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REVIEW PAPER

Translational genetic approaches to substance use disorders: bridging the gap between mice and humans

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Abstract While substance abuse disorders only occur in humans, mice and other model organisms can make valuable contributions to genetic studies of these disorders. In this review, we consider a few specific examples of how model organisms have been used in conjunction with studies in humans to study the role of genetic factors in substance use disorders. In some examples genes that were first discovered in mice were subsequently studied in humans. In other examples genes or specific polymorphisms in genes were first studied in humans and then modeled in mice. Using anatomically and temporally specific genetic, pharmacological and other environmental manipulations in conjunction with histological analyses, mechanistic insights that would be difficult to obtain in humans have been obtained in mice. We hope these examples illustrate how novel biological insights about the effect of genes on substance use disorders can be obtained when mouse and human genetic studies are successfully integrated.

Introduction

In this paper, we explore how genetics studies in mice can be integrated with genetic studies in humans to improve our understanding of the genetics of substance abuse. The field of human genetics has recently focused much of its attention on genome-wide association studies (GWAS), which have provided a powerful tool for identifying genes that influence traits, including several related to substance abuse. While GWAS is not without problems and limitations (Manolio et al. 2009), not the least of which is the requirement for very large sample sizes, they have allowed for great progress without input from animal models. On the horizon are the studies that use next-generation sequencing technologies to identify rare but highly penetrant alleles. In the face of this robust progress, some have questioned whether animal models continue to be relevant for understanding the genetics of substance use disorders.

Studies with model organisms allow experimental manipulations of genotype and environmental factors that are essential to assess causality (Aitman et al. 2011). Thus, genetic associations observed in humans or other animals can be experimentally tested using model organisms. The mouse has been the organism of choice for many such studies, but other organisms have made important contributions as well. Significant advances in the technology of mouse genetics permit several effective approaches, including generation of transgenic animals, homologous recombination to change existing genes, and more recently an international effort to generate null alleles for every gene in the mouse genome (Skarnes et al. 2011). Mouse studies are critical to understand why alleles of particular genes identified in human association studies might alter phenotypes in humans. Studies with mice also allow investigators to test environmental manipulations that would be unethical in humans, such as surgical procedures or administration of unapproved/high doses of drugs. Finally, mouse studies allow control over potential cofounding environmental factors (age, diet, drug exposure, etc.) which can reduce sources of environmental noise.

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While most of the above discussion is very general and applies equally well to all traits, this review will examine substance use disorders, which are especially challenging because of their complexity and heterogeneity. Unlike blood pressure or obesity, which can be readily studied in both mice and humans, substance use disorders are uniquely human and cannot be directly modeled in non-humans. Substance abuse develops slowly over years or decades whereas phenotypes measured in mice typically are performed in days or weeks. While the difference in life span of humans and mice confounds any direct temporal comparisons, in general animal models are intended to study specific aspects of substance use disorders (e.g., locomotor response to a stimulant drug) rather than the full disorder. Several excellent reviews have recently addressed the subject of animal models of substance use disorders (Crabbe et al. 2011; Stephens et al. 2011). In this paper, we will explore examples of genetic studies that have integrated findings from both mice and humans that enrich the understanding beyond what could be attained by studies with either species alone. We have not attempted to be exhaustive, but have instead chosen examples that illustrate different approaches.

Substance abuse is a multi-factorial illness that results from both genetic and environmental risk factors. The genetic risk for substance abuse is likely determined by different neurobiological and genetic vulnerabilities that may be specific to different stages of the disease. Clinical diagnoses such as those in the DSM IV have been defined by the medical community for purposes of diagnosis and treatment. These diagnoses were not designed to facilitate genetic studies and may not correspond to discrete biological phenomena. In this article, we divide substance abuse into six stages (Table 1) which may be influenced by separate, but overlapping genetic factors. Thus, we hypothesize that alleles that are associated with a diagnoses of substance abuse are mediated via their effects on one or more of these stages. This is especially important for translating to animal models because there are behavioral paradigms that attempt to model each of these stages. Therefore, animal models might be used to understand at what stage a particular gene or allele influences the progression toward substance abuse.

