simultaneously. However, the analysis and interpretation of such studies has proven challenging. The integration and interpretation of vast amounts of data obtained from these studies are complicated by a number of factors, including the microarray platform used, the specific features (genes/clones) represented on the array and the statistical analysis and gene selection strategies used to determine significantly changed genes. In human post-mortem brain studies, comparisons across experiments that are performed in different laboratories are further complicated by differences in case selection, as variables such as age, sex, smoking history, drinking history and so on differ among studies. Accurate clinical information is critical to the experimental design. In particular, variables such as the amount of alcohol consumed and/or diagnostic and statistical manual diagnosis of alcohol dependence are used to determine case grouping. Importantly, detailed clinical information can be used to assess individual differences among cases. For example, Liu *et al*. (2006) investigated individual variability in gene expression patterns to discriminate alcoholics from non-alcoholic controls using principal component analysis. Using several functional groups of related genes, controls and alcohol-dependent cases could be predicted with the exception of three misclassified cases. Interestingly, the clinical data indicated that one of these cases was a polydrug user (morphine), one case had been abstinent for 2 years and one case had been treated for depression at the time of death. These findings illustrate the importance of detailed clinical information for accurate assessment of individual variation in gene expression patterns. In addition, concomitant smoking has been shown to alter gene expression in the prefrontal cortex and ventral tegmental area of human alcoholics (Flatscher-Bader and Wilce, 2006; Flatscher-Bader *et al*., 2008).

Brain regional gene expression profiling and alcohol dependence

Several studies have used expression profiling to identify differentially expressed genes in cortical brain regions in response to long-term alcohol consumption (Lewohl *et al*., 2000; Mayfield *et al*., 2002; Sokolov *et al*., 2003; Iwamoto *et al*., 2004a; Liu *et al*., 2004; Flatscher-Bader *et al*., 2005; Liu *et al*., 2006). The prefrontal cortex has been of particular

interest in several gene expression studies because of its susceptibility to damage by alcohol abuse. For example, loss of white matter volume (Harper *et al*., 1985; Kril *et al*., 1997) and neuronal loss in grey matter (Kril and Harper, 1989; Kril *et al*., 1997) have been reported in post-mortem brain of long-term alcohol abusers. Normal function of this brain region is crucial for judgement, decision-making, and other cognitive functions (Godefroy and Rousseaux, 1997; Rahman *et al*., 1999; Ratti *et al*., 2002) that are often impaired in alcoholics. In addition, this brain region is associated with reward systems that are important in the development of alcohol tolerance and dependence (Vetulani, 2001). The neuronal loss outlined above is not as severe in motor, temporal or cingulate cortices, suggesting potential brain region selectivity in alcohol-induced brain damage; thus, two studies compared expression profiles in prefrontal versus motor cortex (Mayfield *et al*., 2002; Liu *et al*., 2004), whereas one study examined gene expression changes in temporal cortex (Sokolov *et al*., 2003). The prefrontal cortex and the nucleus accumbens are components of the mesolimbic dopamine system, which plays a role in mediating the rewarding effects of addictive drugs (Koob, 1992). One study compared differentially expressed genes in these key brain regions (Flatscher-Bader *et al*., 2005).

Early expression profiling studies in prefrontal cortex identified differentially expressed genes involved in myelination, protein trafficking, ubiquitination and mitochondrion function (Lewohl *et al*., 2000; Mayfield *et al*., 2002; Liu *et al*., 2004; Flatscher-Bader *et al*., 2005). Whereas these functional groups of genes were consistently identified, there was variation among individual genes, both in the direction and magnitude of change. This variation was likely due in part to differences in experimental design and array platform. For example, some of these studies used either RNA pooled from several individuals (Lewohl *et al*., 2000; Mayfield *et al*., 2002) or relatively small sample sizes (seven cases per group) (Liu *et al*., 2004). More recently, expression profiling was performed on the prefrontal cortex of 27 individual human cases (14 well characterized alcoholics and 13 matched controls) (Liu *et al*., 2006). Rigorous statistical procedures were applied to identify differentially expressed genes in alcoholics. Similar to the earlier studies, genes generally involved in myelination, ubiquitination, apoptosis, cell adhesion, neurogenesis and neural disease showed altered expression levels. Interestingly, genes involved in neurodegenerative disease such as Alzheimer's were significantly altered (presenilin 1 and transferrin), suggesting a link between alcoholism and other neurodegenerative conditions. In this study, ~230 candidate alcohol-responsive genes were identified and verified by comparing the magnitude and direction of change to other published expression studies utilizing autopsy human brain (Lavoie and Butterworth, 1995; Lewohl *et al*., 2000; Mayfield *et al*., 2002; Sokolov *et al*., 2003; Flatscher-Bader *et al*., 2005). Of the 232 named genes, 27 genes were differentially expressed in other studies. Among those 27 genes, 21 were regulated to a similar extent and in the same direction. Interestingly, myelination-related genes that were downregulated in multiple studies included transferrin (Lewohl *et al*., 2000), UDP glycosyltransferase 8 (Lewohl *et al*., 2000), peripheral myelin protein 22 (Lewohl *et al*., 2000; Flatscher-Bader *et al*., 2005), and proteolipid protein 1 (Lewohl *et al*., 2000). Examples of other differentially expressed genes identified by Liu *et al*. (2006) and confirmed in independent studies included lysosomal-associated membrane protein 2 (Sokolov *et al*., 2003; Flatscher-Bader *et al*., 2005), proteasome subunit, β type 2 (Flatscher-Bader *et al*., 2005), *CANX* (Sokolov *et al*., 2003), *GABBR1* (Flatscher-Bader *et al*., 2005), solute carrier family 12, member 2 (Mayfield *et al*., 2002), and transketolase (Lavoie and Butterworth, 1995). Another striking finding of this study was the identification of a large group of cell adhesion genes (20 genes), 18 of which were downregulated. These molecules play a role in the central nervous system development, synapse formation and immune responses (Lee and Benveniste, 1999; Huntley *et al*., 2002; Milner and Campbell, 2002; Hirano *et al*., 2003; Scheiffele, 2003). Thus, downregulation of genes in this group may contribute to the compromise of neuronal functions in alcoholic human brain. Finally, Liu *et al*. (2006) used principle component analysis of functionally related sets of genes to determine that as few as 12 alcohol-responsive genes could accurately distinguish alcoholic versus control groups, suggesting that specific patterns of expression are associated with alcohol dependence.

As stated above, expression profiles have been compared across different brain regions to determine the regional specificity of alcohol-related reprogramming of gene expression. In comparisons of motor and prefrontal cortex, differentially expressed genes generally fell into the same functional groups as outlined for prefrontal cortex above (Mayfield *et al*., 2002; Liu *et al*., 2004). The Liu *et al*. (2004) study utilized individual cases rather than pooled samples and reported only ~5% overlap of differentially expressed genes between brain regions. A similar degree of overlap (~6) was reported in a comparison of prefrontal cortex and nucleus accumbens (Flatscher-Bader *et al*., 2005), suggesting that there is remarkable regional heterogeneity in gene expression patterns in response to alcohol abuse. The alcohol-responsive transcripts identified in the frontal cortex by Flatscher-Bader and Wilce (2006) included those encoding transcription factors, DNA-binding proteins, mitochondrial proteins and neuroprotection/apoptosis-related proteins. In contrast, in the nucleus accumbens, differentially expressed genes that are associated with synaptic vesicles and cytoarchitecture were significantly downregulated. The authors suggest that these changes in nucleus accumbens gene expression in response to long-term alcohol abuse might result in deficits in normal synaptic transmission and altered plasticity.

