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Implications of genome wide association studies for addiction: Are our *a priori* assumptions all wrong?

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Abstract

Substantial genetic contributions to addiction vulnerability are supported by data from twin studies, linkage studies, candidate gene association studies and, more recently, Genome Wide Association Studies (GWAS). Parallel to this work, animal studies have attempted to identify the genes that may contribute to responses to addictive drugs and addiction liability, initially focusing upon genes for the targets of the major drugs of abuse. These studies identified genes/proteins that affect responses to drugs of abuse; however, this does not necessarily mean that variation in these genes contributes to the genetic component of addiction liability. One of the major problems with initial linkage and candidate gene studies was an *a priori* focus on the genes thought to be involved in addiction based upon the known contributions of those proteins to drug actions, making the identification of novel genes unlikely. The GWAS approach is systematic and agnostic to such *a priori* assumptions. From the numerous GWAS now completed several conclusions may be drawn: (1) addiction is highly polygenic; each allelic variant contributing in a small, additive fashion to addiction vulnerability; (2) unexpected, compared to our *a priori* assumptions, classes of genes are most important in explaining addiction vulnerability; (3) although substantial genetic heterogeneity exists, there is substantial convergence of GWAS signals on particular genes. This review traces the history of this research; from initial transgenic mouse models based upon candidate gene and linkage studies, through the progression of GWAS for addiction and nicotine cessation, to the current human and transgenic mouse studies post-GWAS.

Keywords

Addiction; drug abuse; Genome-wide association study; linkage; nicotine cessation; transgenic; knockout; genetics

1. Introduction

Drug dependence/addiction is a complex disorder which is multi-factorial, involving both environmental and genetic influences. Studies support the view that much of the heritable influence in vulnerability to dependence on addictive substances from different

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pharmacological classes is shared (Karkowski, Prescott, & Kendler, 2000; Kendler, Karkowski, Neale, & Prescott, 2000; Tsuang, et al., 1998). These findings have increased interest in the particular genes that are associated with addiction relevant psychological and physiological traits that may predict risk for the development of drug abuse and addiction. The complex nature of addiction has led some investigators to question the value of genetic research on addictive disorders (Merikangas & Risch, 2003) or the value of GWAS (Agrawal & Lynskey, 2008). The last few years have seen identification of few large association findings for addiction. When viewed as a polygenic trait with substantial allelic and locus heterogeneity, however, there is substantial evidence for convergent GWAS results that suggest value from these experiments. These data have profound implications for the improved understanding and treatment of the complex disorder of addiction, and may have implications for how the genetic basis of other complex diseases might be approached overall.

2. Family based, adoption and twin studies

In considering the relative merit of attempts to identify genes conferring susceptibility to addictive diseases, a necessary first step is to evaluate the evidence concerning the extent to which substance use disorders may be influenced by heritable factors at all. Such evidence can be derived from a range of family-based genetically informative research designs including family, adoption and twin studies (Kendler, et al., 2000; True, et al., 1999; Tsuang, et al., 1998; G.R. Uhl, Elmer, Labuda, & Pickens, 1995). Early family-based studies provided initial clues to potential heritable influences on addictive disorders by examining the risk of substance use disorders in the first-degree relatives of individuals either with or without a substance use disorder. For example, in a study of a large sample of individuals meeting criteria for alcohol dependence and their siblings, Bierut and colleagues (Bierut, et al., 1998) reported that, relative to control individuals, the siblings of alcohol-dependent probands had elevated rates of alcohol dependence (50% for men and 25% for women). Similarly, in a study of adult first-degree relatives of probands with dependence on opioids, cocaine, cannabis and/or alcohol and control probands, Merikangas and colleagues (Merikangas, et al., 1998) reported an eightfold increased risk of drug disorders which was largely independent from the familial aggregation of both alcoholism and antisocial personality disorder. There was also evidence of specificity of familial aggregation of the predominant drug of abuse, suggesting that there may be risk factors that are specific to particular classes of drugs as well as risk factors that underlie substance disorders in general. Similarly, studies have reported both that alcoholism is familial (Kendler, Davis, & Kessler, 1997) and that having an alcoholic parent is associated with a fivefold increase in the risk of alcoholism (Midanik, 1983). Family studies conclude that both alcoholism and other substance use disorders cluster in families, presumably due to heritable factors. However, the family design cannot distinguish whether the causes of familial similarity are genetic or environmental in nature. Further, there appear to be familial influences that confer a non-specific risk for drug dependence.

Adoption studies, on the other hand, are based on a comparison of the concordance or correlation between offspring behavior (i.e. drug dependence) and the characteristics of both the biological and adoptive parents: similarity between offspring and biological parents is suggestive of genetic influences on that behavior, while similarity between offspring and adoptive parents is suggestive of environmental influences. Cadoret and colleagues (Cadoret, Troughton, Ogorman, & Heywood, 1986; Cadoret, Yates, Troughton, Woodworth, & Stewart, 1995, 1996) through their adoption studies suggested that genetic and environmental risk mechanisms operate similarly across genders. Their results also highlighted the etiological importance of some environmental influences— particularly parental divorce and parental psychiatric disorders in the adoptive families—in the

development of drug abuse. These studies were able to isolate the influence of environmental exposures from potential genetic confounds on risk for drug dependence, thus highlighting the utility of adoption and other family designs for elucidating components of environmental risk. However, adoption studies have certain limitations. Firstly, due to the challenges involved with accessing adoption records, these studies are not common. Secondly, biological parents, adoptive parents and adopted children are not, by any means, representative of the population: biological parents are likely to have higher rates of drug dependence while, conversely, adoptive parents are less likely to do so than the population at large. Furthermore, such designs do not negate negative prenatal influences, including prenatal drug exposure, which are more likely to be present in adoptive children than the general population.

The classical twin study design utilizes data from monozygotic (MZ) and dizygotic (DZ) twin pairs, reared together, to attempt to disentangle the role of genetic and environmental influences in population variation in a measured phenotype. Genetic variants are shared completely (100%) between members of MZ twin pairs while DZ twin pairs share, on average, 50% of their genetic variants. There are two sources of environmental variation—those latent environmental factors that members of a twin pair have in common (shared environment) and those environmental factors that are unique to individuals (non-shared environment). Shared environmental factors overlap 100% between members of MZ and DZ twin pairs, under the important assumption of equal environments. Non-shared environment is, by definition, uncorrelated between members of a twin pair. The caveats of a classical twin study include the equal environments assumption and the fact that epigenetic modifications (when not inherited) or sporadic structural change in DNA, which can occur in one member of an MZ pair but not the other, are not captured in a twin study. Reviews of the evidence from twin and adoption studies report that the heritability of alcohol use disorders averages 0.5 to 0.6 (Schuckit, 2009), with estimates ranging from 0.3 to 0.8 for other substances (Agrawal & Lynskey, 2006, 2008; Tsuang, Bar, Harley, & Lyons, 2001; Vink, Willemsen, & Boomsma, 2005).

Although the results of heritability estimates for drug addiction phenotypes are generally around 50%, there is some variability which may relate to differences associated with addiction to specific drugs or to other aspects of addiction criteria used in these studies. In any case, this can only be a starting point, as a demonstration that there are heritable factors that influence individual differences in vulnerability to addiction. The knowledge that a large proportion of abuse liability is genetic does not constitute an understanding of how these genetic factors induce their effects, which genes are involved or how many genes are involved. The first approach that was used in an attempt to identify specific genes that may contribute to addiction liability was the examination of candidate genes, nominated based upon biochemical and other evidence suggesting that those genes should be involved in responses to drugs of abuse or behavioral features of addiction.

3. Candidate Gene Studies of Addiction

The studies discussed above established that there is a substantial genetic basis underlying the predisposition to develop drug addiction, but could not establish the genetic architecture underlying those effects, or the specific genes involved. Other genetic approaches have been used in attempts to identify specific genes involved in addiction, including both linkage and association methods. Linkage involves comparison of genomic markers in related individuals, while association involves comparisons of unrelated individuals. Initial work of this type focussed on specific genes or specific genomic regions, as opposed to genome wide approaches discussed later in this review.

