Review

Genetics of addictions: strategies for addressing heterogeneity and polygenicity of substance use disorders

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Addictions are common psychiatric disorders that exert high cost to the individual and to society. Addictions are a result of the interplay of multiple genetic and environmental factors. They are characterized by phenotypic and genetic heterogeneity as well as polygenicity, implying a contribution of different neurobiological mechanisms to the clinical diagnosis. Therefore, treatments for most substance use disorders are often only partially effective, with a substantial proportion of patients failing to respond. To address heterogeneity and polygenicity, strategies have been developed to identify more homogeneous subgroups of patients and to characterize genes contributing to their phenotype. These include genetic linkage and association studies as well as functional genetic analysis using endophenotypes and animal behavioural experimentation. Applying these strategies in a translational context aims at improving therapeutic response by the identification of subgroups of addiction patients for individualized, targeted treatment strategies. This article aims to discuss strategies addressing heterogeneity and polygenicity of substance use disorders by presenting results of recent research on genetic and environmental components of addiction. It will also introduce the European IMAGEN study that aims to integrate methodical approaches discussed in order to identify the genetic and neurobiological basis of behavioural traits relevant to the development of addictions.

Keywords: addictions; animal models; genetic; heterogeneity; neuroimaging; translational

1. INTRODUCTION

Drug addiction is characterized as a compulsion to take the substance with a narrowing of the behavioural repertoire towards excessive substance intake, and a loss of control in limiting intake (American Psychiatric Association 1994). Addictions are chronic, often relapsing, common psychiatric disorders that exert tremendous cost on the individual and the society. According to the World Health Organization (WHO), there is an estimate of 2 billion alcohol users, 1.3 billion tobacco users and 185 million illicit drug users worldwide. The substantial social and economic costs of addiction are exemplified by the National Health Service of the UK, where £385 million is spent on drug treatment per year (Home Office 2007, Internet communication, <http://www.homeoffice.gov.uk/>). Despite the substantial sum of money spent on treatments for substance use disorder, effect sizes of all available therapies offered are typically modest with a substantial proportion of patients failing to respond. Advancement of understanding in the neurobiology and genetics of addiction is crucial for the future development of better and more effective interventions for addictive behaviour and substance use disorders. Elucidation of genetic factors underlying

inter-individual differences in substance use behaviour will result in the individualization of therapies and targeting of specific clusters of patients using psychotherapeutic or pharmacological approaches, which include the prediction of therapeutic response based on pharmacogenetic profiles. The aim of this review is to evaluate the different approaches that have been employed to elucidate the genetics of addiction.

2. HERITABILITY OF SUBSTANCE USE DISORDER

Addiction is a complex disorder and a genetic component has long been established as a contributor for inter-individual differences in vulnerability. The overall genetic influence for substance use disorders has proved to be substantial and heritabilities for most substance use disorders are estimated to be moderate to high. Family and adoption studies showed that biological offspring of alcoholics are three to five times more likely to develop alcohol dependence than individuals with no family history of alcoholism (Cotton 1979). Heritability for alcoholism is estimated to be between 50 and 60% and is comparable among male and female alcoholics (Heath *et al.* 1997; see Kohnke 2007). Past studies demonstrated that genetic factors not only have influence on substance abuse, but are also involved in the different dimension of substance-taking behaviours (see Schumann 2007). Heritabilities for substance

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Figure 1. Relationship between phenotypic heterogeneity, genetic heterogeneity and polygenicity in alcohol use disorders.

initiation and use are generally lower than for problematic substance use, abuse and dependence (McGue *et al.* 1992; True *et al.* 1997). A twin/sibling study in Colorado ($n=1000$) found a modest to moderate heritability for alcohol initiation or quantity/frequency of alcohol use, but a substantial heritability of 0.78 for alcohol use in adolescents (Rhee *et al.* 2003).

3. SUBSTANCE USE DISORDERS ARE POLYGENIC DISORDERS WITH A COMPLEX INHERITANCE PATTERN AND RESULT FROM INTERACTIONS BETWEEN THE INDIVIDUAL, THE DRUG AND THE ENVIRONMENT

Substance use disorders are results of an interaction between drug, host and environment. They are common, complex disorders that do not conform to a simple Mendelian transmission pattern and involve multiple genes and environment ($G \times E$) interactions (Enoch & Goldman 1999; see Goldman 1993). The complex genetic constitution is partly accounted for by heterogeneity and polygenicity, which are parallel mechanisms that are present to varying degrees in different substance use disorders (figure 1). Heterogeneity assumes that a single or a few genetic variation(s) determine vulnerability and resiliency, but different alleles would lead to the same clinical presentation in different individuals (see Goldman *et al.* 2005). The concept of polygenicity, on the other hand, assumes that a phenotype is a result of simultaneous function of multiple genetic variants. In substance use disorders, polygenicity may include drug-specific genes that contribute to different responses as well as common genes such as comorbidity-related genes and those that alter environmental vulnerability (see Schumann 2007).