Cirrhosis is the result of chronic liver disease that causes scarring and dysfunction of the liver and is a common concomitant condition resulting from long-term alcohol abuse. A number of factors may contribute to abnormal liver function, such as genetic predisposition, viral infection, sustained exposure to environmental toxins, and so on; however, chronic alcohol consumption remains the most common cause of liver dysfunction in Western countries (Grant *et al*., 1988; Tome and Lucey, 2004). The impact of concomitant liver cirrhosis on brain gene expression has been invested by profiling expression patterns in the frontal

cortex of cirrhotic versus non-cirrhotic alcoholics (Liu *et al*., 2007). A striking result of this study was that the magnitude of change in transcript levels between cirrhotic and non-cirrhotic alcoholics was much greater than observed between non-cirrhotic alcoholics and controls. A greater number of over-represented functionally related groups were identified from the list of significantly downregulated genes compared with the upregulated genes. Gene groups involved in cell adhesion, mitochondrial function, and synaptic transmission were over-represented in downregulated genes, whereas genes involved in apoptosis and mitosis were over-represented in upregulated genes. Neurotoxins such as ammonia can pass the blood–brain barrier and affect brain function (Butterworth, 2003), and astrocytes have been suggested to be the main target of the neurotoxin ammonia in cirrhotic patients (Norenberg *et al*., 2004). Thus, the expression levels of astrocyte-specific genes in the cirrhotic alcoholics were compared with those in non-cirrhotic alcoholics to study the possible effects of cirrhosis on glial cells at the transcriptional level (Liu *et al*., 2007). Astrocyte-associated genes such as reticulon 4, ATP-binding cassette, sub-family A, member 1, apolipoprotein E and microsomal glutathione *S*-transferase 1 were all upregulated in cirrhotic alcoholics, whereas genes such as aquaporin 4, transmembrane 4 superfamily member 2 and phosphoprotein enriched with astrocytes 15 were downregulated. The results of this study suggest that concomitant liver cirrhosis may specifically alter gene expression in astrocytes. These cells play important roles in the proper function of the central nervous system by providing basic structural support and by producing trophic factors for neurons. In addition, they maintain the concentrations of ions and neurotransmitters in the extracellular space and remove neurotoxins and cellular debris in the brain (Aschner *et al*., 2002).

In summary, these data suggest that the transcriptional response of the brain to chronic alcohol abuse affects multiple genes in multiple functional systems in different regions of the human brain. These changes at the transcriptional level likely reflect both pre-existing differences in gene expression and those altered as a consequence of alcohol consumption. An important goal for addiction biologists will be to understand the role that these widespread changes in cellular regulation play in alcohol dependence.

Animal phenotypes used for gene mapping and expression

Rodents have been used extensively to study ethanol-related phenotypes and behavioural genetics of alcohol action (Crabbe *et al*., 1999). In particular, selected lines of rats and mice and inbred strains of mice that differ significantly in alcohol-related phenotypes have been used to identify the genetic and environmental factors underlying individual differences in response to alcohol (Crabbe and Phillips, 1998; Bennett *et al*., 2006).

Rodent studies show that differences in alcohol sensitivity among strains have a genetic component, and for most behaviours, the heritability is in the range of 0.2–0.5 (Crabbe *et al*., 1990). Crosses between inbred strains are valuable tools for determining which chromosomal regions determine these genetic differences. This requires a number (for example, 20–80) of different recombinant inbred strains or individual F2 generation animals. These genetic tools are readily available for mice and have more limited availability for rats. The behavioural sensitivity of these strains or individuals as well as differences in DNA sequence (SNPs) among these strains or individuals are determined. This information allows a correlation analysis that defines chromosomal regions linked with the behavioural differences. For alcohol behaviours, there are always multiple regions, so this is a 'quantitative' trait and the regions linked with the trait are quantitative trait loci (QTLs). Such QTL maps have been published for many alcohol-related behaviours, including acute functional tolerance, loss of righting reflex, taste aversion, withdrawal severity, voluntary consumption and conditioned place preference (Risinger and Cunningham, 1998; Crabbe *et al*., 1999; Bergeson *et al*., 2003; Bennett *et al*., 2007).

One goal of QTL mapping is to determine the gene or genes responsible for the QTL, that is, the quantitative trait gene. For the behavioural effects of alcohol, this long and difficult process has only been completed for alcohol withdrawal severity, where multiple PDZ domain protein encoding the multiple PDZ domain protein is differentially expressed in mouse models that vary in severity of alcohol withdrawal (Fehr *et al*., 2002). Multiple lines of evidence suggest that multiple PDZ domain protein is the gene underlying the withdrawal severity QTLs on mouse chromosome 4.

It is generally assumed that at least some of the QTLs reflect differences in the level of gene expression rather than differences in coding region (protein) sequence. Gene expression profiles (from microarray analysis) can be compared for recombinant inbred strains or other genetic tools, and genes from QTLs with differential expression provide promising candidate genes. This was recently accomplished for alcohol-induced loss of righting reflex, alcohol preference and acute functional tolerance (MacLaren *et al*., 2006; Saba *et al*., 2006). Several mouse candidate genes were also human alcohol sensitivity QTLs (MacLaren *et al*., 2006). It is important to note that databases of behavioural, gene expression and gene-sequence differences among recombinant inbred strains are cumulative as the identical strains are being tested in all studies. This provides a platform for *in silico* analysis of relationships among these three variables, and several powerful analysis sites are available, most notably, the WEBQTL section of GeneNetwork (http://www.genenetwork.org/).

Gene expression in animal models of alcoholism

The study of complex gene–environment interactions has been improved greatly by global expression profiling. As outlined earlier, such 'omic' approaches have greatly enhanced our ability to study genetically complex diseases such as cancer, neurodegeneration and drug abuse because they allow an unbiased examination of large numbers of elements (genes, proteins and so on) simultaneously. In addition to the human studies outlined above, expression

profiling has been used to identify alcohol–response genes and pathways in both cell culture and in animal models of alcoholism.

To date, two microarray studies have been published that examined the effects of ethanol on gene expression in neuronal cell cultures. Ethanol treatment was shown to alter the levels of several genes in SH-SY5Y neuroblastoma cells that are involved in the synthesis and metabolism of norepinephrine (Thibault *et al*., 2000). In particular, dopamine-β-hydroxylase, the enzyme required for the conversion of dopamine to norepinephrine, was increased significantly. The norepinephrine system has been shown to play a role in ethanol-related behaviours. For example, local infusions of norepinephrine into the hypothalamus increases ethanol consumption in rats (Hodge *et al*., 1996), and dopamine-β-hydroxylase knockout mice show reduced ethanol preference (Weinshenker *et al*., 2000). Expression profiling studies in SH-SY5Y cells also demonstrated that protein kinase A, mitogen-activated protein/extracellular signal-regulated kinase kinase, and casein kinase II inhibitors blocked the increase in dopamine-β-hydroxylase expression as well as a large subset of additional ethanol-responsive genes (Hassan *et al*., 2003).

Drug-naïve animal studies. An important goal in the addiction field is to identify genes that may underlie drug-related phenotypic variance between strains of mice. Early expression profiling studies examined inbred long-sleep and short-sleep mice (Xu *et al*., 2001), and several inbred strains of mice (C57BL/6J, BALB/c, A/J and DBA/2J) that differ in voluntary ethanol consumption (Murphy *et al*., 2002). Xu *et al*. (2001) identified ~40 genes that differed significantly between inbred long-sleep and short-sleep mice. The identified genes were functionally classified as oncogenes/tumour suppressors, ion channel/transport proteins, transcription factors and ubiquitination related. Murphy *et al*. (2002) reported that only two genes differed between C57BL/6J and BALB/c (c-FMS and cyclin A1). However, other changes in expression were observed, but the differences were small and could not be confirmed by reverse transcription PCR. It should be noted that these early studies utilized different array platforms that contained a relatively small number of features per array. Also, the study designs did not allow a formal statistical analysis; thus, gene selection was based on arbitrary cut-off ratios or qualitative interpretation.