Table 1 Six stages that may constitute independent genetic constructs

Stage	Description
1. Likelihood of initial exposure	Personality or psychiatric factors affecting likelihood of experimenting with drugs
2. Initial responses to drugs	Quality and magnitude of the individuals' first response to a drug influences subsequent use
3. Plastic changes in drug response	Rate and degree of tolerance and sensitization; learning and conditioning
4. Progression and escalation	Likelihood of continuing to use drugs despite mounting negative consequences (e.g., insensitivity to punishment)
5. Withdrawal	Severity of withdrawal following chronic use
6. Relapse	Difficulty in abstaining after cessation of use; susceptibility to factors that precipitate relapse (e.g., cues or stress)

Intermediate phenotypes are traits that are presumed to be genetically determined and that are somehow related to one or more disease (Goldman and Ducci 2007). Endophenotypes (Gottesman and Gould 2003; Gould and Gottesman 2006) are a subset of intermediate phenotypes, for simplicity we will not use the term endophenotype in this review. Like animal models, some intermediate phenotypes specifically target one or more of the stages of substance abuse outlined in Table 1. Intermediate phenotypes may involve laboratory-based manipulations that can be performed in a similar manner in both mice and humans. These properties make intermediate phenotypes more amenable to integration with animal models, and thus of special importance for this review. In this manuscript, we will approach the topic of translational genetics from two perspectives. First we will examine studies in which a particular gene was initially identified in an animal model and was then examined in humans to see if polymorphisms in that same gene were related to analogous phenotypes. Second, we will explore the opposite scenario, in which genes that have been implicated by either candidate gene or GWAS in humans have been further explored using model organisms.

From mouse to human

In this section, we discuss three examples of research studies that have identified a gene in an animal model and then studied the same gene in humans. The same polymorphisms are not expected to exist in both mice and humans. Instead, the unit of translation is the gene; about 99% of genes have reasonably clear human homologs. The assumptions of this approach include: (1) that genes have similar functions with respect to complex traits in mice and humans, (2) that these effects are reasonably robust to genetic context (strain background in mice, and genetic diversity in humans), (3) environmental factors will not confound mouse and human studies and (4) only a small fraction of the genes in the genome have the ability to modulate the sorts of traits that we are interested in studying. The final assumption is important because this discovery of genes using mouse studies will be most valuable if the total number of genes that influence a given trait is small, so that identifying such genes is significantly better than arbitrarily selecting a gene. If these assumptions

are substantially incorrect then this approach will not be very productive.

Multiple PDZ domain protein (*Mpdz*)

Mpdz was identified as a modulator of ethanol and barbiturate withdrawal seizures using classical genetic techniques. A quantitative trait locus (QTL) for withdrawal seizures was initially identified using recombinant inbred lines (Buck et al. 1997, 1999), and was subsequently fine mapped using successively smaller congenic strains to a 1.8-Mb interval that contained just 16 genes. *Mpdz* was identified as the causal gene because it was expressed in brain, contained coding SNPs and was differentially expressed in the relevant two inbred strains. Based on these findings, *Mpdz* was identified as the most likely gene to cause the QTL (Fehr et al. 2002; Shirley et al. 2004). Withdrawal seizures are relevant to substance abuse because the propensity to seizures and other negative symptoms may stem from a common mechanism corresponding to Stage 5 in Table 1. In humans, two candidate gene association studies have subsequently found modest evidence that SNPs in *MPDZ* were associated with risk for alcoholism but interestingly, not alcohol withdrawal seizures (Karpyak et al. 2009, 2011). The failure to demonstrate an association with withdrawal seizures in particular could reflect the imprecise nature of translation between species or could simply reflect poor power to detect such an association in humans, where many environmental and genetic factors also influence this trait. It is also possible that these relatively small studies showing an association between alcoholism and SNPs in *MPDZ* represent false positive findings (Munafo and Flint 2011).

Casein kinase 1 epsilon (*Csnk1e*)

Csnk1e was identified because it was differentially expressed in mice divergently selected for high and low locomotor response to methamphetamine. Moreover, there was a robust expression QTL that co-mapped to a QTL for the locomotor response to methamphetamine (Palmer et al. 2005). Finally, *Csnk1e* modulates the activity of dopamine and adenosine 3′:5′-monophosphate-regulated phosphoprotein of 32 kDa (Darpp-32) which is a critical second messenger pathway that mediates the response to various drugs of abuse (Walaas et al. 2011). The locomotor response to a drug is sometimes equated with the subjectively rewarding effects of a drug in humans (Wise and Bozarth 1987) although this is controversial (Phillips et al. 2008). Inspired by this idea, we made an effort to translate this finding to humans. We examined the association between polymorphisms in *CSNK1E* and the subjectively rewarding effects of stimulant drugs in human volunteers ($N = 96$) who had received placebo or D-amphetamine (10, 20 mg) in a double-blind, within-subjects laboratory-based study. A SNP in *CSNK1E* (rs135745) was associated with the subjectively euphoric effects of amphetamine (Veenstra-VanderWeele et al. 2006), providing some support for the proposed relationship between the locomotor response to stimulant drugs and the subjectively pleasurable effects of drugs in humans and providing a promising link between the animal and human genetic models. This study focuses on Stage 2 in Table 1 and is based on the idea that acute locomotor response in mice is related to subjectively positive drug effects in humans.