Candidate gene approaches have tended to focus upon specific sets of genes based upon *a priori* assumptions about the importance of particular genes in addiction, usually assessing a small number of genomic markers. These assumptions were based in part upon the mechanism of actions of particular drugs of abuse, e.g. dopamine systems (amphetamine, cocaine and other stimulants), opioid systems (heroin and other opiates), GABAergic systems (ethanol and benzodiazepines), etc. Thus, candidate gene studies examined association or linkage of dopaminergic system genes with addiction/dependence for cocaine and amphetamines (Comings, Gonzalez, et al., 1999; Gelernter, Kranzler, & Satel, 1999; Guindalini, et al., 2006; Hong, Cheng, Shu, Yang, & Tsai, 2003; H. C. Liu, et al., 2004; Noble, et al., 1993; Persico, Bird, Gabbay, & Uhl, 1996a; Sery, Vojtova, & Zvolisky, 2001; Tsai, et al., 2002), opioid system genes for opiate addiction/dependence (Bart, et al., 2004; Bond, et al., 1998; Comings, Blake, et al., 1999; Crowley, et al., 2003; Franke, et al., 1999; Li, et al., 2000; Mayer, et al., 1997; Shi, et al., 2002; Szeto, Tang, Lee, & Stadlin, 2001; Tan, Tan, Karupathivan, & Yap, 2003; K. Xu, Liu, Nagarajan, Gu, & Goldman, 2002; Yuferov, et al., 2004; Zimprich, et al., 2000), GABAergic system genes for alcohol dependence (Noble, et al., 1998), and nicotinic cholinergic system genes for nicotine dependence (Saccone, et al., 2007). Additionally, because of the central role that dopaminergic systems are thought to play in drug reward, reinforcement, addiction and dependence, dopaminergic system genes have been investigated in relation to addiction to substances that do not directly affect dopamine, including ethanol (Blum, et al., 1993; Gelernter & Kranzler, 1999; Gorwood, et al., 2003; Noble, 1998a, 1998b; Noble, et al., 1998; Sander, Harms, Podschus, et al., 1997), and opiates (Kotler, et al., 1997; Lawford, et al., 2000). Opioid systems have also been investigated in regard to dependence on drugs other than opiates (Bergen, et al., 1997; A. C. H. Chen, et al., 2002; Dahl, et al., 2005; Franke, et al., 1999; Ide, Kobayashi, et al., 2004; Xuei, et al., 2006). Many genes in these systems have also been examined more broadly in drug dependence to multiple substances or combined groups of individuals addicted to different substances (Agrawal, et al., 2006; Berrettini, Hoehe, Ferraro, DeMaria, & Gottheil, 1997; Blomqvist, Gelernter, & Kranzler, 2000; Comings, Muhleman, Ahn, Gysin, & Flanagan, 1994; Gelernter, Kranzler, & Cubells, 1999; Hoehe, et al., 2000; H. R. Kranzler, Gelernter, O'Malley, Hernandez-Avila, & Kaufman, 1998; Krebs, et al., 1998; Luo, Kranzler, Zhao, & Gelernter, 2003; Smith, et al., 1992; Vandenberg, et al., 2000; Vandenberg, Rodriguez, Miller, Uhl, & Lachman, 1997; H. Zhang, Kranzler, Yang, Luo, & Gelernter, 2008). Serotonergic system genes have also been studied with regard to addiction to a number of substances (Cigler, et al., 2001; Dahl, et al., 2006; Hong, et al., 2003; H. Kranzler, Lappalainen, Nellisery, & Gelernter, 2002; H. R. Kranzler, Hernandez-Avila, & Gelernter, 2002; Patkar, et al., 2001; Sander, Harms, Lesch, et al., 1997). Few studies have looked at additive or interactive effects, although such effects have been noted for the dopamine receptor D2 gene (*DRD2*) and the GABA receptor subunit gene 3 (*GABRA3*) variants for alcoholism (Noble, et al., 1998).

Many of these studies, when producing positive results, did so by assessing multiple markers in multiple ways, and although correcting for the number of comparisons within an analysis, performed multiple analyses, so at best, most results are “nominally” significant. In many instances, allelic variants were more strongly associated with particular drug related phenotypes (Comings, et al., 1994; Gorwood, et al., 2003; Ide, Kobayashi, et al., 2004; Sander, Harms, Lesch, et al., 1997; Shi, et al., 2002), including preference for, or dependence on, particular drug classes (Comings, Gonzalez, et al., 1999; Persico, et al., 1996a), or degree of drug preference (Persico, Bird, Gabbay, & Uhl, 1996b), often when the overall association with drug dependence was not significant. In the Comings et al. (1999) study the dopamine D3 receptor (*DRD3*) was found to be associated with cocaine dependence, but not dependence on amphetamines, opiates or alcohol. More recently, responses to bupropion in nicotine cessation trials have been associated with the dopamine

D4 receptor (*DRD4*) (Leventhal, et al., 2012). Additionally, the apparent effects in some studies have been found to be the result of ethnic stratification (Patkar, et al., 2001).

An important consideration is the nature of most allelic differences relevant to addiction. One study examining the nature of allelic differences found no increase in allelic variants producing changes in the dopamine transporter (DAT) protein sequence and suggested that variation in *DAT* associated with alcohol dependence was likely associated with alterations in levels of expression (Vandenberg, et al., 2000).

More complete summaries of the findings from candidate gene studies have been published previously (Gelernter & Kranzler, 2009; Kreek, Bart, Lilly, Laforge, & Nielsen, 2005) so here we intend only to make a few points based on those studies we have discussed. As can be seen in Table 1, it is apparent that even for individual genes that are particularly well studied (such as *DRD2* and *OPRM1*) less than half of the studies in the literature identify positive associations. Given that there is well-known bias towards publishing positive results, and against publishing negative results, the actual percentage of positive findings is probably substantially less. This might indicate several possibilities. The positive findings may be false positives and entirely spurious. Given the importance of these genes in the actions of drugs of abuse this appears unlikely. Other solutions, however, present themselves, including the possibility that the effects of any particular genetic variant may be rather small, that the variations may be heterogeneously distributed, and that they may be more specifically involved in particular endophenotypes or addictions associated with particular drugs of abuse. These possibilities will be reconsidered below in the context of the findings from GWAS.

The logic behind many candidate gene studies was generally rather sound from the point of view of biological relevance, and the consequences of some polymorphisms appeared to support the logic behind candidate gene studies. For instance, the μ opioid receptor (MOR) A118G SNP produces an amino acid change that alters endogenous ligand binding (Bond, et al., 1998). However, there was no significant association of this polymorphism with opiate dependence. Subsequent studies have both found (Bart, et al., 2004; Szeto, et al., 2001; Tan, et al., 2003) and failed to find (Crowley, et al., 2003; Franke, et al., 2001; Li, et al., 2000; Shi, et al., 2002; Tan, et al., 2003) significant associations for the same polymorphism. Population admixture has been suggested to be involved in positive associations with this polymorphism when they have been observed in some, though not all, cases (Kreek, et al., 2005).

4. Modeling Candidate Genes with Knockout (KO) Mice

Many transgenic mouse models were made specifically made to investigate, under more controlled circumstances, the consequence of direct manipulation of particular genes, and for studying the roles of specific genes in addiction relevant behavioral and physiological traits (G. R. Uhl, Hall, & Sora, 2002; G. R. Uhl, et al., 2000). The first transgenic models, like the first candidate gene association studies, were based upon *a priori* assumptions about the importance of particular genes, including the main target molecules of morphine, amphetamine, and cocaine. This consequently emphasized the importance of monoamine transporters, opioid receptors, and monoamine receptors in addiction. In many cases these studies produced large effects on drug responses and behavior that might be considered to be relevant to addiction, but even these effects were complex and polygenic. As we shall see below, although the effects of opiates were, in many cases, eliminated in MOR KO mice, the case for psychostimulants was much more complex owing to an apparently more complex mechanism of action.

One important point to be considered here is the goal of transgenic studies in the first place – which has not always been expressed particularly explicitly. In most of these early studies the goal was to determine (or perhaps confirm) the site of action of drugs of abuse by eliminating the initial targets using homozygous knockout mice. As models of human genetic variation, these transgenic models are consequently rather poor because it is rare for deletion of entire genes to occur in humans. If the goal is to model human genetic variation then other approaches are necessary, depending on the nature of the human genetic variants to be modeled. Heterozygous knockout mice, generally expressing 50% of the usual RNA and protein levels, may in fact be better models of the range of human variation, but this has not always been done, perhaps because this was not the intended goal of most studies. One problem, overall, with each genetic manipulation, particularly if heterozygous mice are to be studied, is whether a single manipulation can produce a large enough effect to be detectable. If addiction is highly polygenic with each genetic difference contributing only a small amount of the overall variance, this may be a significant problem.

