Review

Neurogenetic studies of alcohol addiction

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Neurogenetic studies of alcohol dependence have relied substantially on genetic animal models, particularly rodents. Studies of inbred strains, selectively bred lines and mutants bearing genes whose function has been targeted for over or under expression are reviewed. Studies focused on gene expression changes are the most recent contributors to this literature, and some genetic effects may work through epigenetic mechanisms. In a few instances, interesting parallels have been revealed between genetic risk in humans and studies in non-human animal models. Future approaches are likely to be increasingly complex.

Keywords: alcohol; genetic animal models; candidate genes

1. INTRODUCTION

There is clear evidence of genetic contributions to alcohol dependence in humans, but studies must cope with diagnostic and aetiologic heterogeneity, as well as comorbidities with other psychiatric disorders and the role of other genetically influenced risk factors such as personality traits (e.g. impulsivity). Genes of influence are manifold, and their individual effects on risk are small. Environmental factors such as major life stressors, work and peer influences are equally important. Although it is more difficult to document, most believe that risk-promoting genes are likely to be of importance only under certain (and possible specific) risk-promoting environmental conditions: that is, there are important gene–environment interactions. Given the genetic heterogeneity of human populations, their long generation time and their unwillingness to cooperate with useful experimental mating schemes, it is a wonder that we have been able to find clear examples of genetic risk. The best such example is the increased frequency of the aldehyde dehydrogenase isoform leading to accumulation of acetaldehyde upon drinking alcohol. Among hundreds of individuals genotyped from the East Asian gene pools where this isoform is predominant, only a single homozygous individual for the slow aldehyde dehydrogenase variant has been reported to earn a diagnosis of alcoholism (Enoch & Goldman 2001).

Research on genetic risk has concentrated on laboratory animals, particularly rodents. Most symptoms important for a diagnosis of alcoholism or an alcohol use disorder as currently defined are behavioural. These include failure to control quantity or frequency of drinking, and continuing to drink in the

face of consequential medical, legal or social/familial problems. It is clearly difficult to envision completely believable rodent models for such behaviours as getting into trouble at work, a limit to the usefulness of such models (Loving & Crabbe 2005). Nonetheless, the tractability of rodent neurobiology, and especially the flowering of mouse genetics and genomics, have made studies of these species attractive. While I will mention other species and attempt to draw parallels with human genetic data where possible, the focus of this review will be on rodent genetic studies. Results from four methods for approaching the neurogenetics of alcoholism will be assessed—inbred strain studies, work with selectively bred lines, candidate gene targeting approaches and gene expression profiling.

I do not believe that there is a rodent model that resembles human alcoholism in all important aspects. The inherent fallacies in attempting such simulacra were eloquently outlined many years ago (McClearn 1979). Rather, I advocate concentrating on specific aspects of the disorder that can be convincingly modelled in rats and mice. These include aspects of the pharmacology of ethyl alcohol (neural sensitivity to the drug, the development of tolerance to its effects with repeated administration, and withdrawal symptoms shared across mammalian species, e.g. seizures or convulsions, autonomic dysregulation). Extensive studies of the neuroadaptations accompanying tolerance development have shown genetic influences to be strong (Kalant *et al.* 1971; Kalant 1998). Recent studies using the powerful genetic manipulations available in invertebrate species have extended the range of genetic influences thought to be responsible (Scholz *et al.* 2000; Cowmeadow *et al.* 2005).

Despite the use of reductionist approaches, the complex behavioural disorder, alcoholism, demands an attempt to model some of the key behavioural features. Foremost among these is self-administration of the drug. Genetic animal model research in

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alcoholism has focused extensively on voluntary oral ethanol self-administration, using the two-bottle preference test. Mice or rats are offered a bottle containing (typically) 10% ethanol and one containing tap water, usually *ad libitum*. The amount of alcohol drunk is taken to indicate the reinforcing effect of the drug. The majority of published studies on the genetics of alcohol in rodents have targeted this phenotype and its variants. This offers the theoretical advantage of allowing comparisons of results across methodological approaches. For example, if gene X is knocked out and found to reduce alcohol consumption, is it also found to be up- or downregulated on a microarray analysis of brain tissue DNA after chronic drinking? I will attempt to identify such consonances in the literature.