Subsequently, another group identified an association between different SNPs in *CSNK1E* (rs1534891-rs6001093-rs135757) and heroin addiction (Levran et al. 2008). Although the SNPs identified in these two studies are in the same gene, they are not in strong linkage disequilibrium (LD) with each other and presumably must represent distinct functional loci. Nevertheless, taken together these data suggest that this gene is important for both the acute responses to drugs of abuse and substance use disorders. Based on the human data suggesting that *CSNK1E* might also influence risk for addiction to opiate drugs, we subsequently examined mice with different alleles of *Csnk1e* and identified a difference in acute sensitivity to the locomotor stimulant effects of the mu-opioid agonist fentanyl (Bryant et al. 2011).

To our knowledge, none of these results have been replicated in any independent samples; however, an association between bipolar disorder and a collection of three unlinked SNPs including rs1534891 has been reported (Shi et al. 2008), providing modest evidence that rs1534891 could have some biologically meaningful effect.

Anaplastic lymphoma kinase (*Alk*)

Alk is regulated by the gene LIM-domain only (*Lmo*), which is implicated in sensitivity to the sedative effects of ethanol in flies (Lasek et al. 2011a). Based on the observation that *Alk* was down-regulated in *Lmo* mutants, *Alk* was examined to determine whether it also influenced the sedative effect of ethanol. Indeed, flies with mutant alleles of *Alk* show diminished behavioral sedation in response to ethanol (Lasek et al. 2011b). Next, this team investigated the mouse homolog of *Alk*, and observed correlations between gene expression and several ethanol-induced behaviors in a recombinant inbred panel of mice. To determine whether these correlations were causative, they generated an *Alk* knock out mouse, which showed diminished sensitivity to the sedative effects of ethanol in the loss of righting reflect task as well as higher voluntary ethanol drinking in the drinking-in-the-dark paradigm (Lasek et al. 2011b). Finally, the same authors studied several SNPs in the human *ALK*

gene and showed a nominally significant association between these SNPs and altered physiological and subjective responses to ethanol in humans (Lasek et al. 2011b). Such responses have been proposed as intermediate phenotypes for alcohol use disorders and are likely important in the early stages of the addiction process (Stage 2 in Table 1). This example illustrates how the same gene appears to have an evolutionarily conserved role in mediating analogous phenotypes across three highly divergent species. The stepwise progress of this study illustrates how model organisms in conjunction with gene-pathway data can be used to identify novel genes.

Summary

These three examples illustrate how studies of specific responses to drugs in model organisms can be used to identify genes that can then be studied in humans. Because of the limited number of genome-wide significant results from GWAS for substance abuse, it is not surprising that the confirmation in humans has come from small, candidate gene-based studies which implicitly assume that these genes have relatively large effects on their corresponding human phenotypes. As mentioned before, if there were only a small number of genes that could influence these traits in humans, then searching for candidates in model organisms might identify a highly enriched set of genes that could be examined in humans. If, on the other hand, a large fraction of genes (say 10%) have the potential to influence the relevant human phenotypes, then enrichment using animal models will only be modestly helpful relative to randomly selecting genes to study in humans, or simply surveying all genes without prior hypotheses, as is done in human GWAS. If the latter situation is correct, then animal models will be most useful for the study genes that have already been shown to influence clinical traits like substance abuse or intermediate phenotypes in humans.

From human to mouse

In this section, we will consider the opposite direction of translation, namely genes that are initially identified in humans, either using a candidate gene approach or as a result of GWAS, and are then examined in mice. Although human genetics studies can be very effective in gene identification, they offer little information regarding the mechanisms by which the genes affect behavior. If the only goal of genetic studies of substance abuse was to identify markers that predict risk for substance abuse, there would be little reason to model human susceptibility alleles in mice. However, another goal of these studies is to identify the underlying biological substrates for substance use disorders. An improved understanding of the biological mechanisms is needed to design effective treatments for substance abuse. Thus, a major benefit of genetic studies in humans is the ability to perform mechanistic studies; to realize this goal, mice and other models organisms are necessary.