Nonetheless, and putting these issues aside for the time being, these studies have helped to identify the mechanisms of action of drugs of abuse underlying their behavioral and physiological effects, including abuse liability and important adverse consequences, such as toxicity and lethality. These models have also been used to confirm the potential for these genes to contribute to addiction based on nomination from human genetic studies. This literature has been reviewed a number of times recently (e.g. (Moriya, Hall, & Sora, 2013; Sora, Li, Igari, Hall, & Ikeda, 2010)) so we will only briefly describe this work here, in order to illustrate the logic that initiated these studies, and focus primarily upon the KO mice that were developed for the main molecular targets of opiates, cocaine and amphetamine-like drugs. Again, the important point here is that these studies were initiated in the mid-1990s based upon *a priori* assumptions about the importance of certain genes in addiction, including data from candidate gene studies. These studies thus began in the pre-genomic era, and as the ability to examine the association of all genes with addiction developed during the genomic era, it became obvious that many of these assumptions, at least from the perspective of genetic variation underlying addiction in humans, appeared to be wrong.

Phenotypic analysis of transgenic mice, in particular those utilizing KO mice, have greatly contributed to our understanding of the molecular mechanisms of drugs of abuse. As initial candidate gene studies began to find evidence supporting the roles of a number of monoaminergic genes, in particular, in the genetic component of addiction liability, KO studies were conducted, in part, to confirm the importance of these genes in the effects of drugs of abuse, and by implication, addiction. Thus, early efforts to examine the roles of genes in addiction using transgenic techniques targeted what were thought to be the main targets of opiates (MOR), cocaine (DAT) and amphetamine (the vesicular monoamine transporter; VMAT2).

4.1 Monoamine Transporter KO Mice and Cocaine

Dopaminergic systems, and in particular the mesolimbic dopamine system, have long been considered to have a central role in drug reward, and by implication, addiction. Numerous pieces of evidence lead to this central tenant of addiction theory, including the recognition that all drugs of abuse, either directly or indirectly, stimulate brain dopaminergic pathways (Wise & Bozarth, 1987). Indeed, dopaminergic systems, and the neural circuitry with which they integrate, have been shown to be involved in a wide variety of reward related behavior that might be expected to be involved in addiction, including reward prediction error (Schultz, 2006), cue learning (Ito, Dalley, Howes, Robbins, & Everitt, 2000), incentive motivation (Berridge & Robinson, 1998), the induction and expression of behavioural

sensitization (Cador, Bjijou, & Stinus, 1995), habit formation (Yin & Knowlton, 2006) and reinstatement of drug seeking behavior (Shaham, Shalev, Lu, De Wit, & Stewart, 2003).

Thus, when the mechanisms underlying the rewarding and reinforcing effects of cocaine were first considered in knockout mice, expectations were based upon a preponderance of data indicating that dopamine was the primary mediator of the rewarding effects of cocaine, despite the fact that cocaine inhibits reuptake via the serotonin transporter (SERT) and the norepinephrine transporter (NET) as well (Rothman & Baumann, 2003). This was supported by an apparent correlation between reinforcing efficacy and the ability of various compounds to bind to DAT (Kuhar, Ritz, & Boja, 1991). Thus, the first publication examining the behavioral effects of cocaine in DAT KO mouse described these mice as being “indifferent” to cocaine (Giros, Jaber, Jones, Wightman, & Caron, 1996), consistent with expectations. This was based upon the observation that DAT KO mice did not exhibit locomotor stimulant effects of cocaine. However, subsequent studies demonstrated that DAT KO mice could develop both a conditioned place preference for cocaine and self-administer cocaine, at least under some conditions, using strains developed at the the Molecular Neurobiology Branch (MNB) at the National Institute on Drug Abuse, (USA) and at Duke University, respectively (Rocha, et al., 1998; Sora, et al., 1998). It must be noted however, that although DAT KO mice did develop a significant conditioned place preference for cocaine at the high dose, there was a slight diminution in sensitivity to cocaine in that there was not a significant place preference at the low dose as there was in wildtype (WT) mice. In any case, the result was so surprising that the evidence that dopamine might partially mediate cocaine reward was largely ignored because it had been demonstrated that it was not *necessary* for drug reward as had been presumed. This issue was revisited recently and it was shown that although DAT KO mice do self-administer cocaine, when subsequently challenged under circumstances that demand more effort to receive the cocaine, such as a progressive ratio, they respond far less (Thomsen, Hall, Uhl, & Caine, 2009).

In any case the ability of cocaine to have any rewarding effects at all in these mice suggests that some other mechanisms must be involved, and several potential possibilities were suggested, including mediation by one of the other two main targets of cocaine. Although deletion of either SERT or NET was shown to increase cocaine conditioned place preference (Sora, et al., 1998; F. Xu, et al., 2000), perhaps due to elimination of aversive effects of cocaine (G. R. Uhl, et al., 2002), one possibility was that, in the absence of DAT, the additional deletion of SERT or NET would eliminate the rewarding effects of cocaine. Combined deletion of DAT and SERT was found to eliminate the rewarding effects of cocaine in the conditioned place preference paradigm (Sora, Hall, et al., 2001). Combined deletion of NET and DAT was lethal (Hall, Sora and Uhl, unpublished observation). Furthermore, the selective SERT and NET blockers fluoxetine and nisoxetine were shown to have rewarding effects in DAT KO mice that they did not have in WT mice (Hall, et al., 2002), while peripheral administration of fluoxetine or the NET blocker reboxetine increased dopamine release in the nucleus accumbens of DAT KO mice, but not in WT mice (Carboni, et al., 2001; Shen, et al., 2004). These findings suggest that cocaine is rewarding in DAT KO mice because of actions at SERT (and perhaps NET). This might be considered to be consistent with an “occult” reuptake hypothesis (Y. J. Liu & Edwards, 1997; G. R. Uhl, et al., 2000), whereby cocaine continues to produce rewarding effects in DAT KO mice by elevating dopamine levels in the striatum, but by actions at SERT and NET rather than DAT. However, this does not appear to be the case as administration of cocaine, fluoxetine or nisoxetine into the striatum fail to affect dopamine levels or alter dopamine clearance in DAT KO mice (Budygin, John, Mateo, & Jones, 2002; Mateo, Budygin, John, Banks, & Jones, 2004; Mateo, Budygin, John, & Jones, 2004; Shen, et al., 2004). By contrast, local injection experiments have shown the locus of the fluoxetine and cocaine effects in DAT

KO mice to be in the ventral tegmental area (Mateo, Budygin, John, & Jones, 2004), consistent with an excitatory serotonergic input that must be somewhat different in DAT KO mice from the usual circumstance in wildtype mice.

In addition to the apparent changes in serotonergic and noradrenergic contributions to the effects of cocaine in DAT KO mice, there are other profound changes in these mice. As a consequence of the deletion of DAT in DAT KO mice, basal extracellular dopamine levels are extremely high in the dorsal striatum and nucleus accumbens (Rocha, et al., 1998; Shen, et al., 2004), apparently a result of dramatically reduced dopamine clearance (Mateo, Budygin, John, Banks, et al., 2004), while other aspects of dopamine function are substantially reduced, including levels of postsynaptic receptors, autoreceptor function and dopamine synthesis (Gainetdinov, Jones, & Caron, 1999; Giros, et al., 1996; Jones, et al., 1999; Sora, et al., 1998). However, one of the more interesting consequences of DAT deletion is that not all dopamine regions are affected in the same manner. Extracellular dopamine levels in the prefrontal cortex are unaffected in DAT KO mice (Shen, et al., 2004) or which is consistent with NET being the primary mediator of dopamine uptake in the prefrontal cortex (Mazei, Pluto, Kirkbride, & Pehek, 2002). One of the results of this circumstance would therefore be that the ratio of cortical to subcortical dopaminergic activity is altered in DAT KO mice, so that DAT KO mice are essentially hypofrontal. Indeed, deficits in prepulse inhibition of startle in DAT KO mice (Barr, et al., 2004; Ralph, Paulus, Fumagalli, Caron, & Geyer, 2001; Yamashita, et al., 2006) can be reversed by administration of NET blockers in the prefrontal cortex (Arime, Kasahara, Hall, Uhl, & Sora, 2012), which elevate dopaminergic tone and is associated with activation of prefrontocortical glutamatergic projections to the nucleus accumbens. Consistent with this hypothesis, a recent brain imaging study demonstrated reduced activity of an apparent corticostriatal circuit in DAT KO mice (X. Zhang, et al., 2010).