2. INBRED STRAINS

In a seminal study, inbred C57BL/6 mice were compared with four other inbred strains for two-bottle preference for ethanol (McClearn & Rodgers 1959). They drank more than the other strains, one of which (DBA/2) refused to drink ethanol nearly completely. These strains have been repeatedly characterized over the years, and C57BL/6 and DBA/2 have nearly the highest and lowest preference levels, respectively, of the more than 40 inbred strains subsequently tested in almost all studies. The pattern of strain differences has been replicated many times over the past 45 years, with highly reliable results (Wahlsten *et al.* 2006).

An inbred strain results when brothers and sisters are mated, generation after generation. In each generation, half the allelic genetic variability is lost, until after 20 generations, all same-sex animals have two copies of the same allele for any gene—they are obligatory homozygotes throughout their genome (Falconer & Mackay 1996). However, each inbred strain is genetically unique, and different from all others. The degree of difference depends upon the pedigree history of the strain's derivation, and there are seven major lineages for the more than 100 standard inbred strains that are commercially available (Petkov *et al.* 2004). If a panel of inbred strains is tested under controlled environmental conditions, the differences in a behaviour (or a neurochemical phenotype, for example) are taken to be of allelic genetic origin. To the extent that they exceed the average individual differences within a strain, they serve as an estimate of the aggregate effect of genetics on the phenotype.

Within an inbred strain, however, there are always individual differences. Most C57BL/6J mice show increasing alcohol drinking over a period of two to three weeks, for example, which was noted in the earliest paper (McClearn & Rodgers 1959). The source of these differences cannot be allelic, and must derive from environmental factors (e.g. social dominance in group-housed animals, food, how well they slept the night before testing). Alternatively, they may be epigenetically based, whether or not they are transmitted to their offspring (Francis *et al.* 1999, 2003).

What have we learned from inbred strain studies of alcohol-related phenotypes since 1959? When the inbred strain literature on behavioural responses (Phillips & Crabbe 1991) and neurochemical strain

differences (Allan & Harris 1991) were first comprehensively reviewed in 1991, only a handful of studies were published and these were nearly all limited to comparisons of the C57BL/6J and DBA/2J strains, previously identified as outliers for alcohol drinking and for the severity of alcohol withdrawal (Kakihana 1979). However, the development of recombinant inbred (RI) strains (Bailey 1971) extended the use of inbred strains to a new purpose. RI strains are derived from re-inbreeding after intercrossing two inbred strains to obtain an F2 generation. The F2 population shows a mosaic pattern of DNA segments on each chromosome due to crossovers during meiosis. After inbreeding to form a new RI strain is complete, the order of genetic segments randomly reshuffled in the genome of the F2 animals is preserved in future generations with the same fidelity as in standard inbreds (Silver 1995). With the advent of high-throughput genomics enabled by the Human Genome Project, genomic markers were rapidly developed. These started with restriction fragment length polymorphisms, soon thereafter succeeded by microsatellite repeat markers, and most recently by single nucleotide polymorphisms, or SNPs. The stability of inbred strains thus allowed genome scientists to build a mouse genome map in parallel with the human genome map.

The mouse map was completed in 2002, but long before that, the genetic marker map was sufficiently dense to allow mapping the location of genes whose alleles were correlated with high or low values on any measured phenotype. Thus, it was possible to study the 26 B×D RI strains then in existence, each derived from crossing (high-drinking strain) C57BL/6J mice with (low-drinking strain) DBA/2J mice. These B×D RI strains differed markedly in their preference for ethanol, and because each possessed a stable mixture of marker alleles that were inherited from either C57BL/6J or DBA/2J progenitors, the location of segments of DNA associated with high and low drinking could be 'read' directly from the patterns of RI strain phenotypic differences (Phillips *et al.* 1998). These locations are initially deemed 'loci,' called 'quantitative trait loci' (QTL) for two reasons: first, because the trait, drinking, is influenced by many genes, its inheritance pattern is not all or none (Mendelian), but rather graded or quantitative; and second, because any QTL comprises a substantial stretch of chromosomal DNA and includes multiple genes. The stability of inbred strains across generations and laboratories has allowed several groups to combine similar analyses, and several QTL affecting preference drinking have been firmly supported in multiple studies (Rodriguez *et al.* 1994; Melo *et al.* 1996; Phillips *et al.* 1998; Tarantino *et al.* 1998). However, none of these QTL for drinking has as yet been resolved to the level of a single quantitative trait gene (QTG). This is for several reasons, including (but not limited to) the difficulty of manipulating all the candidate genes in the QTL interval to rule in or out their role; the possibility that multiple genes may reside in a single QTL interval; and the small effect size for any QTL.