As outlined above, C57BL/6J (B6) and DBA/2J (D2) mice differ markedly in a number of ethanol-related behaviours. Ethanol and other drugs of abuse activate the mesolimbic dopamine pathway (Koob, 1992). Gene expression patterns have been compared across the major components of this system (nucleus accumbens, prefrontal cortex, ventral tegmental area) in control (ethanol-naïve) and acute ethanol-treated (see 'Ethanol-treated animals' below) B6 and D2 inbred mouse strains (Kerns *et al*., 2005). In control animals, a large number of expression differences were identified between strains (>750), the majority of which were observed in the prefrontal cortex. The genes that differed between strains mapped to regions of chromosomes 1 and 4 that are linked to QTLs for ethanol traits such as locomotor activation, acute withdrawal and preference (Melo *et al*., 1996; Buck *et al*., 1997; Tarantino *et al*., 1998; Crabbe *et al*., 1999; Demarest *et al*., 2001). One gene that was differentially expressed between strains was multiple PDZ domain protein, which mapped to a narrow region of chromosome 4. Multiple PDZ domain protein is a confirmed quantitative trait gene associated with *Alcw2* (alcohol withdrawal 2) (Shirley *et al*., 2004). These findings underscore the strength of gene expression studies in the search for genes that underlie QTLs for complex traits.

Expression profiling has also been used to identify transcriptome differences in mice selectively bred for differences in acute functional tolerance (high acute functional tolerance; low acute functional tolerance) (Tabakoff *et al*., 2003). This study employed rigorous filtering criteria for the selection of differentially expressed genes. These filters included multiple statistical procedures, and the selected genes had to be localized in QTLs associated with acute functional tolerance. Six genes were identified that may be involved in a signal transduction pathway involved in neuroadaptation (glutamate receptor, ionotropic, delta 2, ephrin-B3, glutamate receptor, ionotropic, *N*-methyl D-aspartate 1, zinc-finger protein 179, transcription elongation factor and peroxiredoxin 5). The authors hypothesize that these genes play a role in acute functional tolerance by a mechanism involving NMDA receptor phosphorylation and trafficking to the synaptic membrane.

The hippocampus is important for the development of ethanol tolerance (Ludvig *et al*., 2001). Gene expression has been examined in the hippocampus of alcohol-naïve inbred alcohol-preferring and alcohol-non-preferring rats (Edenberg *et al*., 2005). Numerous (~130) expression differences were identified between lines. Functionally related groups of genes included those involved in cell growth and adhesion, protein trafficking, regulation of gene expression, metabolic pathways, cellular signalling systems and synaptic function. These genes may contribute to differences in sensitivity to ethanol and/or in the development of tolerance that is observed in these animals. Gene expression has also been examined in the frontal cortex of rat strains genetically selected for alcohol self-administration preference, AA (Alko, alcohol) and P (Indiana, preferring), or avoidance, ANA (Alko, non-alcohol) and NP (Indiana, non-preferring) (Worst *et al*., 2005). In this study, the expression pattern of six genes differed significantly between AA and ANA rats (glutamate receptor, metabotropic 3, calcium channel, voltage-dependent, alpha 2/delta subunit 1, vesicle-associated membrane protein 2, syntaxin 1A, syntaxin 1B and mammalian homologue of the *unc-18* gene). These genes are involved in neurotransmitter-release machinery and vesicle fusion; thus, normal neurotransmission may differ between these strains. Of these genes, only vesicle-associated membrane protein 2 was differentially expressed in P versus NP rats, suggesting that these alterations are not a universal feature of all animal models of alcohol consumption. Interestingly, there was no overlap in these genes compared with those identified in the hippocampus of inbred rats (Edenberg *et al*., 2005), and little overlap with genes identified in various brain regions of AA versus ANA rats (Helsinki, Finland) (Arlinde *et al*., 2004), demonstrating the diversity of brain

regional patterns of expression between different strains of animals.

A recent comprehensive rat expression study examined innate differences in gene expression in different brain regions of inbred alcohol-preferring and alcohol-non-preferring rats (Kimpel *et al*., 2007). Differences in expression were found in each of the regions studied (nucleus accumbens, amygdala, frontal cortex, caudate-putamen and hippocampus); and, in general, the genes were related functionally to neurotransmission, neuroplasticity, intracellular messaging and regulation of transcription. Of the regions studied, the greatest number of differences were found in the amygdala (~50). A number of these genes are related to neuroplasticity (cell division cycle 42, fibroblast growth factor receptor 1, vascular endothelial growth factor A, neuritin 1, BH3 interacting (with *BCL2* family) domain, apoptosis agonist, p21 (*CDKN1A*)-activated kinase 2, SH3-domain kinase binding protein 1). In addition, 13 of the 54 differences in the amygdala were located within established alcohol QTLs. A statistical analysis of between-strain differences (collapsed across brain region) identified ~300 differentially expressed genes that were included in 17 over-represented GO categories. As inbreeding randomly fixes genes that are not associated with the selection phenotype, the authors make the important point that some of the differences in expression may not be relevant to alcohol preference.

Ethanol-treated animals. Several groups have used microarray profiling to identify strain-specific changes in gene expression in response to ethanol. In each of these studies, expression patterns were compared in B6 and D2 strains of mice; however, the studies differed with respect to the brain region and ethanol administration method used. Two early studies examined strain differences in whole brain gene expression in response to acute high doses of ethanol (Murphy *et al*., 2002; Treadwell and Singh, 2004) or to chronic ethanol administration (Murphy *et al*., 2002). The interpretation of both of these studies is limited, as gene selection was based on arbitrary cut-off ratios or qualitative analysis and as brain regional differences in ethanol-responsive expression patterns are likely masked in whole brain studies. The earliest expression profiling study examined the effects of acute and chronic ethanol withdrawal on gene expression in the hippocampus (Daniels and Buck, 2002). Whereas a somewhat limited number of genes were represented on the arrays used in this study, differentially expressed genes fell into several important signal transduction pathways. For example, in D2 animals, acute and chronic withdrawal changed genes belonging to the mitogen-activated protein kinase, the Janus kinase/signal transducers and activators of transcription, and the Akt/phosphatidylinositol 3-kinase pathways. In contrast, in B6 mice, chronic withdrawal altered a different set of genes in the MAP kinase pathway. These finding demonstrate that there are major differences in the cellular adaptations to ethanol withdrawal between these strains. Strain differences in gene expression in response to a singe acute dose of ethanol has been studied in different regions of the mesolimbic dopamine system (nucleus accumbens, prefrontal cortex, ventral tegmental area) (Kerns *et al*., 2005). Ethanol regulation of ~300 genes was identified across brain regions of B6 or D2 animals. In general, acute ethanol altered a larger number of genes in D2 compared to B6 animals. A striking finding in this study was that significantly more genes were up- than downregulated by ethanol in the nucleus accumbens and prefrontal cortex of D2 mice, while a greater number of genes were downregulated in these brain regions of B6 mice. In general, the majority of ethanol-regulated genes are involved in neuroplasticity; however, the regulation of discrete functional groups and pathways tended to be regionally specific (prefrontal cortex: glucocorticoid signalling, neurogenesis, and myelination; nucleus accumbens: neuropeptide signalling and developmental genes including factor brain-derived neurotrophic factor; and ventral tegmental area: retinoic acid signalling). These findings illustrate the high degree of complexity in brain regional gene regulation in animals with divergent alcohol-related phenotypes.