These adaptations in DAT KO mice could be considered from a couple of different perspectives. On the one hand they might be interpreted as being uninformative as regards the usual mechanisms that underlie the rewarding effects of cocaine. In this respect it is important to note that a transgenic line was created in which DAT maintains much of its normal uptake function, but is insensitive to cocaine, the DAT-CI transgenic line (R. Chen, et al., 2006). These mice show neither cocaine-induced elevation of dopamine levels in the nucleus accumbens nor cocaine conditioned place preference. Although this line clearly demonstrates that elimination of DAT as a target for cocaine can have profound effects on cocaine reward, the DAT KO mouse does demonstrate that under some circumstances other mechanisms may mediate the rewarding effects of cocaine. Indeed, in dopamine deficient mice in which transgenic manipulations produce an almost complete elimination of dopamine (Zhou & Palmiter, 1995), cocaine continues to produce rewarding effects that are also serotonin-mediated (Hnasko, Sotak, & Palmiter, 2007).

These studies have demonstrated that a number of important and unexpected mechanisms underlie, or can underlie, the rewarding effects of psychostimulant drugs. However, the question remains as to what these studies may tell us about the genetic mechanisms underlying addiction in humans. That manipulation of these genes can alter responses to psychostimulant drugs, or produce behavioral phenotypes that might be expected to be associated with addiction, does not necessarily mean that variation in those genes in humans has an important influence on addiction liability. This may not necessarily be because those genes cannot affect those phenotypes, but rather because the allelic variation simply does not exist in humans for other reasons. Indeed, the most dramatic effects observed in transgenic mouse models are the result of rather extreme alterations in function that are not seen in humans (homozygous deletion of these genes or complete elimination of dopamine). When heterozygous KO mice have been examined, which would roughly model the range of

variation of these genes that is observed in humans (approximately a 50% reduction) they have rarely been shown to exhibit the changes that are observed in homozygous KO mice.

4.2 VMAT2 and amphetamine-like compounds

The mechanisms of action of amphetamine-like compounds involve effects at both plasma membrane transporters and vesicular transporters (Seiden & Sabol, 1993; Sulzer, Sonders, Poulsen, & Galli, 2005). The presence of VMAT2 in dopaminergic, noradrenergic, serotonergic, histaminergic, and potentially trace aminergic neurons invites consideration of a wider role for monoaminergic neurotransmission in the effects of amphetamine-like compounds (Eiden & Weihe, 2011). While the different amphetamine compounds share affinity for VMAT2, the affinity of these compounds for the monoamine plasma membrane transporters differs substantially (Rothman & Baumann, 2003). This is an important aspect of their function as they primarily enter the cell via the plasma membrane transporters to assert their actions at VMAT2. Thus, some amphetamine-like compounds, such as methamphetamine (METH) and 3,4-methylenedioxymethamphetamine (MDMA) act fairly selectively on DAT and SERT respectively, while d-amphetamine itself is rather non-selective. Recently there has been a proliferation of illicit use of novel amphetamine analogues, including methcathinone and methylenedioxymethcathinone, among others. These compounds differ from other amphetamine-like compounds in their subjective effects, their potential for adverse effects and their selectivity for various monoamine transporters (Martinez-Clemente, Escubedo, Pubill, & Camarasa, 2012; Schifano, et al., 2011). Because amphetamine-like compounds have diverse effects, potentially determined by multiple monoamine transporters in addition to VMAT2, KO studies have been used in an attempt to determine which monoamine systems are critical components of the actions of different amphetamine analogues.

At about the same time, three groups independently reported the generation of VMAT2 KO mice (Fon, et al., 1997; Takahashi, et al., 1997; Y. M. Wang, et al., 1997), demonstrating very similar patterns of effects. Perhaps not surprisingly, given the potential impact of VMAT2 deletion on all monoaminergic neurotransmission, deletion of both copies of VMAT2 was mostly lethal within a few days after birth and completely lethal by 2 weeks of age, due to substantially reduced feeding behavior (indeed general reductions in behaviour overall). Homozygous VMAT2 KO mice had very low whole brain monoamine levels despite increased synthesis rates (Fon, et al., 1997; Y. M. Wang, et al., 1997). Heterozygous KO mice were shown to have somewhat reduced monoamine levels and reduced exocytotic release (Fon, et al., 1997; Takahashi, et al., 1997; Y. M. Wang, et al., 1997), although the extent of these reductions differed by brain region and neurotransmitter across the three reports. Indeed, Takahashi et al. (1997) found elevated levels of both dopamine and DOPAC in the striatum, although this was in the context of a variety of other changes, including reduced DAT levels and elevated expression of tyrosine hydroxylase. Wang et al., (1997) found reduced striatal dopamine levels, but in the context of increased DOPAC levels. Both serotonin and 5-HIAA were reduced in the frontal cortex in the Takahashi et al., (1997) study. Overall these initial studies indicate that even heterozygous deletion of the VMAT2 gene produces profound changes in monoaminergic function that differentially impacts the three main monoamine systems in a regionally dependent manner, but are generally indicative of reduced availability of the monoamines for exocytotic release.

Because of the early lethality in homozygous VMAT2 KO mice, behavioral studies have examined heterozygous VMAT2 KO mice. Although this might be considered to be problematic, this again depends upon the goal of the study, heterozygous mice being much more near the normal range of variation for the expression of most genes. Heterozygous VMAT2 KO mice are surprisingly normal in terms of motor co-ordination, locomotor activity, weight gain, fertility and some simple forms of learning (Takahashi, et al., 1997).

Despite reduced amphetamine-induced striatal dopamine release in heterozygous VMAT2 KO mice (Y. M. Wang, et al., 1997), the acute locomotor stimulant effects of amphetamine and cocaine are increased (Takahashi, et al., 1997; Y. M. Wang, et al., 1997). This is surprising but might be the result of alterations in the affinity states of postsynaptic receptors (Seeman, Hall, & Uhl, 2007). These compensatory changes are not sufficient to normalize all functions however, as heterozygous VMAT2 KO mice do not develop behavioural sensitization to amphetamine (Y. M. Wang, et al., 1997) and exhibit reduced conditioned place preference for amphetamine (Takahashi, et al., 1997).

Because of the interactions between DAT and VMAT2 in determining the dynamics of dopamine release, the interactive effects of heterozygous (+/-) deletion of the two genes was recently examined on methamphetamine locomotion and sensitization of locomotion (Fukushima, et al., 2007). As might be expected from previous studies with amphetamine (Spielewoy, et al., 2001; Takahashi, et al., 1997), the acute locomotor stimulant effects of methamphetamine were increased in VMAT2 +/- mice and decreased in DAT +/- mice. Similarly, sensitization of these effects was also increased in VMAT2 +/- mice and reduced in DAT +/- mice, in contrast to a previous study using amphetamine (Y. M. Wang, et al., 1997). It might be noted that the initial response of VMAT2 +/- mice was so high that there may have been ceiling effects. No interactive effects were observed in the Fukushima et al. (2007) study between the two genetic manipulations. The identification of effects in heterozygous mice may make it more likely that genetic differences in humans that alter gene expression by similar magnitudes might have observable effects on sensitivity to amphetamine-like drugs.

Based on the findings noted above, variations in the expression of DAT (within the human range) may have greater effects on responses to amphetamine-like drugs than responses to cocaine. The acute locomotor stimulating effects of cocaine are largely eliminated in homozygous DAT KO mice (Giros, et al., 1996; Morice, Denis, Giros, & Nosten-Bertrand, 2004; Sora, et al., 1998) but heterozygous mice are largely unaffected. Similarly, sensitization is not observed in DAT KO mice even after extended periods of habituation to normalize activity (Mead, Rocha, Donovan, & Katz, 2002). In another paradigm, homozygous DAT KO mice exhibit neither conditioned locomotion nor context-dependent sensitization (Hall, et al., 2009), yet heterozygous DAT KO mice are not different from wildtype mice.