QTL mapping has led to at least one QTG. Acute withdrawal from ethanol and pentobarbital has been studied in standard inbreds and the B×D RI strains,

and provisional QTL identified (Buck *et al.* 1997). Subsequent studies winnowed one QTL on chromosome 4 to a few genes (Fehr *et al.* 2002). Finally, comparisons of purpose-bred mice and standard inbred strains ruled out all but a single gene, *Mpdz*, as the source of the effects on withdrawal severity (Shirley *et al.* 2004). This gene encodes a scaffolding protein that participates in neurotransmitter–receptor interactions, and current studies are exploring possible specific partners affected by the *Mpdz* polymorphism. There are many new methods for mapping QTL and isolating the specific genes of importance. Inbred strains can be surveyed directly for associations between markers and phenotypes (Grupe *et al.* 2001; Liao *et al.* 2004), although the statistical mapping power of such analyses has been questioned due to the close pedigree relationships among standard inbred strains, leading to large haplotype similarities. Mapping studies are more difficult, but more powerful, in outbred stocks (Mott *et al.* 2000; Flint *et al.* 2005; Valdar *et al.* 2006).

Another feature of inbred strains that is very useful is their cumulative power. A recent effort has sought to enter phenotypic information for many of the widely used strains into a database. The Mouse Phenome Database (MPD) <http://phenome.jax.org/pub/cgi/phenome/mpdcgi?rtn=docs/home> contains nearly 1200 phenotypes as of this writing (May 2008) which have been collected on at least eight inbred strains (average of 18 strains/phenotype), and targets 36 strains as high priority. The phenotypes range from behavioural to neurochemical, anatomical and physiological and are derived from 68 projects; some data are available for 598 strains. Owing to the inherent replicability of inbred strain data (Wahlsten *et al.* 2006), strain mean data can be correlated across phenotypes, and a significant correlation across strain means suggests that some genes affect both phenotypes. The increasing availability of SNP data in the MPD and elsewhere further strengthens the usefulness of this resource.

For example, several studies have explored the negative genetic relationship suggested by McClearn's group between high drinking (McClearn & Rodgers 1959) and low withdrawal (Kakihana 1979). A meta-analysis of strain differences on these two phenotypes found them to be significantly negatively genetically correlated. This correlation was obtained despite differences in the specific preference phenotypes examined (short versus long exposure ethanol preference tests), and acute versus chronic ethanol withdrawal (Metten *et al.* 1998). A later study surveyed strains for their sensitivity to ethanol's effectiveness in conditioning a taste aversion (CTA) to a paired, novel flavour. Ethanol's efficacy in the CTA paradigm was correlated with high withdrawal and low preference drinking (Broadbent *et al.* 2002). A recent meta-analysis of dozens of published studies with genetically defined rats and mice has included assessment of panels of inbred mouse strains (Green & Grahame 2008). This analysis confirmed the high preference–low withdrawal–low CTA triad of association, which was also seen in lines selectively bred for drinking (see §3). The authors argue that genetically high drinkers appear to be more sensitive to some aversive

properties of ethanol, which limit preference drinking. Interestingly, although there was a strong and consistent association between home cage drinking and intravenous self-administration across genotypes, there was a weaker association between these consummatory responses and the efficacy of ethanol to condition a place preference. The place preference data are complicated by a species difference: whereas mice prefer ethanol-associated locations, rats generally learn to avoid them, for reasons as yet not understood (Green & Grahame 2008). An older study of several inbred mouse strains documented differences in their ability to withhold a rewarded nose poke response for a few seconds. There was a strong correlation between this behavioural analogue of impulsivity and the preference of the strains for alcohol (Logue *et al.* 1998). A study of impulsive action in multiple mouse strains is finding their response inhibition to a no-go signal in a go/no-go task to be well correlated with the pre-signal impulsive nose-poke data (S. H. Mitchell *et al.* 2008, personal communication).