Microarray studies examining direct strain-dependent differences in gene expression in response to ethanol in rats are limited. A recent study utilizing a functional genomics approach to identify alcohol responsive genes (Rodd *et al*., 2007) is outlined below (see Informatics Approaches). With repeated cycles of intoxication and withdrawal, rats develop a marked and long-lasting increase in voluntary ethanol intake (Rogers *et al*., 1979; Roberts *et al*., 2000). This drinking paradigm was used to identify alcohol-responsive genes in the cingulate cortex and amygdala of Wistar rats (Rimondini *et al*., 2002). A small set of changed genes was reported that were primarily upregulated in this model. The identified genes are associated with pathways associated with alcohol dependence (for example, glutamatergic, endocannabinoid, and monoamine neurotransmission). In addition, pathways not previously thought to be alcohol responsive, such as members of the mitogen-activated protein kinase pathway, were identified. In a separate study, genes involved in ethanol-induced oxidative stress and protein trafficking were identified in the hippocampus of inbred Lewis rats chronically exposed to ethanol (Saito *et al*., 2002).

Informatics approaches

As outlined above, microarray studies have provided valuable new insight into gene regulation in genetically complex diseases such as alcoholism. A strategy commonly used among addiction researchers is to identify expression differences between strains of animals selectively bred for divergent drug-related phenotypes. Limitations to this approach include the availability of resources to survey large numbers of genetically characterized strains and the lack of statistical power to identify small but reliable differences in gene expression. Access to large databases of expression data has provided researchers the tools to overcome these obstacles. The 'meta-analysis' approach of combining expression data has been used successfully in the cancer field (Rhodes *et al*., 2004) and been applied recently to the alcohol field (Mulligan *et al*., 2006; Rodd *et al*., 2007).

Mulligan *et al*. (2006) used a meta-analysis approach to identify candidate genes that contribute to ethanol

consumption by combining databases of expression data from genetic mouse models of voluntary alcohol consumption. In this study, 13 different strains of mice from five independent experiments that were performed in three different laboratories were compared. The studies utilized only drug-naïve animals and included selected lines bred for high and low drinking, inbred strains that differ in voluntary alcohol consumption, and a hybrid strain that shows the highest voluntary alcohol intake of any mouse genotype to date (Blednov *et al*., 2005). Approximately 3800 genes that were differentially expressed between mice displaying high and low levels of ethanol drinking were identified. The top 75 genes, ranked by effect size, fell into broad categories of cellular homeostasis and neuronal function. Examples of genes that were expressed higher in alcohol-preferring models included the following: β-2-microglobulin, mannosidase, alpha, class 2B, member 1, sodium channel, voltage-gated, type IV, β, microtubule-associated protein, RP/EB family, member 1, protein kinase C, ε and somatostatin, which are functionally associated with immunity/cellular defence, glycosylation, ion-channel activity, microtubule, intracellular signalling and neuronal signalling, respectively. In addition, expression data from a mouse line congenic for chromosome 9, which contains genes associated with alcohol intake (Belknap and Atkins, 2001), were used as a filter to identify candidate genes for an alcohol-drinking QTL. This analysis identified 20 genes that may represent novel quantitative trait genes underlying ethanol preference.

Multiple expression data sets and informatic approaches were used to identify candidate genes for alcoholism in rats (Rodd *et al*., 2007). In this analysis, expression data sets were obtained from multiple brain regions (frontal cortex, amygdala, caudate-putamen, nucleus accumbens and hippocampus) from three experimental paradigms including drug-naïve and ethanol-treated inbred alcohol-preferring and alcohol-non-preferring rats. Ethanol was administered using chronic free-choice consumption and intracranial self-administration (into ventral tegmental area) models. Overlapping expression data were then filtered using human genetic linkage data, human tissue data (post-mortem brain, lymphocytes and fibroblasts) and biological roles data. Analysis of gene expression data identified $\sim$3000 significantly changed genes across brain regions and experimental paradigms. The list of candidate genes was further reduced by identifying those changed in all three experiments and those that were changed in at least two out of three experiments. An empirical probability scoring system was derived that combined expression data with the additional filters listed above to identify high-priority candidate genes. The highest-ranking genes (those changed in all three experiments) included CD81 molecule, nucleoporin like 1, phosphatidylethanolamine-binding protein and aldehyde dehydrogenase 6 family, member A1.

The studies outlined in this section demonstrate that large data sets of gene expression data can be combined with behavioural and genetic data to identify genes or functional pathways that underlie ethanol-related phenotypes and other complex traits.

Looking into the future

Increasingly sophisticated genetic tools (haplotype and SNP maps, mapping arrays, expression arrays and so on) are being applied to complex diseases ranging from cancer to schizophrenia. What is the desired or imagined outcome for such studies? For most diseases, genetic or genomic assessment of risk or susceptibility is a goal. Breast cancer prediction is an area where genetics have been aggressively developed and marketed, although the accuracy of such markers remains controversial. For alcoholism, family history and personal history are strong predictors of risk and it is not clear that genetic markers of risk will be a practical or useful contribution. Areas of more importance for alcoholism are 'genetic medicines' and genomic/proteomic biomarkers for alcohol abuse. The success (or lack thereof) for naltrexone in the treatment of alcohol dependence depends in part on a polymorphism in the μ opioid receptor, and this gives the possibility of genotype-based selection of pharmacotherapy for alcoholism (Oslin *et al*., 2006). Another likely application of 'omics' to addiction medicine is selection of biomarkers for alcohol and drug dependence or abuse based on changes in gene expression or protein levels in blood samples. Sensitive and selective biomarkers can only be defined after measuring many different transcripts or proteins with array technologies. This review presents many 'candidate' genes for alcohol and drug dependence and a plethora of changes in gene expression that might, or might not, be responsible for development of dependence. When will we move past 'candidates' to 'defined' genes? The history of genetics of complex diseases brings great excitement about new techniques with large increases in genetic power (for example, selected lines, recombinant inbred strains, QTL analysis, gene expression arrays, SNP maps and so on). But application of these new approaches reveals that the complexity of the disease and the genetics of the organism are much greater than we appreciated. This leads to the development of new approaches, which reveal new complexities. The immediate future may bring the realization that we will not be able to define the genetics of dependence until we better understand how genes interact with environmental variables to influence drug responses and related behaviours.

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Conflict of interest

The authors state no conflict of interest.

References

Anni H, Israel Y (2002). Proteomics in alcohol research. *Alcohol Res Health* **26**: 219–232.

Arlinde C, Sommer W, Bjork K, Reimers M, Hyytia P, Kiianmaa K *et al.* (2004). A cluster of differentially expressed signal transduction genes identified by microarray analysis in a rat genetic model of alcoholism. *Pharmacogen J* **4**: 208–218.

Aschner M, Sonnewald U, Tan KH (2002). Astrocyte modulation of neurotoxic injury. *Brain Pathol* **12**: 475–481.

Atz M, Walsh D, Cartagena P, Li J, Evans S, Choudary P *et al.* (2007). Methodological considerations for gene expression profiling of human brain. *J Neurosci Methods* **163**: 295–309.