Other amphetamine-like compounds may involve mechanisms mediated by other monoamine systems. Thus, work with methylenedioxymethamphetamine (MDMA) has focused on SERT. The locomotor stimulant effects of MDMA are abolished in SERT KO mice (Bengel, et al., 1998), as is MDMA-induced serotonin release and MDMA self-administration (Trigo, et al., 2007). Surprisingly, and perhaps indicating non-SERT mediated mechanisms in some of the effects of MDMA, increases in extracellular dopamine levels in the nucleus accumbens in SERT KO mice after administration of MDMA are unchanged (Trigo, et al., 2007) or reduced (Hagino, et al., 2011). In the latter study, the effects of MDMA on dopamine release were only eliminated in combined DAT/SERT KO mice. Other evidence supports a role of dopamine in the effects of MDMA, as well as more generally supporting one of the major themes that has emerged from many of these KO studies, that there are substantial interactions between dopamine and serotonin systems in the rewarding effects of psychostimulants. For example, male dopamine receptor D₁ KO mice show significant increases in MDMA-induced hyperactivity compared to WT mice, while DRD2 KO mice exhibit reductions in MDMA-induced hyperactivity (Risbrough, et al., 2006).

As for the cocaine studies discussed in the previous section, studies of the effects of other psychostimulants in VMAT2 KO and other gene KO mouse strains have supported, to some extent, the evidence from candidate gene studies in that manipulations of these genes do affect responses to drugs of abuse. However, as in the previous case, most of these effects required homozygous deletion, not something that is observed in humans. On the other hand, in the case of VMAT2 +/- and DAT +/- KO mice, which would potentially model the range of variation in these genes that is actually observed in humans, effects upon a number of the behavioral responses to amphetamine-like drugs were observed.

4.3 MOR KO mice and opiates

Perhaps one of the most unsurprising series of studies using transgenic mice was the demonstration that the effects of most opiates are eliminated in MOR KO mice. It must be noted that these mice were exceedingly useful in examining the specificity of opiate actions at MOR and in identifying more complex sorts of interactions between opioid receptor subtypes. As for DAT and VMAT2, several groups constructed MOR KO mice at about the same time (Loh, et al., 1998; Matthes, et al., 1996; Sora, et al., 1997; Tian, et al., 1997). The first of these strains demonstrated that MOR KO mice did not exhibit morphine induced conditioned place preference, analgesia or tolerance (Matthes, et al., 1996). However, two important differences from studies of psychostimulants were observed in these strains of MOR KO mice: heterozygous KO mice showed far more effects than many other KO strains, and deletion of MOR affected responses to a much wider range of addictive drugs than most other KO strains that have been studied. This last idea was summarized in a review previously (Hall & Uhl, 2006) so those findings will be addressed only briefly here, concentrating on studies most relevant to addiction.

Conditioned place preference induced by morphine or heroin is eliminated in homozygous MOR KO mice (Contarino, et al., 2002; Hall, et al., 2003; Matthes, et al., 1996; Sora, Elmer, et al., 2001). The rewarding effects of buprenorphine, on the other hand were shown to have a partially non-MOR dependent component (Ide, Minami, et al., 2004). Heterozygous mice on a mixed genetic background have increased morphine conditioned place preference (Sora, Elmer, et al., 2001), while no difference was observed in congenic MOR KO mice on a C57Bl6/J background (Hall, et al., 2003). The rewarding effects of buprenorphine were equally affected in heterozygous MOR KO mice as they were in homozygous MOR KO mice (Ide, Minami, et al., 2004). Morphine self-administration was eliminated in homozygous MOR KO mice (Becker, et al., 2000; Sora, Elmer, et al., 2001), but also almost completely abolished in heterozygous MOR KO mice (Sora, Elmer, et al., 2001). That heterozygous MOR KO mice are unaffected under some conditions, but under other conditions are as affected as homozygous MOR KO mice, was suggested to be the result of differential receptor reserve in different MOR circuits (Sora, Elmer, et al., 2001). In any case, an important point is that heterozygous MOR KO mice, which are in the range of normal levels of variation in the expression of MOR in humans (G. R. Uhl, Sora, & Wang, 1999), exhibit differences in addiction related phenotypes.

The effects of MOR KO on drug reward were not limited to opiates. The conditioned place preference induced by Δ^9 -tetrahydrocannabinol (THC) (Ghozland, et al., 2002), ethanol (Hall, Sora, & Uhl, 2001), nicotine (Berrendero, Kieffer, & Maldonado, 2002) and cocaine (Becker, et al., 2002; Hall, Goeb, Li, Sora, & Uhl, 2004) are reduced or eliminated in MOR KO mice. Some studies have failed to observe effects of MOR deletion on conditioned place preference for MDMA (Robledo, et al., 2004), cocaine (Contarino, et al., 2002) or ethanol (Becker, et al., 2002), although limited dose ranges, the sex of the subjects and differences in breeding strategies may have affected the results of those studies (see discussion in (Hall & Uhl, 2006)). Not all studies have examined heterozygous MOR KO mice, but the conditioned place preferences induced by ethanol and cocaine were as affected in

heterozygous MOR KO mice as in homozygous MOR KO mice (Hall, et al., 2004; Hall, et al., 2001). Ethanol consumption has also been shown to be reduced in MOR KO mice (Becker, et al., 2002), although this reduction was sex-dependent in another study (Hall, et al., 2001). It is interesting to note that in the Becker et al., (2002) study consumption was initially similar, but MOR KO mice did not increase their consumption over the period of study as did the WT mice. Operant self-administration of ethanol is largely eliminated in homozygous MOR KO mice (Roberts, et al., 2000) and cocaine self-administration is substantially reduced (Mathon, Lesscher, et al., 2005). This latter effect was associated with reduced midbrain dopamine cell firing (Mathon, Ramakers, Pintar, & Marinelli, 2005), perhaps due to increased GABAergic inhibition (Mathon, Lesscher, et al., 2005).

These studies in MOR KO mice demonstrate several important points. Firstly, to some degree, they confirm the results of earlier candidate gene studies for MOR in that manipulation of MOR can affect both opiate and non-opiate drug responses that are relevant to addiction. Secondly, this occurs in heterozygous mice, which are much more likely to reflect a range of variation in MOR expression that is within the human range.

5. GWAS for drug addiction/dependence

Based on the results of the human candidate gene and mouse KO studies discussed above, it might be thought that those genes are clearly implicated in addiction. Based on the marked alterations in survival of several of these KO strains, it was also unlikely that variations that produced marked alterations in these genes would survive to become common in the population. Thus, there were thoughts that GWAS might identify some of those genes, and also that many were unlikely to harbour variants that altered their functions to the extent found in KO mice. In conducting genome wide studies, as for candidate gene studies, both linkage and association methods can be used. Initial genome-wide linkage scans for alcohol dependence found possible linkage at as many as four loci (Foroud, et al., 2000; Long, et al., 1998; Reich, et al., 1998). Generally, these effects were relatively modest (LOD scores between 1.5 and 4) and the same loci were not identified in all samples. A genome-wide linkage scan for nicotine dependence identified only one locus with a strong linkage signal (Straub, et al., 1999). However, in further analysis the authors identified a number of regions in which multiple consecutive positive markers could be found, and the authors concluded that the difficulty was identifying signals from genes that each likely had a small effect and contributed heterogeneously to the phenotype. The stringency of the statistical criteria applied to the analysis, discussed in more detail below, may have been a critical factor in the limited number of positive effects identified in these studies. Indeed, a genome-wide linkage scan for opioid dependence identified only weak linkage signals (Gelernter, et al., 2006), and a genome-wide linkage scan for nicotine dependence identified only one “genome-wide” result that was significant (Gelernter, et al., 2007). As will be discussed below, there are statistical issues with regard to “genome-wide” significance, but because linkage involves fewer recombination events, it may lack the resolution necessary for these studies.