Opioid receptor, mu 1 (*OPRM1*)

The *OPRM1* gene contains a missense mutation in exon 1 that is one of the most heavily studied polymorphisms in the genetics of substance abuse; this polymorphism causes a A-G substitution at position 118 (rs1799971 a.k.a. A118G, N40D, and Asn40Asp) (Mague and Blendy 2010; Yuferov et al. 2010). Although several in vitro studies have suggested that this polymorphism has functional consequences (Kroslak et al. 2007), the direction of the effect has been disputed. Early studies suggested that the minor (G) allele was associated with increased agonist affinity (gain-of-function), but more recent studies have suggested lower mRNA and protein expression (Beyer et al. 2004; Zhang et al. 2005), and lower receptor binding potential (Ray et al. 2011), suggesting a loss-of-function in G allele carriers. Associations have been published with many traits, including substance abuse and pain sensitivity, however, they remain controversial (Mague and Blendy. 2010; Yuferov et al. 2010; Walter and Lotsch 2009; Arias et al. 2006; Ray et al. 2011). To examine the effects of this polymorphism on behavior while maintaining an isogenic environmental and genetic background, a mutation was made at the equivalent position in the mouse *Oprm1* gene (Mague et al. 2009). These G/G mice showed reduced *Oprm1* mRNA and protein levels (consistent with the loss-of-function hypothesis); reduced locomotor sensitivity in response to morphine and reduced anti-nociception on the hot plate test in response to morphine; these differences were consistent with some previous observations in humans. Taking a slightly different approach, a mouse with the SNP containing exon knocked in was also generated (Ramchandani et al. 2011). This study focused on the modulation of dopamine release following ethanol administration in both humans and mice. Using positron emission tomography and [11C]-raclopride displacement in humans and micro-dialysis in mice, they showed that the G allele was associated with greater dopamine release in response to ethanol in both species. Interestingly, Ramchandani et al. (2011) did not observe a difference in receptor binding between the two lines. Taken together, these findings suggest that this SNP is especially likely to influence the initial response to multiple different drugs of abuse, thus altering the early stages of the addiction process (Stage 2 in Table 1). Mouse models have been a valuable tool for establishing the importance of this SNP

both in terms of functional/anatomical intermediates and in terms of the behavioral endpoints.

This study exemplifies the ability of animal models to establish causality by recreating a specific mutation observed in humans and establishing that it is sufficient to alter gene expression and behavior. While the mechanism by which this SNP impacts risk for substance abuse is not known, the modulation of morphine-induced locomotor activation and ethanol-induced dopamine release suggests that it may modulate sensitivity to the reinforcing effects of drugs. The focus by Ramchandani et al. (2011) on measures of dopamine release in both mice and humans circumvents the need to identify equivalent *behavioral* measures in both mice and humans.

Nicotinic receptor cluster alpha-3, alpha-5, beta-4 (*CHRNA3-CHRNA5-CHRNB4*)

A candidate-gene association study that examined SNPs in 348 genes identified a cluster of nicotinic receptor subunits (*CHRNA3-CHRNA5-CHRNB4*) on chromosome 15 as being involved in nicotine dependence (Saccone et al. 2007). Since then, this region has become the most significant and best replicated of any GWAS of substance use disorders. In particular, the number of cigarettes smoked per day shows an especially strong signal at this locus (Thorgeirsson et al. 2008, 2010; Amos et al. 2008a; Liu et al. 2010; Tobacco and Genetics Consortium. 2010), and nicotine dependence is also associated with this locus (Thorgeirsson et al. 2008; Saccone et al. 2007). Finally, this locus is also associated with increased risk for lung cancer and peripheral arterial disease (Thorgeirsson et al. 2008; Amos et al. 2008b; Hung et al. 2008; Truong et al. 2010). Because *CHRNA3*, *CHRNA5* and *CHRNB4* are members of a single haplotype block, it has been challenging to determine which SNP(s) in which gene(s) cause the association and whether the association is due to coding differences (e.g., rs16969968 which causes D398N substitution in *CHRNA5*) or expression differences, which might be due to either coding or non-coding SNPs. Indeed, statistical analyses suggest that there is likely to be more than one functionally significant allele at this locus (Saccone et al. 2010). Animal models have been used to address this issue. Fowler et al. (2011) observed a large increase in nicotine self-administration in mice that were homozygous for null alleles of the *Chrna5* gene. Importantly, they were able to reverse this effect by replacing *Chrna5* expression in the habenula, and to replicate the effect of the null allele by knocking-down *Chrna5* in the habenula, suggesting that *Chrna5* expression in this region was necessary and sufficient for normal nicotine self-administration behavior. Similarly, Frahm et al. (2011) showed that overexpression of *Chrnb4* decreased nicotine aversion and that this effect was reversed by viral-mediated expression of the *Chrna5* D398N variant in the habenula. Thus, mouse models have helped to elucidate possible roles for two of the genes in the haplotype. Both of these studies examined nicotine self-administration, which is thought to be comparable to self-administration as measured by cigarettes smoked per day in humans. In addition, using a model system, it was possible to manipulate *Chrna5* and *Chrnb4* expression in the habenula, which provided mechanistic insights about the importance of this brain region, which would have been difficult to obtain using human subjects.