Barr CS, Newman TK, Becker ML, Champoux M, Lesch KP, Suomi SJ *et al.* (2003). Serotonin transporter gene variation is associated with alcohol sensitivity in rhesus macaques exposed to early-life stress. *Alcohol Clin Exp Res* **27**: 812–817.

Belknap JK, Atkins AL (2001). The replicability of QTLs for murine alcohol preference drinking behavior across eight independent studies. *Mamm Genome* **12**: 893–899.

Bennett B, Downing C, Carosone-Link P, Ponicsan H, Ruf C, Johnson TE (2007). Quantitative trait locus mapping for acute functional tolerance to ethanol in the L x S recombinant inbred panel. *Alcohol Clin Exp Res* **31**: 200–208.

Bennett B, Downing C, Parker C, Johnson TE (2006). Mouse genetic models in alcohol research. *Trends Genet* **22**: 367–374.

Bergeson SE, Kyle Warren R, Crabbe JC, Metten P, Gene Erwin V, Belknap JK (2003). Chromosomal loci influencing chronic alcohol withdrawal severity. *Mamm Genome* **14**: 454–463.

Bierut LJ, Dinwiddie SH, Begleiter H, Crowe RR, Hesselbrock V, Nurnberger Jr JI *et al.* (1998). Familial transmission of substance dependence: alcohol, marijuana, cocaine, and habitual smoking. *Arch Gen Psychiatry* **55**: 982–988.

Blalock EM, Geddes JW, Chen KC, Porter NM, Markesbery WR, Landfield PW (2004). Incipient Alzheimer's disease: microarray correlation analyses reveal major transcriptional and tumor suppressor responses. *Proc Natl Acad Sci USA* **101**: 2173–2178.

Blednov YA, Metten P, Finn DA, Rhodes JS, Bergeson SE, Harris RA *et al.* (2005). Hybrid C57BL/6J × FVB/NJ mice drink more alcohol than do C57BL/6J mice. *Alcohol Clin Exp Res* **29**: 1949–1958.

Buck KJ, Metten P, Belknap JK, Crabbe JC (1997). Quantitative trait loci involved in genetic predisposition to acute alcohol withdrawal in mice. *J Neurosci* **17**: 3946–3955.

Butterworth RF (2003). Hepatic encephalopathy. *Alcohol Res Health* **27**: 240–246.

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington HL *et al.* (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* **301**: 386–389.

Colantuoni C, Jeon OH, Hyder K, Chenchik A, Khimani AH, Narayanan V *et al.* (2001). Gene expression profiling in postmortem Rett Syndrome brain: differential gene expression and patient classification. *Neurobiol Dis* **8**: 847–865.

Cotton NS (1979). The familial incidence of alcoholism: a review. *J Stud Alcohol* **40**: 89–116.

Crabbe JC, Phillips TJ, Harris RA, Arends MA, Koob GF (2006). Alcohol-related genes: contributions from studies with genetically engineered mice. *Addict Biol* **11**: 195–269.

Crabbe JC, Phillips TJ (1998). Genetics of alcohol and other abused drugs. *Drug Alcohol Depend* **51**: 61–71.

Crabbe JC, Phillips TJ, Buck KJ, Cunningham CL, Belknap JK (1999). Identifying genes for alcohol and drug sensitivity: recent progress and future directions. *Trends Neurosci* **22**: 173–179.

Crabbe JC, Phillips TJ, Kosobud A, Belknap JK (1990). Estimation of genetic correlation: interpretation of experiments using selectively bred and inbred animals. *Alcohol Clin Exp Res* **14**: 141–151.

D'Souza D, Gil R, Madonick S, Perry E, Forselius-Bielen K, Braley G *et al.* (2006). Enhanced sensitivity to the euphoric effects of alcohol in schizophrenia. *Neuropsychopharmacology* **31**: 2767–2775.

Daniels GM, Buck KJ (2002). Expression profiling identifies strain-specific changes associated with ethanol withdrawal in mice. *Genes Brain Behav* **1**: 35–45.

Davies AG, Pierce-Shimomura JT, Kim H, VanHoven MK, Thiele TR, Bonci A *et al.* (2003). A central role of the BK potassium channel in behavioral responses to ethanol in *C.elegans*. *Cell* **115**: 655–666.

Demarest K, Koyner J, McCaughran Jr J, Cipp L, Hitzemann R (2001). Further characterization and high-resolution mapping of quantitative trait loci for ethanol-induced locomotor activity. *Behav Genet* **31**: 79–91.

Dick DM, Aliev F, Wang JC, Saccone S, Hinrichs A, Bertelsen S *et al.* (2007a). A systematic single nucleotide polymorphism screen to fine-map alcohol dependence genes on chromosome 7 identifies association with a novel susceptibility gene ACN9. *Biol Psychiatry* [Epub ahead of print].

Dick DM, Bierut L, Hinrichs A, Fox L, Bucholz KK, Kramer J *et al.* (2006). The role of GABRA2 in risk for conduct disorder and alcohol and drug dependence across developmental stages. *Behav Genetics* **36**: 577–590.

Dick DM, Rose RJ, Viken RJ, Kaprio J, Koskenvuo M (2001). Exploring gene–environment interactions: socioregional moderation of alcohol use. *J Abnorm Psychol* **110**: 625–632.

Dick DM, Wang JC, Plunkett J, Aliev F, Hinrichs A, Bertelsen S *et al.* (2007b). Family-based association analyses of alcohol dependence phenotypes across DRD2 and neighboring gene ANKK1. *Alcohol Clin Exp Res* **31**: 1645–1653.

Duncan TE, Duncan SC, Hops H, Stoolmiller M (1995). An analysis of the relationship between parent and adolescent marijuana use via generalized estimating equation methodology. *Multivariate Behav Res* **30**: 317–339.

Duranceaux NCE, Schuckit MA, Eng MY, Robinson SK, Carr LG, Wall TL (2006). Associations of variations in alcohol dehydrogenase genes with the level of response to alcohol in non-Asians. *Alcohol Clin Exp Res* **30**: 1470–1478.

Dutta R, McDonough J, Yin X, Peterson J, Chang A, Torres T *et al.* (2006). Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. *Ann Neurol* **59**: 478–489.

Edenberg HJ, Dick DM, Xuei X, Tian H, Almasy L, Bauer LO *et al.* (2004). Variations in GABRA2, encoding the á2 subunit of the GABA-A receptor are associated with alcohol dependence and with brain oscillations. *Am J Hum Genet* **74**: 705–714.

Edenberg HJ, Strother WN, McClintick JN, Tian H, Stephens M, Jerome RE *et al.* (2005). Gene expression in the hippocampus of inbred alcohol-preferring and -nonpreferring rats. *Genes Brain Behav* **4**: 20–30.

Edenberg HJ, Xuei X, Chen H-J, Tian H, Flury Wetherill L, Dick DM *et al.* (2006). Association of alcohol dehydrogenase genes with alcohol dependence: a comprehensive analysis. *Hum Mol Gen* **15**: 1539–1549.

Ehlers CL, Gilder DA, Wall TL, Phillips E, Feiler H, Wilhelmsen KC (2004). Genomic screen for loci associated with alcohol dependence in Mission Indians. *Am J Med Genet B Neuropsychiatr Genet* **129**: 110–115.

Erblich J, Earleywine M (1999). Children of alcoholics exhibit attenuated cognitive impairment during an ethanol challenge. *Alcohol Clin Exp Res* **23**: 476–482.

Ernouf D, Copagnon P, Lothion P, Narcisse G, Benard JY, Daoust M (1993). Platelets 3H 5-HT uptake in descendants from alcoholic patients: a potential risk factor for alcohol dependence. *Life Sci* **52**: 989–995.