Beginning in the late 1990s the MNB at NIDA began a series of GWAS examining drug dependence. The first GWAS to examine drug (polysubstance) dependence, and one of the first studies to take this approach in any complex disorder, used less than 1500 single nucleotide polymorphisms (SNPs) in European-American and African-American drug dependent subjects (G. R. Uhl, Liu, Walther, Hess, & Naiman, 2001). This marker density was obviously not sufficient to identify many individual genes, so the authors spoke of “chromosomal” regions. Nonetheless, this analysis identified a number of regions consistent with previous linkage-based genome scans (Foroud, et al., 2000; Long, et al., 1998; Reich, et al., 1998; Straub, et al., 1999) and a number of loci that were not identified in previous studies. Part of the strategy used in this, and many subsequent MNB studies, was to do

comparisons in both European-American and African-American subjects and to look for replication in terms of identification of the same chromosomal regions between these independent samples (Drong, et al., 2011; Q. R. Liu, et al., 2006; G. R. Uhl, Liu, & Walther, 2001). Other studies examined other samples including drug dependent individuals from the Baltimore site of the Epidemiological Catchment Area study (Johnson, et al., 2008), alcohol dependent samples from the Collaborative Study on the Genetics of Alcoholism (Johnson, et al., 2006), 2 samples of methamphetamine dependent individuals (G. R. Uhl, Drong, Liu, et al., 2008), and nicotine dependent individuals (G. R. Uhl, et al., 2007).

Subsequent studies following the initial Uhl et al. (2001) study increased the SNP marker density to 10 thousand (Q. R. Liu, et al., 2005), 100 thousand (Johnson, et al., 2006), 500 thousand (Q. R. Liu, et al., 2006) and 1 million (Drong, et al., 2011; Drong, et al., 2010; G. R. Uhl, et al., 2007). Subsequent studies have also conducted genome-wide association with copy number variants (Drong, Montoya, et al., 2009). Beginning with the 2005 study, the marker density was sufficient to begin to discuss individual “candidate” genes. Even at this stage many more positive markers than expected by chance were associated with the initial Uhl et al. (2001) study or with previous whole-genome linkage studies, a pattern which continued to develop throughout the series of studies. As these studies progressed, it was necessary to consider more carefully, and much more explicitly than the field was often ready to accept, the tradeoff between false positive and false negative rates in the criteria used to identify genes as being “positive” in any particular analysis (for a detailed consideration of this issue see (Sebastiani, Timofeev, Dworkis, Perls, & Steinberg, 2009)). Several aspects of the approach used in this series of studies mitigated concerns about false positives, including the identification of not just single SNP associations, but clusters of positive SNPs in specific genes (Q. R. Liu, et al., 2006). Furthermore, the initial strategy of identifying positive findings in both European-American and African-American subjects was extended to other drug dependent populations (Johnson, et al., 2006). Additionally, the original two populations, although not necessarily the same subjects, were assessed in a series of studies using increasing marker densities. Thus, it is important to consider the findings as a whole, and the degree of replication across those studies, and other studies that have been completed subsequently. As discussed in Drong et al. (2011), the definition of “replication” in GWAS for major gene effects that involve the same allele is relatively straightforward, but this is not the circumstance for phenotypes that have a polygenic genetic architecture, high allelic heterogeneity, and high locus heterogeneity, and when the arrangement of linkage disequilibria differs across populations or samples. Thus, “replication” should be defined based on identification of the same chromosomal regions; which effectively means regions of individual genes defined by high linkage disequilibrium once the marker density in GWAS is sufficiently dense. Based on these criteria, there was substantial replication of results across these studies despite their differences, which produced a substantial overlap in the genes identified, as discussed in more detail below. Furthermore, comparison of the results of GWAS and murine quantitative trait locus studies for ethanol, methamphetamine and barbiturates indicates substantial overlap as well (G. R. Uhl, Drong, Johnson, Fatusin, et al., 2008).

Since modern GWAS involve a level of multiple comparisons that are unprecedented in biology, it is important to consider the nature of the trade-off between false-positive and false-negative results. As discussed in Drong et al. (2011), many GWAS using so-called “template” approaches that demand individual SNPs with p values $<10^{-8}$ to achieve “genome-wide significance” are so stringent in attempts to reduce false positive findings that they are often unable to identify any significant effects at all (e.g. providing a large number of false negative results instead). Changing the statistical criteria or approach to reduce these false negatives, but in the context of identification of multiple SNP markers in each locus, and replication across multiple samples and multiple marker densities, provides

confidence that the loci that are repeatedly identified are in fact truly associated with drug dependence (for a discussion of these alternative approaches such as the clustering strategy see (Drgon, et al., 2010)). The result of this approach has been a high degree of replication across studies. Table 2 presents an analysis from Uhl et al. (2008) which identified 50 genes that had been highly replicated in studies of drug dependence and related phenotypes. The p values in the table (from Uhl et al. (2008)) represent the probabilities of the repeated identification of these genes in so many studies based on Monte Carlo simulations. This list forms a stark contrast to the limited number of genes, of weak effect, generally identified in the linkage studies discussed above. With regard to the fact that each gene is not identified repeatedly in each study 100% of the time, it is important to note that 100 percent concordance should not be expected across studies for reasons mentioned above – that is, it is likely that the set of genes involved in drug dependence in different populations, in dependence on different substances, in different levels of dependence, and so forth, may not completely overlap. As this field progresses it is likely that we will be able to demonstrate different sets of genes associated with each of these modifying conditions.

Some of the first attempts to address this partial overlap for different aspects of addiction have compared the results of the drug dependence studies mentioned above with those of nicotine cessation studies (Drgon, Johnson, et al., 2009; Drgon, Montoya, et al., 2009; G. R. Uhl, et al., 2007). These studies have identified a highly significant overlap between the set of genes associated with nicotine dependence and nicotine cessation and the set of genes identified in four previous GWAS for drug dependence. Furthermore, there was a substantial, though far from complete, overlap between genes associated with nicotine dependence and nicotine cessation. Interestingly, as in previous GWAS for drug dependence, cell adhesion molecules were over-represented in the set of genes identified as being associated with nicotine dependence with respect to their representation in the genome as a whole (see Table 2). Importantly, and in contrast to other GWAS for drug dependence, a number of other genes that had been previously associated with nicotine or drug dependence using candidate gene or genome-wide linkage approaches were apparent in this list including a number of nicotinic acetylcholine system genes, GABAergic genes, serotonergic system genes, opioidergic system genes and other neuropeptide associated genes. This is not true of comparison to drug dependence phenotypes as a whole and may represent specific contributions of these genes to nicotine dependence. A subsequent analysis of 3 nicotine cessation (quit-success) samples (G. R. Uhl, Liu, et al., 2008) identified a substantial overlap in the set of genes associated with nicotine cessation, and characterized by clustered positive SNPs. Once again, cell adhesion molecule genes were prominent in this analysis, along with genes coding for enzymes, transcriptional regulation and smaller numbers of genes associated with other functions, including some neurotransmitter receptors. As for most drug dependence GWAS (except perhaps nicotine dependence) monoamine system genes were conspicuous for their absence.

GWAS for nicotine cessation mentioned above examined different nicotine cessation methods: denicotinized cigarettes (Drgon, Johnson, et al., 2009), mecamylamine/nicotine replacement (G. R. Uhl, et al., 2007) and bupropion (G. R. Uhl, Liu, et al., 2008). Using data from these studies a “quit success” genotype score was developed to assess the cumulative genetic contribution to quit success (Rose, Behm, Drgon, Johnson, & Uhl, 2010). This study found that there was an interaction between this quit success score, nicotine dose during pre-cessation treatment, and the degree of nicotine dependence. Those individuals with high quit-success genotype scores had relatively high abstinence rates (20-25 % at 10 weeks) independent of nicotine replacement dose. Those individuals with low quit-success genotype scores had highly differential responses to nicotine doses depending on level of dependence – low dependence individuals had far greater abstinence rates at the lower nicotine dose (33% at the low dose vs. 10% at the high dose), while high dependence individuals had

greater abstinence rates at the higher nicotine dose (9% at the low dose vs. 22% at the high dose). This study suggests that the use of genetic information, in combination with assessment of the degree of physiological dependence, may greatly increase the effectiveness of nicotine cessation treatments. Furthermore, analysis of clinical trial data has indicated that the use of genetic information may enhance statistical power and reduce costs of nicotine cessation clinical trials (G. R. Uhl, Drgon, Johnson, & Rose, 2009).

A study that examined methamphetamine dependence (G. R. Uhl, Drgon, Liu, et al., 2008) also found a substantial overlap of positively associated genes with studies that examined drug dependence (defined by addiction to one or more addictive substances). Like those studies, genes for cell adhesion molecules, enzymes, transcriptional regulation, cell structure and RNA, DNA and protein handling/modifying genes were overrepresented. This included *Csmd1* and *Cdh13*, genes which are among the most commonly identified genes in GWAS for drug dependence (see Table 2).