These studies focus on alleles associated with both risk for dependence as well as the number of cigarettes smoked per day, which are components of Stages 3 and 4 as outlined in Table 1. However, the human polymorphisms could also influence other stages as well (e.g., withdrawal and relapse). In contrast to the studies of *OPRM1* discussed above, which follow up on polymorphism implicated by candidate gene studies, the *CHRNA3-CHRNA5-CHRNB4* gene cluster was discovered by GWAS and is extremely statistically robust. Another contrast with the *OPRM1* studies is that neither of these mouse models of nicotinic subunits introduced a particular human polymorphism into the mice. Thus, the data speak more to the importance of these nicotinic subunits and of the habenula rather than specific confirmation of the causal role of any particular polymorphism's importance in humans.

Catechol-*O*-methyltransferase (*COMT*)

Among the most heavily studied polymorphisms in all of psychiatric genetics is the so-called warrior/worrier Val158Met amino acid substitution caused by rs4680, which was first described by Lachman et al. (1996). This SNP appears to alter the enzymatic activity of COMT such that Met carriers have lower activity. This polymorphism has been associated with numerous diseases and putative intermediate phenotypes, including pain sensitivity, anxiety and substance abuse (Tunbridge. 2010; Bousman et al. 2009). To clarify the importance of the coding SNP versus polymorphisms that might be in LD with rs4680, Papaleo et al. (2008) generated mice that were transgenic for the human Val allele of COMT. Presence of the Val allele altered the effects of amphetamine on working memory tasks in a manner that paralleled earlier observations in humans (Mattay et al. 2003).

As mentioned above, the *COMT* Val158Met polymorphism is associated with a broad array of traits, including anxiety, pain sensitivity, and psychiatric disease susceptibility in humans. It is interesting to note that an unrelated polymorphism in the corresponding mouse gene (*Comt1*), causes differences in *Comt1* gene expression and was associated with a number of behavioral differences in mice

by three independent groups (Palmer and Dulawa. 2010; Kember et al. 2010; Li et al. 2010; Segall et al. 2010).

Summary and future challenges

Human genetics is uniquely suited for associating disease states with specific genes and polymorphisms. Model organisms can identify genes that can alter disease-related phenotypes. Model organisms can also be used to test whether the polymorphisms found in humans are plausible causes of the human phenotypes and can provide mechanistic insights that would be hard to obtain using humans. For psychiatric traits such as substance abuse, it is difficult to relate the human and mouse phenotypes, for this reason it is important to understand the relationship between mouse behavioral paradigms and the various component processes that contribute to substance abuse. Intermediate phenotypes in humans such as those outlined in Table 1 can be helpful for connecting disease diagnoses to animal behavioral paradigms. In this review, we have focused on single genes as the unit of analysis and translation. This decision was partially dictated by the published literature that we are reviewing. It is easy to imagine that biological pathways could also be used such that interacting neighbors of an implicated gene might also be investigated in the complimentary species; as we discussed above, the identification of *Alk* in flies illustrates this concept (Lasek et al. 2011b). Interactions among genes that belong to a common pathway offer one possible explanation for the small effects of SNPs identified by GWAS relative to the large estimates of heritability (Manolio et al. 2009). This so-called 'missing heritability' or 'dark matter' has yet to be fully explained. One possibility is a significant portion of trait heritability results from epistatic interactions between genes. Recent studies have investigated this possibility and have identified evidence for interactions among genes. For example, Nikolova et al. (2011) computed a multi-locus genetic profile score that represented the cumulative impact of five functional polymorphic loci on DA signaling and used this profile to identify reward-related activation of the ventral striatum even though none of the loci had significant main effects. Similarly, Grucza et al. (2010) identified interactions among over 300 candidate genes as well as age of onset of smoking to identify epistatic interactions that have been missed due to prior emphasis of main effects. These sorts of studies might be an important next step for GWAS. It should be possible to explore epistatic relationships using animal models where four homozygous groups that are isogenic except at the putatively interacting alleles could be used to directly test these relationships and explore their molecular origins. Finally, as attention is increasingly focused on the study of rare alleles, animal models will be an invaluable tool to validate the importance of these genes for human psychiatric disorders (e.g., Bevilacqua et al. 2010), including substance abuse disorders, because they provide an easy means of generating large cohorts of mice with the desired genotypes.

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