4. SUBSTANCE USE DISORDERS COMPRISE A VARIETY OF BEHAVIOURAL AND ENVIRONMENTAL CHARACTERISTICS THAT RESULT IN PHENOTYPICAL HETEROGENEITY

Phenotypic heterogeneity is extensive in the manifestation of alcoholism, with alcoholics differing in magnitudes such as age of onset of problems, alcohol

symptoms, drinking history and comorbid disorders (see Dick & Foroud 2003). One of the approaches to reduce phenotypic heterogeneity is by classification of more genetically or neurobiologically homogeneous phenotypes. Based on an adoption study and a neurobiological learning model, alcoholism is differentiated into two subtypes of more genetically homogeneous phenotypes (Cloninger 1987a,b). Type I alcoholism is characterized by late onset of alcohol problems and low inheritance, as well as a low degree of novelty seeking and psychological dependence, which are coupled with guilt and fear about alcoholism. Type II alcoholism is associated with early onset (prior to the age of 25), the presence of antisocial behaviour, elevated novelty seeking and reduced harm avoidance (Bau *et al.* 2001). This subtyping of alcoholism into more homogeneous groups has shown to reduce some phenotypic heterogeneity. However, identification of single genes that account for a large amount of variance within a phenotype still remains challenging. Therefore, geneticists have turned to endophenotypes as a way of dealing with the substantial heterogeneity involved in substance use disorder.

5. ENDOPHENOTYPES CHARACTERIZE HOMOGENEOUS GROUPS OF INDIVIDUALS BASED ON NEUROBIOLOGICAL CRITERIA

Endophenotypes are defined as the measurable intermediates between an observed disorder and the biological processes responsible for the manifestation of that disorder (Gottesman & Gould 2003). As suggested by Gottesman & Gould, endophenotypes may be heritable intermediate phenotypes that are neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive or neuropsychological. The deployment of endophenotypes in exploring the aetiology of disease is justified by being more homogeneous in nature and provides simpler clues to genetic underpinning than the disease syndrome itself. Endophenotypes allow the identification of the 'downstream' traits of clinical phenotypes as well as the 'upstream' effects of genes and are likely to be influenced by variation at fewer genes (figure 2). By

Figure 2. Relationship between endophenotypes, disease (phenotype) and aetiological factors including genes, environment and gene–environment interaction. A_1 and A_2 represent, respectively, the alcohol dehydrogenase 2 (*ADH2*) and aldehyde dehydrogenase 2 (*ALDH2*) polymorphisms that are associated with reduced alcohol consumption in the southern Asian population. A_3 represents the association of *CHRM2* with low amplitude of P300 event-related potential (ERP). A_n , additive genetic factors; $A \times E$, gene–environment interaction; E_{1-m} , environmental factors.

decomposing or deconstructing psychiatric diagnoses into their intermediate phenotypes, the complexity of genetic and phenotype analysis can be reduced. While the concept of endophenotype in psychiatry was developed 35 years ago (Gottesman & Shields 1973), technological advances have served to increase its relevance in recent years.

The search for endophenotypes of substance use disorder has been extensive in the past years with some fine successes. In alcoholism, several endophenotypes have been identified including the low-amplitude P300 event-related potential (ERP) robustly related to alcoholism and the observation of facial flushing syndrome predominantly in Southeast Asians (see Enoch *et al.* 2003). The P300, also known as P3, is an evoked electroencephalographic brain potential that is thought to index the physiologic correlate of attention allocation, basic information processing and the activation and maintenance of working memory (Polich & Herbst 2000). Reduced amplitude of P300 was observed in offspring of alcoholic families, regardless of whether the offspring are themselves alcoholics (Begleiter *et al.* 1984). The potential use of P300 amplitude as an endophenotype of alcoholism was further endorsed by a carefully characterized study using data from the Collaborative Study on the Genetics of Alcoholism (COGA) that consists of more than 1800 families, representing over 12 000 individuals (Hesselbrock *et al.* 2001). A reduced level of P300 amplitude was observed in alcoholics, unaffected relatives of alcoholics and unaffected offspring of an alcoholic father, when compared correspondingly with non-alcoholics, unaffected relatives of controls and offspring of controls. The use of electrophysiological data-based endophenotype has led to the subsequent identification of a muscarinic cholinergic receptor gene, *CHRM2*, as a gene associated with predisposition to alcohol dependence (Dick *et al.* 2006). Another useful example of endophenotype is the observation of the facial flushing syndrome

mainly in Southeast Asians, which discourages alcohol intake. This observation not only led to the discovery of genetic variation of alcohol metabolizing genes, alcohol dehydrogenase 2 and 3 genes (*ADH2* and *ADH3*) and aldehyde dehydrogenase 1 and 2 genes (*ALDH1* and *ALDH2*), but the reduced alcohol consumption in half of the southern Asian population was also in part explained by the presence of special functional variants of *ADH2* (Arg47His) and *ALDH2* (Glu487Lys), which lead to the reduced metabolism of alcohol and thus confer a protective effect against the development of alcoholism (Thomasson *et al.* 1991; Radel & Goldman 2001).