One of the most important conclusions from this series of studies is that most of the genes expected, on an *a priori* basis, to be associated with drug dependence were not those which were most consistently identified in GWAS. Indeed, in analyzing the 96 genes in which clusters of positive SNPs were identified in the Liu et al. (2006) study, almost no monoaminergic system genes were found, whereas 28% of the genes associated with drug dependence were cell adhesion molecules, much higher than the overall representation of these genes in the genome. It must be noted that another possibility that might influence the sorts of genes in which allelic variation contributes to addiction could be the ability of particular genes to accumulate variation that might impact upon addiction. That is, certain genes may be too important, and consequently variation too deleterious, to accumulate variations that would impact upon addiction phenotypes, even if variation in those genes has the *possibility* of doing so as demonstrated in KO studies in mice. In this context it is important to note the deleterious effects of DAT KO and VMAT2 KO noted above.

Although GWAS have generally failed to identify many of the genes that were previously identified by candidate gene studies, this is not to say that those findings are necessarily erroneous. It would appear that they may be involved in particular endophenotypes and particular populations. In some cases, these effects may be quite strong. Thus, genetic variation in the *Oprm1* gene is generally associated with sensitivity to opiates, including both analgesic and addictive properties of this class of drugs, as well as others (for review see (Ikeda, et al., 2005)). The genetic differences underlying these associations are likely to be quite heterogeneous as more than 100 polymorphisms in the *Oprm1* gene have been demonstrated, many of which produce functional consequences on OPRM1 expression, binding affinity or other aspects of OPRM1 function.

This does not mean that such genes account for the majority of the genetic load associated with addiction; indeed, this is the real implication of GWAS. Even prior to the publication of the Liu et al (2006) study, the realization that the types of genes being identified in GWAS for drug dependence involved classes of genes likely to be involved in synaptic plasticity led to the suggestion that addiction is fundamentally a problem of altered mnemonic processes (G. R. Uhl, 2004). This finding is particularly interesting in the context of the emphasis on addiction phenomena such as craving and habit that are primarily conditioned responses (for review see (Robbins, Ersche, & Everitt, 2008)) and the evidence for synaptic changes after exposure to a variety of classes of addictive drugs (for review see (Badiani & Robinson, 2004)). The genes that have been identified in GWAS for drug dependence may be involved either in adjusting brain “wiring” prior to drug experiences or subsequent to drug experiences, or both. Based on this analysis, and consistent with the high comorbidity of drug dependence with other psychiatric disorders, it was suggested that GWAS for a number

of psychiatric conditions will identify overlapping sets of genes with pleiotropic influences (G. R. Uhl, Drgon, Johnson, Li, et al., 2008). This was confirmed for substance dependence and bipolar disorder in one analysis (Johnson, Drgon, McMahon, & Uhl, 2009) and is discussed in detail in Uhl et al. (2008). Furthermore, analysis of the genes associated with smoking cessation identified a plausible Bayesian network which could not be produced by random selection of SNPs (G. R. Uhl, et al., 2010). Analysis of genes associated with smoking initiation, nicotine dependence and smoking cessation found that these genes were enriched in particular in certain enzymatic and neuroanatomical pathways (J. Wang & Li, 2010). However, it must be noted that this was based upon both GWAS and candidate gene association studies which would be likely to bias the results towards *a priori* expectations (such as dopaminergic systems).

6. Conclusions

GWAS for drug-dependence have attained a high degree of replication, e.g. the same genes or gene loci being associated with drug dependence across a large number of studies. This concordance is far from 100%, but such an expectation would be untenable given the likelihood of a high degree of both gene and loci heterogeneity, and substantial differences in subject pools in terms of features of their underlying addictions (drugs of preference, degree of dependence, psychiatric comorbidities, core behavioral symptoms, etc.). Furthermore, complete concordance between studies (rather than a probabilistic overlap) should not be expected because it has become abundantly apparent that addiction, like most common diseases perhaps, is highly polygenic. These studies have also shown that when the genes which have been identified are considered as a whole, they are not the genes which, *a priori*, would have been thought to be involved in addiction – e.g. the overabundance of cell adhesion molecules. These genes thus represent a vastly untapped resource for understanding addiction, and comorbid pleiotropically mediated psychiatric conditions, as well as for developing new treatments for these conditions. With regard to the fundamental question posed by the title of this article, although GWAS have generally failed to identify many of the genes that were previously identified by candidate gene studies, this is not to say that those findings are necessarily erroneous. It would appear that they may be involved in particular endophenotypes and particular populations, but at the same time these genes do not account for the majority of the genetic influence on addiction liability.

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Abbreviations

CI	cocaine insensitive
DRD2	dopamine D ₂ receptor
DRD3	dopamine D ₃ receptor
DRD4	dopamine D ₄ receptor
DAT	dopamine transporter
DZ	dizygotic
GABRA3	GABA receptor subunit gene 3
GWAS	genome wide association studies
KO	knockout

METH	methamphetamine
MDMA	methylenedioxymethamphetamine
MNB	Molecular Neurobiology Branch
MZ	monozygotic
MOR	μ opioid receptor
NET	norepinephrine transporter
SERT	serotonin transporter
VMAT2	vesicular monoamine transporter 2
WT	wildtype

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Table 1

Summary of Candidate Gene Association Studies for Addiction

Study	Gene	Condition	Positive Association?
Cigler et al 2001	<i>HTR1B</i>	cocaine/alcohol dependence	No
Kranzler et al 2002	<i>HTR1B</i>	drug dependence	No
Vandenbergh et al 1997	<i>COMT</i>	drug dependence	Yes
Sander et al 1997 ⁽¹⁾	<i>DAT</i>	alcohol dependence	No
Guindalini et al 2006	<i>DAT</i>	cocaine dependence	Yes
Hong et al 2003	<i>DAT</i>	methamphetamine dependence	No
Liu et al 2004	<i>DAT</i>	methamphetamine dependence	No
Blum et al 1993	<i>DRD2</i>	alcohol dependence	Yes
Noble et al 1998	<i>DRD2</i>	alcohol dependence	Yes
Blomqvist et al 2000	<i>DRD2</i>	alcohol/drug dependence	No
Comings et al 1994 ⁽¹⁾	<i>DRD2</i>	alcohol/drug dependence	Yes
Gelernter and Kranzler 1999	<i>DRD2</i>	alcoholism	No
Comings et al 1999 ⁽⁶⁾	<i>DRD2</i>	cocaine dependence	Yes
Gelernter, Kranzler and Satel 1999	<i>DRD2</i>	cocaine dependence	No
Noble et al 1993	<i>DRD2</i>	cocaine dependence	Yes
Berrettini and Persico 1996	<i>DRD2</i>	drug dependence	No
Persico et al 1996	<i>DRD2</i>	drug dependence	Yes/No
Sery et al 2001	<i>DRD2</i>	methamphetamine dependence	No
Tsai et al 2002	<i>DRD2</i>	methamphetamine dependence	No
Lawford et al 2000	<i>DRD2</i>	opiate dependence	Yes
Comings et al 1999 ⁽⁶⁾	<i>DRD3</i>	cocaine dependence	Yes
Krebs et al 1998 ⁽²⁾	<i>DRD3</i>	substance abuse	Yes
Vandenbergh et al 2000	<i>DRD4</i>	drug dependence	Yes
Tsai et al 2002	<i>DRD4</i>	methamphetamine dependence	No
Kotler et al 1997	<i>DRD4</i>	opiate dependence	Yes
Agrawal et al 2006	<i>GABRA2</i>	drug dependence	Yes
Noble et al 1998	<i>GABRB3</i>	alcohol dependence	Yes
Zhang et al 2008	<i>OPRD1</i>	alcohol/drug dependence	Yes
Franke et al 1999	<i>OPRD1</i>	opiate dependence	No
Mayer et al 1997	<i>OPRD1</i>	opiate dependence	Yes
Xu et al 2002	<i>OPRD1</i>	opiate dependence	No
Xuei et al 2006	<i>OPRK1</i>	alcohol dependence	Yes
Zhang et al 2008	<i>OPRK1</i>	alcohol/drug dependence	Yes
Yuferov et al 2004	<i>OPRK1</i>	heroin dependence	No
Bergen et al 1997	<i>OPRM1</i>	alcohol dependence	No