Evans SJ, Choudary PV, Neal CR, Li JZ, Vawter MP, Tomita H *et al.* (2004). Dysregulation of the fibroblast growth factor system in major depression. *Proc Natl Acad Sci USA* **101**: 15506–15511.

Fehr C, Shirley RL, Belknap JK, Crabbe JC, Buck KJ (2002). Congenic mapping of alcohol and pentobarbital withdrawal liability loci to a <1 centimorgan interval of murine chromosome 4: identification of Mpdz as a candidate gene. *J Neurosci* **22**: 3730–3738.

Flatscher-Bader T, van der Brug M, Hwang JW, Gochee PA, Matsumoto I, Niwa S *et al.* (2005). Alcohol-responsive genes in the frontal cortex and nucleus accumbens of human alcoholics. *J Neurochem* **93**: 359–370.

Flatscher-Bader T, Wilce PA (2006). Chronic smoking and alcoholism change expression of selective genes in the human prefrontal cortex. *Alcohol Clin Exp Res* **30**: 908–915.

Flatscher-Bader T, Zuvela N, Landis N, Wilce PA (2008). Smoking and alcoholism target genes associated with plasticity and glutamate

transmission in the human ventral tegmental area. *Hum Mol Genet* **17**: 38–51.
Franke P, Nöthen MM, Wang T, Knapp M, Lichtermann D, Neidt H *et al.* (2000). DRD4 exon III VNTR polymorphism—susceptibility factor for heroin dependence? Results of a case-control and a family-based association approach. *Mol Psychiatry* **5**: 101–104.
Gelernter J, Kranzler H (1999). D2 dopamine receptor gene (DRD2) allele and haplotype frequencies in alcohol dependent and control subjects: no association with phenotype or severity of phenotype. *Neuropsychopharmacology* **20**: 640–649.
Godefroy O, Rousseaux M (1997). Novel decision making in patients with prefrontal or posterior brain damage. *Neurology* **49**: 695–701.
Goldman D (1998). General and specific inheritance of substance abuse and alcoholism (commentary). *Arch Gen Psychiatry* **55**: 964–965.
Goodwin DW, Schulsinger F, Moller N, Hermansen L, Winokur G, Guze SB (1974). Drinking problems in adopted and nonadopted sons of alcoholics. *Arch Gen Psychiatry* **31**: 164–169.
Gottesman II, Gould TD (2003). The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry* **160**: 636–645.
Grant BF, Dufour MC, Harford TC (1988). Epidemiology of alcoholic liver disease. *SeM Liver Dis* **8**: 12–25.
Harper CG, Kril JJ, Holloway RL (1985). Brain shrinkage in chronic alcoholics: a pathological study. *BMJ (Clin Res Ed)* **290**: 501–504.
Hassan S, Duong B, Kim KS, Miles MF (2003). Pharmacogenomic analysis of mechanisms mediating ethanol regulation of dopamine beta-hydroxylase. *The J Biol Chem* **278**: 38860–38869.
Heath AC, Madden PA, Bucholz KK, Dinwiddie SH, Slutske WS, Bierut LJ *et al* (1999). Genetic differences in alcohol sensitivity and the inheritance of alcoholism risk. *Psychol Med* **29**: 1069–1081.
Hinckers AS, Laucht M, Schmidt MH, Mann KF, Schumann G, Schuckit MA *et al.* (2006). Low level of response to alcohol as associated with serotonin transporter genotype and high alcohol intake in adolescents. *Biol Psychiatry* **60**: 282–287.
Hirano S, Suzuki ST, Redies C (2003). The cadherin superfamily in neural development: diversity, function and interaction with other molecules. *Front Biosci* **8**: d306–d355.
Hodge CW, Slawecki CJ, Aiken AS (1996). Norepinephrine and serotonin receptors in the paraventricular nucleus interactively modulate ethanol consumption. *Alcohol Clin Exp Res* **20**: 1669–1674.
Hoffman PL, Tabakoff B (1996). Alcohol dependence: a commentary on mechanisms. *Alcohol Alcohol* **31**: 333–340.
Hu X, Oroszi G, Chun J, Smith TL, Goldman D, Schuckit MA (2005). An expanded evaluation of the relationship of four alleles to the level of response to alcohol and the alcoholism risk. *Alcohol Clin Exp Res* **29**: 8–16.
Huntley GW, Gil O, Bozdagi O (2002). The cadherin family of cell adhesion molecules: multiple roles in synaptic plasticity. *Neuroscientist* **8**: 221–233.
Iwamoto K, Bundo M, Yamamoto M, Ozawa H, Saito T, Kato T (2004a). Decreased expression of NEFH and PCP4/PEP19 in the prefrontal cortex of alcoholics. *Neurosci Res* **49**: 379–385.
Iwamoto K, Kakiuchi C, Bundo M, Ikeda K, Kato T (2004b). Molecular characterization of bipolar disorder by comparing gene expression profiles of postmortem brains of major mental disorders. *Mol Psychiatry* **9**: 406–416.
Jackson ES, Wayland MT, Fitzgerald W, Bahn S (2005). A microarray data analysis framework for postmortem tissues. *Methods* **37**: 247–260.
Johnson C, Drgon T, Liu QR, Walther D, Edenberg H, Rice J *et al.* (2006). Pooled association genome scanning for alcohol dependence using 104 268 SNPs: validation and use to identify alcoholism vulnerability loci in unrelated individuals from the collaborative study on the genetics of alcoholism. *Am J Med Genet B Neuropsychiatr Genet* **141**: 844–853.
Jones KA, Porjesz B, Almasy L, Bierut L, Goate A, Wang JC *et al.* (2004). Linkage and linkage disequilibrium of evoked EEG oscillations with CHRM2 receptor gene polymorphisms: implications for human brain dynamics and cognition. *Int J Psychophysiol* **53**: 75–90.
Kendler KS, Jacobson KC, Prescott CA, Neale MC (2003). Specificity of genetic and environmental risk factors for use and abuse/dependence of cannabis, cocaine, hallucinogens, sedatives, stimulants, and opiates in male twins. *Am J Psychiatry* **160**: 687–695.
Kerns RT, Ravindranathan A, Hassan S, Cage MP, York T, Sikela JM *et al.* (2005). Ethanol-responsive brain region expression networks: implications for behavioral responses to acute ethanol in DBA/2J versus C57BL/6J mice. *J Neurosci* **25**: 2255–2266.
Kimpel MW, Strother WN, McClintick JN, Carr LG, Liang T, Edenberg HJ *et al.* (2007). Functional gene expression differences between inbred alcohol-preferring and -non-preferring rats in five brain regions. *Alcohol* **41**: 95–132.
Koob GF (1992). Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol Sci* **13**: 177–184.
Koob GF, Le Moal M (2001). Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology* **24**: 97–129.
Kril JJ, Halliday GM, Svoboda MD, Cartwright H (1997). The cerebral cortex is damaged in chronic alcoholics. *Neuroscience* **79**: 983–998.
Kril JJ, Harper CG (1989). Neuronal counts from four cortical regions of alcoholic brains. *Acta Neuropathol* **79**: 200–204.
Lappalainen J, Krupitsky E, Remizov M, Pchelina S, Taraskina A, Zvartau E *et al.* (2005). Association between alcoholism and gamma-amino butyric acid alpha2 receptor subtype in a Russian population. *Alcohol Clin Exp Res* **29**: 493–498.
Lavoie J, Butterworth RF (1995). Reduced activities of thiamine-dependent enzymes in brains of alcoholics in the absence of Wernicke's encephalopathy. *Alcohol Clin Exp Res* **19**: 1073–1077.
Lee SJ, Benveniste EN (1999). Adhesion molecule expression and regulation on cells of the central nervous system. *J Neuroimmunol* **98**: 77–88.
Lewohl JM, Wang L, Miles MF, Zhang L, Dodd PR, Harris RA (2000). Gene expression in human alcoholism: microarray analysis of frontal cortex. *Alcohol Clin Exp Res* **24**: 1873–1882.
Li T, Zhu Z-H, Liu X, Hu X, Zhao J, Sham PC *et al.* (2000). Association analysis of polymorphisms in the DRD4 gene and heroin abuse in Chinese subjects. *Am J Med Genet* **96**: 616–621.
Li TK (2000). Pharmacogenetics of responses to alcohol and genes that influence alcohol drinking. *J Stud Alcohol* **61**: 5–12.
Liu J, Lewohl JM, Dodd PR, Randall PK, Harris RA, Mayfield RD (2004). Gene expression profiling of individual cases reveals consistent transcriptional changes in alcoholic human brain. *J Neurochem* **90**: 1050–1058.
Liu J, Lewohl JM, Harris RA, Dodd PR, Mayfield RD (2007). Altered gene expression profiles in the frontal cortex of cirrhotic alcoholics. *Alcohol Clin Exp Res* **31**: 1460–1466.
Liu J, Lewohl JM, Harris RA, Iyer VR, Dodd PR, Randall PK *et al.* (2006). Patterns of gene expression in the frontal cortex discriminate alcoholic from nonalcoholic individuals. *Neuropsychopharmacology* **31**: 1574–1582.
Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H *et al.* (2002). Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nature Med* **8**: 500–508.
Loring JF, Wen X, Lee JM, Seilhamer J, Somogyi R (2001). A gene expression profile of Alzheimer's disease. *DNA Cell Biol* **20**: 683–695.
Ludvig N, George MA, Tang HM, Gonzales RA, Bungay PM (2001). Evidence for the ability of hippocampal neurons to develop acute tolerance to ethanol in behaving rats. *Brain Res* **900**: 252–260.
Luo X, Kranzler H, Zuo L, Wang S, Blumberg H, Gelernter J (2005). CHRM2 gene predisposes to alcohol dependence, drug dependence and affective disorders: results from an extended case-control structured association study. *Hum Mol Gen* **14**: 2421–2434.
Luthar S, Anton S, Merikangas K, Rounsaville BJ (1992). Vulnerability to drug abuse among opioid addicts' siblings: individual, familial, and peer influences. *Compr Psychiatry* **33**: 190–196.
MacLaren EJ, Bennett B, Johnson TE, Sikela JM (2006). Expression profiling identifies novel candidate genes for ethanol sensitivity QTLs. *Mamm Genome* **17**: 147–156.
Mayfield RD, Lewohl JM, Dodd PR, Herlihy A, Liu J, Harris RA (2002). Patterns of gene expression are altered in the frontal and motor cortices of human alcoholics. *J Neurochem* **81**: 802–813.
McGough JJ, McCracken JT, Hariri AR, Lewis DA (2006). Genetics and the future of clinical psychiatry. *Am J Psychiatry* **163**: 1676–1678.