Inbred strains will continue to provide useful data in the search for genetic influences on alcohol responses. Their use is insured by their usefulness as stable backgrounds for placement of spontaneous mutants, or those engineered (e.g. as knockouts, see §4) or induced, e.g. by ethylnitrosourea (ENU) mutagenesis (see Hamre *et al.* 2007). They are also increasingly used in gene expression profiling studies (e.g. Letwin *et al.* 2006; see §5).

3. SELECTIVE BREEDING

The technique of selective breeding is the oldest in behavioural genetics, and was refined through many years of usage in agricultural settings. When extremely high-scoring individuals are selected from a population and mated together, the effect is to increase the frequency of alleles in their offspring that support high scores. Over many generations, the population under directional selection shows a gradual increase in their scores. The rate of response to selection is proportional to the degree of genetic influence, or heritability. Selected lines are extremely useful for studying the biological mechanisms underlying the selected response, and any trait not directly selected which changes over generations in parallel to the selected response increases is demonstrably influenced by the selected alleles. This is called a correlated response to selection.

Starting in the 1940s, Mardones successfully bred rats for high ethanol preference, proving that the trait was heritable (Mardones & Segovia-Riquelme 1983). The basic experimental design has been used many times since to create other high-preferring rat and mouse lines. High-preferring mice or rats may reach high blood alcohol levels (BALs), but usually only after many months of drinking. More typically, they stop drinking at BALs of approximately 60 mg%. In other words, rodents appear to have some internal controls limiting intake which are not shared by susceptible humans. Neurobiological and genetic findings from these selected lines are discussed in a series of recent reviews (Murphy *et al.* 2002;

Bell *et al.* 2006; Colombo *et al.* 2006; Quintanilla *et al.* 2006; Sommer *et al.* 2006). UChB (versus UChA), P (versus NP), AA (versus ANA), HAD (versus LAD), sP (versus sNP) and msP (versus msNP) rats (and HAP versus LAP mice) were all bred for the same trait using a nearly identical protocol; one could reasonably expect that strong genetic correlates of high alcohol preference should have emerged from these multiple replications.

Much of the early work with the selectively bred rat lines was focused on assessments of neurotransmitter levels, their activity, their interactions with brain receptors and the neuroanatomical differences in these brain systems, in comparisons with naive animals. There is substantial agreement across models that low limbic system levels of serotonin and other serotonergic differences characterize high drinkers (Murphy *et al.* 2002). Interestingly, dopaminergic differences have not been reported as consistently (Sommer *et al.* 2006). Differences in endocannabinoid, opioid, GABA, glutamate and other systems remain to be established clearly in multiple models. Somewhat more clarity has evolved from behavioural comparisons of the multiple pairs of selected lines. High drinkers are more sensitive to low-dose alcohol and less sensitive to higher-dose, sedative effects in some studies, but these results have not been generalized consistently across studies. There is good agreement that high drinkers tend to develop tolerance more readily than non-drinkers, and that it persists longer. As noted in analyses of inbred strain panels, the relationship between ethanol-induced CTA and drinking is shared across selections (Phillips *et al.* 2005; Green & Grahame 2008). Finally, mice were bred from the F2 cross of C57BL/6J×DBA/2J in a short-term selection for high- or low-preference drinking. Mice from the high drinking line were found to be more impulsive in a go/no-go response inhibition task than the low drinkers, but the lines did not differ in a task of impulsive choice, delay discounting (Wilhelm *et al.* 2007).

Rats have also been bred for their intake of ethanol in a limited access paradigm. HARF rats drink substantial amounts of ethanol in a 20 min access period, versus the low-drinking LARF line (Lê *et al.* 2001). However, these animals have been rarely studied (Shram *et al.* 2004; Turek *et al.* 2005). To overcome some limitations of the older selected lines, we have developed a new model for binge drinking. C57BL/6J mice had been shown to self-administer substantial ethanol in a limited access paradigm during the circadian dark (Sharpe *et al.* 2005). Mice drink and eat mostly during their circadian dark period. In our paradigm, C7BL/6J mice drinking in the dark (DID) readily self-administered approximately 7–8 g ethanol kg⁻¹ body weight in 4 hours and became intoxicated (Rhodes *et al.* 2005). We later showed that inbred strains differed in DID and the response was both reliable and heritable (Rhodes *et al.* 2007). We are currently selectively breeding mice for this trait: after nine selected generations, average BAL has doubled (because average intake has increased 150%) and the high DID (HDID-1) mice also become intoxicated (Crabbe *et al.* submitted). These mice should be useful for mechanistic studies of the biological and genetic