Study	Gene	Condition	Positive Association?
Gelernter, Kranzler and Cubbells 1999	<i>OPRM1</i>	alcohol/drug dependence	No
Kranzler et al 1998	<i>OPRM1</i>	alcohol/drug dependence	Yes
Berrettini et al 1997	<i>OPRM1</i>	cocaine/opiate dependence	No
Hoehe et al 2000	<i>OPRM1</i>	drug dependence	Yes
Luo et al 2003 ⁽⁴⁾	<i>OPRM1</i>	drug dependence	Yes/No
Ide et al 2003 ⁽¹⁾	<i>OPRM1</i>	methamphetamine dependence	No
Bart et al 2004	<i>OPRM1</i>	opiate dependence	Yes
Bond et al 1998	<i>OPRM1</i>	opiate dependence	Yes
Crowley et al 2003	<i>OPRM1</i>	opiate dependence	No
Franke et al 2001	<i>OPRM1</i>	opiate dependence	No
Li et al 2000	<i>OPRM1</i>	opiate dependence	No
Shi et al 2002	<i>OPRM1</i>	opiate dependence	No
Szeto et al 2001	<i>OPRM1</i>	opiate dependence	Yes
Tan et al 2003	<i>OPRM1</i>	opiate dependence	Yes/No
Xuei et al 2006 ⁽³⁾	<i>PDYN</i>	alcohol dependence	Yes
Chen et al 2002	<i>PDYN</i>	cocaine dependence	Yes
Dahl et al 2005	<i>PDYN</i>	cocaine dependence	Yes
Zimprich et al 2000	<i>PDYN</i>	opiate dependence	No
Comings et al 1999	<i>PENK</i>	opiate dependence	Yes
Kranzler et al 2002	<i>SERT</i>	alcohol dependence	No
Sander et al 1997 ⁽¹⁾	<i>SERT</i>	alcohol dependence	No
Patkar et al 2001	<i>SERT</i>	cocaine dependence	No
Hong et al 2003	<i>SERT</i>	methamphetamine dependence	No
Dahl et al 2006	<i>TPH2</i>	cocaine dependence	No

Summary of candidate gene studies for different drug dependence samples. The last column denotes whether a positive association was identified. Yes/No denotes a qualified positive finding, pertaining to positive finding in part of the sample but not others.

Abbreviations: *HTR1B*, serotonin receptor 1B; *COMT*, catechol-O-methyl transferase; *DAT*, dopamine transporter; *DRD2*, dopamine receptor D2; *DRD3*, dopamine receptor D3; *DRD4*, dopamine receptor D4; *GABRA2*, GABA receptor 2; *GABRB3*, GABA receptor 3; *OPRD1*, opioid receptor; *OPRK1*, opioid receptor; *OPRM1*, μ opioid receptor; *PDYN*, preprodynorphin; *PENK*, preproenkephalin; *SERT*, serotonin transporter; *TPH2*, tryptophan hydroxylase 2

⁽¹⁾ Association primarily with specific addiction related phenotypes

⁽²⁾ Schizophrenic substance abusers

⁽³⁾ Significant association only in 1 of 3 Asian ethnic groups

⁽⁴⁾ Significant in European Americans, but not African Americans

⁽⁵⁾ Association with psychostimulant preference

⁽⁶⁾ Association with cocaine dependence but not dependence on other drugs

Table 2

Genes repeatedly identified in GWAS

Gene	Description	Class	# samples with clustered positive SNPs	P value
<i>BAI3</i>	brain-specific angiogenesis inhibitor	CAM	7	p<0.00001
<i>CDH13</i>	cadherin 13	CAM	16	p<0.00001
<i>CLSTN2</i>	calsyntenin 2	CAM	14	p<0.00001
<i>CNTNAP2</i>	contactin-associated protein-like 2	CAM	13	p<0.00001
<i>CSMD1</i>	CUB and Suxhi multiple domains 1	CAM	18	p<0.00001
<i>CTNNA2</i>	catenin 2	CAM	12	p<0.00001
<i>DAB1</i>	diablated homolog 1	CAM	13	p<0.00001
<i>DSCAM</i>	Down Syndrome cell adhesion molecule	CAM	13	p<0.00001
<i>NRXN1</i>	neurexin 1	CAM	12	p<0.00001
<i>PTPTD</i>	receptor protein tyrosine phosphatase D	CAM	13	p<0.00001
<i>SGCZ</i>	sarcoglycan zeta	CAM	15	p<0.00001
<i>ASTN2</i>	astrotactin 2	CAM	14	p<0.00007
<i>CNTN4</i>	contactin 4	CAM	11	p<0.00011
<i>CNTN6</i>	contactin 6	CAM	10	p<0.00012
<i>LRP1B</i>	low-density lipoprotein-related protein 1B	CAM	14	p<0.00015
<i>NRG1</i>	neuregulin 1	CAM	8	p<0.00024
<i>ITGB8</i>	integrin 8	CAM	6	p<0.00026
<i>PTPRM</i>	receptor protein tyrosine phosphatase M	CAM	12	p<0.00029
<i>ROR1</i>	receptor tyrosine kinase-like orphan rec 1	CAM	9	p<0.00029
<i>TRIO</i>	triple functional domain/PTPRF interact	CAM	7	p<0.00069
<i>CSMD2</i>	CUB and Suxhi multiple domains 2	CAM	11	p<0.00083
<i>CNTN5</i>	contactin 5	CAM	12	p<0.00098
<i>CTNNA3</i>	catenin 3	CAM	11	p<0.00109
<i>LRRN6C</i>	leucine rich repeat neuronal 6C	CAM	14	p<0.00134
<i>CTTND2</i>	catenin 2	CAM	8	p<0.00327
<i>ANKS1B</i>	ankyrin repeat sterile domain 1B	CAM	8	p<0.00341
<i>SEMA3C</i>	semaphorin 3C	CAM	4	p<0.00631
<i>RYR3</i>	ryanodine receptor 3	CHA	14	p<0.00001
<i>CAMK1D</i>	calcium/calmodulin-dependent protein kinase 1D	ENZ	11	p<0.00001
<i>CHN2</i>	chimerin 2	ENZ	10	p<0.00001
<i>FHIT</i>	fragile histidine triad gene	ENZ	16	p<0.00001
<i>PRKG1</i>	cGMP-dependent protein kinase I	ENZ	17	p<0.00001
<i>SERPINA1</i>	serpin peptidase inhibitor A 1	ENZ	6	p<0.00001
<i>LARGE</i>	like-glycosyltransferase	ENZ	11	p<0.00002
<i>UST</i>	uronyl-2-sulfotransferase	ENZ	7	p<0.00017

Gene	Description	Class	# samples with clustered positive SNPs	P value
<i>DAPK1</i>	death-associated protein kinase 1	ENZ	8	p<0.00024
<i>GALNTL4</i>	UDPNac- D-galactosamine:polyptide Nacgalactosaminyltransferase-like 4	ENZ	9	p<0.00040
<i>CAPN13</i>	calpain 13	ENZ	7	p<0.00042
<i>PDE4D</i>	cAMD-specific phosphodiesterase 4D	ENZ	10	p<0.00055
<i>AKAP13</i>	protein kinase A anchor protein 13	ENZ	9	p<0.00062
<i>ST6GALNAC3</i>	NAcneuraminy1-2,3- galactosyl-1,3- Nacgalactosaminide 2,6-sialyltransferase 3	ENZ	11	p<0.00080
<i>SERPINA2</i>	serpin peptidase inhibitor A 2	ENZ	4	p<0.00138
<i>DGKB</i>	diacylglycerol kinase	ENZ	9	p<0.00138
<i>FGF14</i>	fibroblast growth factor 14	LIGAND	10	p<0.00001
<i>GRM7</i>	metabotropic glutamate receptor 7	REC	11	p<0.00001
<i>GPR39</i>	G protein-coupled receptor 39	REC	6	p<0.00033
<i>ABCC4</i>	ATP-binding cassette C4	TRANSP	7	p<0.00001
<i>SLC24A4</i>	solute carrier family 24 member 3	TRANSP	10	p<0.00033
<i>XKR4</i>	XK family member 4	TRANSP	9	p<0.00091
<i>SLC9A9</i>	solute carrier family 9 member 9	TRANSP	10	p<0.00134

Summary of genes repeatedly identified in GWAS for drug dependence and related phenotypes, after Uhl et al., (2008). P values represent Monte Carlo analyses of the likelihood of repeatedly seeing each gene in multiple GWAS.