Melo JA, Shendure J, Pociask K, Silver LM (1996). Identification of sex-specific quantitative trait loci controlling alcohol preference in C57BL/ 6 mice. *Nature Genet* **13**: 147–153.

Merikangas KR, Mehta RL, Molnar BE, Walters EE, Swendsen JD, Aguilar-Gaziola S *et al.* (1998a). Comorbidity of substance use disorders with mood and anxiety disorders: results of the International Consortium in Psychiatric Epidemiology. *Addict Behav* **23**: 893–907.

Merikangas KR, Stolar M, Stevens DE, Goulet J, Preisig MA, Fenton B *et al.* (1998b). Familial transmission of substance use disorders. *Arch Gen Psychiatry* **55**: 973–979.

Milner R, Campbell IL (2002). The integrin family of cell adhesion molecules has multiple functions within the CNS. *J Neurosci Res* **69**: 286–291.

Mirin SM, Weiss RD, Griffin ML, Michael JL (1991). Psychopathology in drug abusers and their families. *Compr Psychiatry* **32**: 36–51.

Mirnics K, Middleton FA, Lewis DA, Levitt P (2001). Analysis of complex brain disorders with gene expression microarrays: schizophrenia as a disease of the synapse. *Trends Neurosci* **24**: 479–486.

Mirnics K, Middleton FA, Marquez A, Lewis DA, Levitt P (2000). Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. *Neuron* **28**: 53–67.

Mulligan MK, Ponomarev I, Hitzemann RJ, Belknap JK, Tabakoff B, Harris RA *et al.* (2006). Toward understanding the genetics of alcohol drinking through transcriptome meta-analysis. *Proc Natl Acad Sci USA* **103**: 6368–6373.

Murphy BC, Chiu T, Harrison M, Uddin RK, Singh SM (2002). Examination of ethanol responsive liver and brain specific gene expression, in the mouse strains with variable ethanol preferences, using cDNA expression arrays. *Biochem Genet* **40**: 395–410.

Nestler EJ (2000). Genes and addiction. *Nature Genet* **26**: 277–281.

Norenberg MD, Jayakumar AR, Rama Rao KV (2004). Oxidative stress in the pathogenesis of hepatic encephalopathy. *Metab Brain Dis* **19**: 313–329.

Oslin DW, Berrettini WH, O'Brien CP (2006). Targeting treatments for alcohol dependence: the pharmacogenetics of naltrexone. *Addict Biol* **11**: 397–403.

Pongrac JL, Middleton FA, Peng L, Lewis DA, Levitt P, Mirnics K (2004). Heat shock protein 12A shows reduced expression in the prefrontal cortex of subjects with schizophrenia. *Biol Psychiatry* **56**: 943–950.

Prescott CA, Kendler KS (1999). Genetic and environmental contributions to alcohol abuse and dependence in a population-based sample of male twins. *Am J Psychiatry* **156**: 34–40.

Purcell AE, Jeon OH, Zimmerman AW, Blue ME, Pevsner J (2001). Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. *Neurology* **57**: 1618–1628.

Rahman S, Sahakian BJ, Hodges JR, Rogers RD, Robbins TW (1999). Specific cognitive deficits in mild frontal variant frontotemporal dementia. *Brain* **122**: 1469–1493.

Ratti MT, Bo P, Giardini A, Soragna D (2002). Chronic alcoholism and the frontal lobe: which executive functions are imparied? *Acta Neurol Scand* **105**: 276–281.

Rausch JL, Monteiro MG, Schuckit MA (1991). Platelet serotonin uptake in men with family histories of alcoholism. *Neuropsychopharmacology* **4**: 83–86.

Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D *et al.* (2004). Large-scale meta-analysis of cancer microarray data identifies common transcriptional profiles of neoplastic transformation and progression. *Proc Natl Acad Sci USA* **101**: 9309–9314.

Rimondini R, Arlinde C, Sommer W, Heilig M (2002). Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. *FASEB J* **16**: 27–35.

Risinger FO, Cunningham CL (1998). Ethanol-induced conditioned taste aversion in BXD recombinant inbred mice. *Alcohol Clin Exp Res* **22**: 1234–1244.

Roberts AJ, Heyser CJ, Cole M, Griffin P, Koob GF (2000). Excessive ethanol drinking following a history of dependence: animal model of allostasis. *Neuropsychopharmacology* **22**: 581–594.