contributions to excessive drinking. They have not been tested for any other traits. However, the DID phenotype is proving to be a useful way to explore mechanisms underlying binge-like drinking. DID was shown to be reduced by intraperitoneal naltrexone doses that did not affect water or sugar–water intake (Kamdar *et al.* 2007). Another group has shown that the GABA_B agonist baclofen reduced ethanol DID without affecting water drinking, while two GABA_A agonists, THIP and muscimol, reduced drinking non-specifically (Moore *et al.* 2007). A CRF₁ receptor antagonist also reduced ethanol DID without affecting sucrose drinking (Sparta *et al.* 2008). Finally, infusion of urocortin 1 into lateral septum selectively reduced ethanol DID, while CRF reduced intake of both ethanol and water (Ryabinin *et al.* 2008).

Many other lines have been selectively bred for differential responses to alcohol. These include the long-sleep (LS) and short-sleep (SS) mice, which were selected for differences in the duration of the loss of the righting reflex after high-dose ethanol. These mice have been the subject hundreds of papers, and have been used effectively to map QTL underlying this response (Bennett *et al.* 2006). A large panel of RI strains has been developed from an LS×SS cross, and these RI strains have also been used to map QTL for ethanol-induced locomotor activation and acute functional tolerance development (Downing *et al.* 2006; Bennett *et al.* 2007). The locomotor activating response to ethanol is taken as an analogue of ethanol's euphoric effects in humans. Mice selected for high ethanol-induced activity (FAST) have been compared with low responders (SLOW) in many studies. These findings have been reviewed elsewhere (Palmer & Phillips 2002; Cunningham & Phillips 2003). Selection for differences in the severity of withdrawal after chronic ethanol treatment has been the subject of multiple selective breeding efforts. The Withdrawal Seizure-Prone and -Resistant selected lines remain under active investigation in several laboratories. Early work with these lines has been comprehensively reviewed (Metten & Crabbe 1996) and much of the recent work has focused on the role of neurosteroid systems in withdrawal (Finn *et al.* 2004; Beckley *et al.* 2008).

The use of selective breeding has seen a good deal of use in recent years since Belknap noted its efficacy in the aid of gene mapping efforts (Belknap *et al.* 1997). Especially when starting with a simple cross of two inbred strains, where there are only two possible alleles at each gene, intense directional selection based on phenotype usually produces very rapid changes in both the phenotype and the underlying important gene frequencies. The parallel changes in phenotype and genotype have provided strong support for several QTL analyses (Buck *et al.* 1997). More recently, interest has turned towards using genome-wide expression analyses to identify genes of interest. Here, too, short-term selective breeding has rapidly uncovered genes important for the phenotype (Mulligan *et al.* 2006). An interesting use of selected lines has recently employed the oldest selected rat line, high-drinking UChB rats. Individuals from East Asian gene pools possessing alleles coding for relatively inactive ALDH 2*2 activity experience aversive side effects when they drink alcohol

because they generate high levels of blood acetaldehyde. These subjects are highly protected from developing alcohol dependence. The Chilean group developed an anti-*Aldh2* antisense gene, injected it into UChB rats and saw pronounced suppression of ethanol intake (Ocaranza *et al.* 2008).

4. CANDIDATE GENES

The first study reporting the effects of a targeted gene deletion found that serotonin 1B receptor null mutants drank more ethanol, were less sedated by an acute dose of ethanol and developed tolerance to a lesser extent than wild-types (Crabbe *et al.* 1996). This finding has proven to depend upon genotype at other loci, an example of epistasis or gene–gene interaction (Phillips & Belknap 2002). A review 10 years hence found that 141 published reports assessed 93 targeted genes (Crabbe *et al.* 2006). Seventy-six mutants were tested for two-bottle preference. One quarter drank more than wild-types, one-third less and 40% about the same. The genes showing a major effect were not clustered in any pattern that offered clues to the underlying neurobiology.

Since the initial review, 34 additional papers have appeared, adding information about alcohol responses for several previously targeted genes, but also introducing 14 new genes to the mix. Two studies used ENU mutagenesis to generate novel mutants, some of which were found to differ in alcohol responses (Pawlak *et al.* 2005; Hamre *et al.* 2007). In one study, cerebellar tissue from ethanol-treated mice with or without a protein kinase C (PKC) gamma null mutation was compared in a gene expression profiling analysis. Several genes of potential relevance to the failure of PKC gamma mutants to develop ethanol tolerance were identified (Bowers *et al.* 2006). With no disrespect intended to the authors of the new studies, the increase in knowledge since 2006 has not revealed any new striking findings (not reviewed here). Gene targeting will remain a very useful tool for confirming the importance of a gene for which there are parallel lines of evidence for its importance. However, it is unlikely to be a sufficient tool for proving gene involvement on an alcohol response.

A clear example of how a strong case can be built for the involvement of a specific gene product in alcohol responses is offered by studies of mice engineered to lack the PKC epsilon isoform. Several previous studies had consistently established that PKC epsilon null mutants drank less ethanol than wild-types, and this effect was postulated to reflect the insensitivity of these mutants to ethanol's enhancement of GABA_A receptor function in neurochemical and electrophysiological assays (Crabbe *et al.* 2006). The null mutants were also shown to self-administer less ethanol in an oral operant paradigm (Olive *et al.* 2000). Mice had been shown to develop either a conditioned place preference (CPP) or aversion (CPA) for a location paired with an ethanol injection, depending on whether the injection is before or after each daily training session, respectively (Cunningham *et al.* 1998). In dose–response studies, PKC epsilon mutants were found to be more sensitive to ethanol-induced CPA and less sensitive to CPP than

wild-types (Newton & Messing 2007). In studies attempting to regulate ethanol preference drinking through administration of diazepam, zolpidem or L-655,708, PKC epsilon mutants, unlike wild-type controls, were insensitive to all three GABA_A receptor modulators. The authors concluded that PKC epsilon is necessary for ethanol's modulatory effects on GABA_A receptors (Besheer *et al.* 2006). Because no drug was available that selectively inhibited PKC epsilon activity over other PKC isoforms, the authors used a chemical strategy to generate an ATP analogue-sensitive PKC epsilon mutant to selectively inhibit catalytic activity of PKC epsilon (Qi *et al.* 2007). Using this mutant, they showed that PKC epsilon affects GABA_A receptors by phosphorylating the $\gamma 2$ subunit, thereby affecting receptors with the $\alpha 1\beta 2\gamma 2$ subunit composition, an abundant variant in brain. This may explain the low ethanol withdrawal severity of PKC epsilon mutants (Olive *et al.* 2001) and the increased withdrawal in $\gamma 2$ hemizygotes (Hood *et al.* 2006).

5. GENE EXPRESSION ANALYSES

The area of genetic analysis changing most rapidly in alcohol studies is exploration of the role of gene expression. Studies of gene expression changes, particularly following chronic ethanol administration, have been pursued for many years. The limiting nature of gene-by-gene expression assessment technology (e.g. Northern analysis of mRNA, RNase protection analyses) kept progress to a slow pace. However, many useful findings were carefully documented (for a review, see Reilly *et al.* 2001). Early studies used banked tissue from human brain to compare alcoholics and controls (Lewohl *et al.* 2000) or cultured cells to which alcohol was applied (Thibault *et al.* 2000). With the advent of gene chip technologies, it became possible to survey nearly the entire genome for expression differences, and some similarities emerged between microarray analyses and earlier studies (Lewohl *et al.* 2000; Liu *et al.* 2006). Microarrays also facilitated the spread of global expression analyses to their use in genetically defined rodent lines, such as the LS and SS mice (Xu *et al.* 2001) and the PKC gamma mutants just discussed (Bowers *et al.* 2006).

Gene expression profiling analyses typically discover numerous genes differentially expressed between control and treatment tissues, or between genotypes with high and low sensitivity to alcohol. Increasingly, the resulting lists of genes are being replaced with more sophisticated bioinformatics analyses of the output that seek gene clusters, gene families and/or similarity across multiple studies. For example, data banks containing the whole brain gene expression outputs from three pairs of lines selectively bred for high- or low-preference drinking were explored in conjunction with data from six inbred or isogenic strains of mice. A meta-analysis found nearly 4000 genes consistently differentially expressed in high versus low drinkers (Mulligan *et al.* 2006). Functional groups were proposed based on analysis of the proteins coded by those genes (the transcriptome). Using congenic strains, which differed from the background strain only

in one small piece of DNA from chromosome 9 that contained a QTL for preference drinking first reported in an analysis of B×D RI strains (Phillips *et al.* 1994), more microarray analyses were performed. Comparison of the datasets reduced the number of candidate genes within the QTL to 16 plus 4 expressed sequence tags (ESTs). These candidates included *Scn4b*, encoding a sodium channel.