Rodd ZA, Bertsch BA, Strother WN, Le-Niculescu H, Balaraman Y, Hayden E *et al.* (2007). Candidate genes, pathways and mechanisms for alcoholism: an expanded convergent functional genomics approach. *Pharmacogenomics J* **7**: 222–256.

Rogers J, Wiener SG, Bloom FE (1979). Long-term ethanol administration methods for rats: advantages of inhalation over intubation or liquid diets. *Behav Neural Biol* **27**: 466–486.

Rounsaville BJ, Kosten TR, Weissman MM, Prusoff R, Pauls D, Anton SF *et al.* (1991). Psychiatric disorders in relatives of probands with opiate addiction. *Arch Gen Psychiatry* **48**: 33–42.

Saba L, Bhave SV, Grahame N, Bice P, Lapadat R, Belknap J *et al.* (2006). Candidate genes and their regulatory elements: alcohol preference and tolerance. *Mamm Genome* **17**: 669–688.

Saito M, Smiley J, Toth R, Vadasz C (2002). Microarray analysis of gene expression in rat hippocampus after chronic ethanol treatment. *Neurochemical research* **27**: 1221–1229.

Scheiffele P (2003). Cell-cell signaling during synapse formation in the CNS. *Annu Rev Neurosci* **26**: 485–508.

Schuckit MA (2002). Vulnerability factors for alcoholism. In: Davis, K. (ed). *Neuropsychopharmacology: The Fifth Generation of Progress*. Lippincott Williams & Wilkins Co: Baltimore. ch 98, pp 1399–1411.

Schuckit MA, Danko GP, Smith TL (2004a). Patterns of drug-related disorders in a prospective study of men chosen for their family history of alcoholism. *J Stud Alcohol* **65**: 613–620.

Schuckit MA, Edenberg HJ, Kalmijn J, Flury L, Smith TL, Reich T *et al.* (2001). A genome-wide search for genes that relate to a low level of response to alcohol. *Alcohol Clin Exp Res* **25**: 323–329.

Schuckit MA, Mazzanti C, Smith TL, Ahmed U, Radel M, Iwata N *et al.* (1999). Selective genotyping for the role of 5-HT2A, 5-HT2C, and GABAα6 receptors and the serotonin transporter in the level of response to alcohol: a pilot study. *Biol Psychiatry* **45**: 647–651.

Schuckit MA, Smith TL (2000). The relationships of a family history of alcohol dependence, a low level of response to alcohol and six domains of life functioning to the development of alcohol use disorders. *J Stud Alcohol* **61**: 827–835.

Schuckit MA, Smith TL (2006). An evaluation of the level of response to alcohol, externalizing symptoms, and depressive symptoms as predictors of alcoholism. *J Stud Alcohol* **67**: 215–227.

Schuckit MA, Smith TL, Danko GP, Anderson KG, Brown SA, Kuperman S *et al.* (2005a). Evaluation of a level of response to alcohol-based structural equation model in adolescents. *J Stud Alcohol* **66**: 174–184.

Schuckit MA, Smith TL, Kalmijn J (2004b). The search for genes contributing to the low level of response to alcohol: Patterns of findings across studies. *Alcohol Clin Exp Res* **28**: 1449–1458.

Schuckit MA, Wilhelmsen K, Smith TL, Feiler HS, Lind P, Lange LA *et al.* (2005b). Autosomal linkage analysis for the level of response to alcohol. *Alcohol Clin Exp Res* **29**: 1976–1982.

Shirley RL, Walter NA, Reilly MT, Fehr C, Buck KJ (2004). Mpdz is a quantitative trait gene for drug withdrawal seizures. *Nature Neurosci* **7**: 699–700.

Slutske WS, Heath AC, Dinwiddie SH, Madden PAF, Bucholz K, Dunne MP *et al.* (1998). Common genetic risk factors for conduct disorder and alcohol dependence. *J Abnorm Psychology* **107**: 363–374.

Sokolov BP, Jiang L, Trivedi NS, Aston C (2003). Transcription profiling reveals mitochondrial, ubiquitin and signaling systems abnormalities in postmortem brains from subjects with a history of alcohol abuse or dependence. *J Neurosci Res* **72**: 756–767.

Sullivan P, Kendler K, Neale M (2003). Schizophrenia as a complex trait. *Arch Gen Psychiatry* **60**: 1187–1192.

Tabakoff B, Bhave SV, Hoffman PL (2003). Selective breeding, quantitative trait locus analysis, and gene arrays identify candidate genes for complex drug-related behaviors. *J Neurosci* **23**: 4491–4498.

Tarantino LM, McClearn GE, Rodriguez LA, Plomin R (1998). Confirmation of quantitative trait loci for alcohol preference in mice. *Alcohol Clin Exp Res* **22**: 1099–1105.

Thibault C, Lai C, Wilke N, Duong B, Olive MF, Rahman S *et al.* (2000). Expression profiling of neural cells reveals specific patterns of ethanol-responsive gene expression. *Mol Pharmacol* **58**: 1593–1600.

Tome S, Lucey MR (2004). Review article: current management of alcoholic liver disease. *Aliment Pharmacol Ther* **19**: 707–714.

Tomita H, Vawter MP, Walsh DM, Evans SJ, Choudary PV, Li J *et al*. (2004). Effect of agonal and postmortem factors on gene expression profile: quality control in microarray analyses of postmortem human brain. *Biol Psychiatry* **55**: 346–352.

Treadwell JA, Singh SM (2004). Microarray analysis of mouse brain gene expression following acute ethanol treatment. *Neurochem Res* **29**: 357–369.

Tsuang MT, Lyons MJ, Meyer JM, Doyle T, Eisen SA, Goldberg J *et al*. (1998). Co-occurrence of abuse or different drugs in men. *Arch Gen Psychiatry* **55**: 967–972.

Vetulani J (2001). Drug addiction. Part II. Neurobiology of addiction. *Pol J Pharmacol* **53**: 303–317.

Volavka J, Czobor P, Goodwin D, Gabrielli Jr WF, Penick EC, Mednick SA *et al*. (1996). The electronencephalogram after alcohol administration in high-risk men and the development of alcohol use disorders 10 years later: preliminary findings. *Arch Gen Psychiatry* **53**: 258–263.

Wall TL, Shea SH, Luczak SE, Cook TAR, Carr LG (2005). Genetic associations of alcohol dehydrogenase with alcohol use disorders and endophenotypes in white college students. *J Abnorm Psychol* **114**: 456–465.

Weinshenker D, Rust NC, Miller NS, Palmiter RD (2000). Ethanol-associated behaviors of mice lacking norepinephrine. *J Neurosci* **20**: 3157–3164.

Whitfield JB, Nightingale BN, Bucholz KK, Madden PAF, Heath AC, Martin NG (1998). ADH genotypes and alcohol use and dependence in Europeans. *Alcohol Clin Exp Res* **22**: 1463–1469.

Winokur G, Coryell W, Endicott J, Keller M, Akiskal H, Solomon D (1996). Familial alcoholism in manic-depressive (bipolar) disease. *Am J Med Genet* **67**: 197–201.

Worst TJ, Tan JC, Robertson DJ, Freeman WM, Hyytia P, Kiianmaa K *et al*. (2005). Transcriptome analysis of frontal cortex in alcohol-preferring and nonpreferring rats. *J Neurosci Res* **80**: 529–538.

Xu Y, Ehringer M, Yang F, Sikela JM (2001). Comparison of global brain gene expression profiles between inbred long-sleep and inbred short-sleep mice by high-density gene array hybridization. *Alcohol Clin Exp Res* **25**: 810–818.