Several other expression-based analyses have recently appeared. AA and ANA rats show many differentially expressed genes whose patterns of function have been discussed (Sommer *et al.* 2006). Candidate genes for preference drinking were explored in NP rats with a congenic interval containing a QTL for high preference from the P line. Several genes in several brain regions were differentially expressed (Carr *et al.* 2007). QTL effects can also be based on differences in gene expression rather than sequence. Several studies are actively pursuing these loci, deemed 'expression QTL' or 'eQTL'. eQTL are often likely to be caused by transcription factors in the interval, and those transcription factors can either act on an adjacent or nearby gene (deemed 'cis-acting') or on genes on other chromosomes (deemed 'trans-acting'). Most such studies are integrating the results from sequence-based and eQTL results to obtain a more complete picture of genetic influences (Hitzemann *et al.* 2004; Chesler *et al.* 2005; Letwin *et al.* 2006; Peirce *et al.* 2006; Tabakoff *et al.* 2008). One recent study has combined results from studies of alcohol and other drugs of abuse (Li *et al.* 2008).

6. EPIGENETIC CHANGES

Epigenetic modification of chromatin has been proposed to translate environmental stimuli into persistent 'cellular memories'. The acetylation state can be pharmacologically modulated by histone deacetylases (HDACs) and histone acetyltransferases that catalyse reversible histone acetylation. There are several studies reporting chromatin remodelling and regulation of transcription after chronic ethanol through dynamic histone acetylation. One study has shown changes in histone deacetylation or DNA methylation in chronic alcoholics (Bonsch *et al.* 2004a). Chromatin remodelling was also shown in rat brain after chronic alcohol (Mahadev & Vemuri 1998; Kim & Shukla 2006). A recent study in *Drosophila* showed that a single anaesthetic dose of benzyl alcohol could induce tolerance and showed a pattern of histone H4 acetylation across the promoter region of a gene (*slo*) of known importance for the phenotype. This gene encodes the pore-forming region of BK potassium channels and an HDAC inhibitor produced parallel effects on histone acetylation, *slo* expression, and produced a tolerance-mimicking phenotype, probably through a CREB transcription site (Wang *et al.* 2007).

Recent work from Nestler's group has shown that chronic cocaine administration leads to increases in histone modifications at genes known to be crucial for cocaine's chronic effects. The *Bdnf* and *Cdk5* genes showed H3 hyperacetylation in their promoter regions after chronic, but not acute, cocaine. Furthermore,

HDAC inhibitor treatment affected chronic cocaine responses (Kumar *et al.* 2005). Other work has shown that HDAC5 epigenetically controls behavioural adaptations to chronic emotional stimuli and histone acetylation plays a role in the behavioural response to cocaine (Renthal *et al.* 2007).

7. CONCLUSIONS

The pace of accumulation of information regarding genetic modulation of responses to and oriented towards alcohol continues to accelerate. I have reviewed progress derived from several methods for linking specific genes to alcohol-related behaviours. I conclude with selected examples of directions where, I think, promising leads are being developed. In each case, the developing story has used multiple methods to further the theorized link between gene and behaviour.

One area of promise is the link between endogenous opioids and alcohol dependence. Substantial data from animal models implicate common genetic contributors to endogenous opioid function and alcohol self-administration or dependence (Town *et al.* 2000). Numerous gene targeting studies suggest a specific role for the mu opioid receptor in ethanol preference, as its deletion reduces intake in many studies (Crabbe *et al.* 2006). However, there does not appear to be a straightforward homology with the human receptor. A meta-analysis of many studies seeking to test an association between the specific Asn40Asp polymorphism in the mu opioid receptor, OPRM1, and substance dependence failed to find a significant elevation in relative risk (Arias *et al.* 2006), even in the several studies of alcohol-dependent subjects. Nonetheless, naltrexone remains an effective therapy for some alcoholics (Heilig & Egli 2006), and a recent study reported an association between response to naltrexone therapy and the Asn40Asp polymorphism in a population of alcoholics (Anton *et al.* 2008). It should be noted that a smaller study of patients in several Veterans Affairs hospitals saw no similar association (Gelernter *et al.* 2007).

Animal studies have provided a convincing link between the stress axis and ethanol drinking. Mice lacking functional CRF-1 receptors show normal ethanol preference, but the increase in preference following stress may be attenuated (Sillaber *et al.* 2002). A similar effect is seen when a CRF-1 receptor antagonist is administered to normal mice (Lowery *et al.* 2008), and, as noted above, an antagonist also attenuates ethanol DID (Sparta *et al.* 2008). Also as noted above, intra-septal CRF and urocortin 1 both reduce DID (Ryabinin *et al.* 2008). Ethanol intake can also be potentiated following induction of physical dependence (Lopez & Becker 2005), and these increases are also blocked by CRF₁ antagonists (Chu *et al.* 2007; Finn *et al.* 2007; Richardson *et al.* 2008) or are absent in the knockout (Chu *et al.* 2007). A recent study has suggested that a polymorphism in the human CRHR1 gene is associated with heavy drinking in a sample of German 15-year olds in some measures, only if associated with more stressful life events

(Blomeyer *et al.* 2008). It should be noted that two previous studies with adult samples have failed to find significant associations (Dahl *et al.* 2005; Treutlein *et al.* 2006).

Finally, I mention the interesting story of alpha synuclein. QTL mapping studies in crosses between P and NP rats identified a region of rat chromosome 4, among others (Bice *et al.* 1998; Carr *et al.* 1998). A subsequent gene expression profiling analysis compared tissue from multiple brain areas from inbred P and NP rats, and found many genes and ESTs to be differentially expressed (Liang *et al.* 2003). Although alpha synuclein had not been mapped in the rat, it was selected as a candidate gene based on its location in the homologous portion of mouse chromosome 6, and it was shown to be expressed more highly in P hippocampus than in NP. Levels of mRNA in the blood of alcohol self-administering cynomolgus monkeys were higher than in controls (Walker & Grant 2006). Subsequent studies reported increased alpha synuclein mRNA (Bonsch *et al.* 2004b) and protein (Bonsch *et al.* 2005a) levels in the blood of alcoholics versus that of controls. This group also reported increased levels of homocysteine in plasma of alcoholics, and further that these patients showed a correlated increase in levels of DNA methylation (Bonsch *et al.* 2004a). Increased plasma homocysteine levels were also correlated with cognitive deficits in early withdrawal (Wilhelm *et al.* 2006). The DNA hypermethylation is pronounced in the area of the alpha synuclein, but not the presenilin 1, promoter (Bonsch *et al.* 2005b). Thus, the alpha synuclein story may depend upon epigenetic modifications of the expression of other genes as yet unidentified.

This story, like all others, has its qualifications. A study of 219 multiplex families reported both a SNP and a haplotype association of alpha synuclein polymorphisms with alcohol craving, but not with alcohol dependence (Foroud *et al.* 2007). However, a subsequent study of two American Indian populations found scattered, weak associations with addiction-related variables, but none survived correction for multiple comparisons (Clarimon *et al.* 2007). In addition it must be noted that the original difference between P and NP rats was not seen in comparisons of the two pairs of HAD and LAD lines selected for the same phenotype. Nor has any association with the region of mouse chromosome 6 that harbours the gene ever emerged from the many QTL studies in the literature.

In conclusion, we continue to gain ground in our understanding of the effects of specific genes on alcoholism risk. The goal of genomically personalized therapies remains beyond our reach at the moment. The most hopeful sign is the increasing cooperative assembly and evaluation of large phenotypic and genetic datasets. It seems likely that a disease as genetically complex as alcohol dependence will require such a large-scale effort to solve its pathophysiology and aetiology. I have discussed elsewhere the parallel need for thinking carefully about the many behavioural assays employed with rodent animal model work (Crabbe & Morris 2004; Wahlsten & Crabbe 2007). We certainly are in need of continuing development of more relevant phenotypes than those currently available. In particular, the lack of

parallelism between phenotypes studied in human genetic and animal model research is distressing, and there is room for much work on this front.

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