One of the technological advancements that greatly increases the use of endophenotypes in the assessment of $G \times E$ interaction in psychiatry is functional neuroimaging including functional magnetic resonance imaging (fMRI), positron emission tomography and magnetic encephalography. These technologies permit the measurement of information processing in discrete brain circuits that might not be immediately observable phenotypically but may be linked to the functional expression of disorder states (see Hariri & Weinberger 2003). These unique properties of functional neuroimaging have been used in the studies investigating $G \times E$ interactions. For example, the potential association between the activation of specific brain regions to negative affective stimuli and selected genetic variations has been investigated extensively in fMRI studies. In these studies, brain activity is measured by metabolic activity represented by changes in blood flow in specific brain areas following the presentation of a stimulus (BOLD response). These findings are analysed for association with genetic variations that typically are selected for a known biochemical effect on neurobiological pathways relevant to substance use disorders. An association of a serotonin transporter (*5HTT*) genotype *ll* with increased amygdala activation during the presentation of aversive but not pleasant stimuli has been reported by several individual groups (Hariri *et al.*

Figure 3. Neurotransmitter systems and environmental factors that have been associated with alcohol use disorders.

2002; Heinz *et al.* 2005). Moreover, an additive effect of this *l/l* genotype, an A/G substitution of the *5HTT* and a Val158Met polymorphism of the catechol-O-methyltransferase (*COMT*) gene have been reported to be responsible for approximately 40% of the inter-individual variance in the averaged BOLD response of amygdala, hippocampal and limbic cortical regions elicited by unpleasant stimuli (Smolka *et al.* 2005, 2007). Findings from these genetic–neuroimaging studies demonstrate the great potential of functional brain imaging in assessing G×E interaction on the function of neuronal networks.

The use of endophenotypes in complex diseases has been supported by a recent study that compared the effect sizes of genes in complex diseases with those that have been discovered to influence intermediate phenotypes. As predicted, genes influencing endophenotypes demonstrated a greater effect size, and thus endophenotypes are more powerful for discovering potential genes involved in the regulation of brain activity (Goldman & Ducci 2007). These discoveries have prompted the development of integrated approaches to identify the genetic and neurobiological basis of behavioural traits relevant to the development of substance use. An example of such an integrated approach is the IMAGEN project (www.imagen-europe.com): reinforcement-related behaviour in normal brain function and psychopathology, which is funded as part of the sixth framework programme of the European Commission. The goal of this study is to identify the genetic and neurobiological basis of traits that influence individual differences in brain responses to reward, punishment and emotional cues in adolescents, and mediate risk for mental disorders with a major public health impact. Functional and structural genetic–neuroimaging study will be performed on a cohort of more than two thousand 14-year-old adolescents who are being recruited at seven centres in the UK, Germany, France and Ireland. Endophenotypes of risk for adolescent mental illness will be explored based on cognitive, behavioural, clinical and neuroimaging data using 3T scanners. To determine the predictive value of intermediate phenotypes and genetics for the development of mental disorders, the cohort will be psychometrically assessed

during recruitment and longitudinally at year 4 (age 16–18) of the project. Neuroimaging permits the measurement of specific brain functions implicated in the aetiology of mental disorders and links them to genetic variations and behavioural characteristics relevant to disease processes. In addition, DNA samples and a phenotype database for the cohort will create a powerful resource for the present and future genetic investigations. In this study, a whole-genome scan in humans will be performed and the results will be compared with the transcriptional activation patterns of animals selected for extreme phenotypes of impulsivity and other relevant behavioural traits. Results obtained will be validated in 1000 siblings from the Canadian Saguenay youth neuroimaging study. The IMAGEN study will help elucidate the neural basis of substance use disorders as well as the environmental factors influencing pathological processes that contribute to addictions. It will, thus, lay the groundwork for the development of treatments that target specific neurobiological mechanisms rather than heterogeneous categories of mental illness.

6. GENE×ENVIRONMENT INTERACTIONS: GENETIC VARIATIONS MEDIATE ENVIRONMENTAL INFLUENCES ON SUBSTANCE USE DISORDERS

Adoption studies have demonstrated that individuals who have the most susceptibility to alcohol abuse are those with both genetic and familial environmental risks, namely an alcohol-dependent biological parent and an alcoholic adoptive parent (Cloninger *et al.* 1981; Cadoret *et al.* 1986). Findings from past studies have revealed the association of multiple genetic and environmental factors with alcohol use disorders (figure 3). A Finnish twin study of alcohol use among adolescents further exhibited the importance of G×E interaction in alcoholism by demonstrating a varied magnitude of genetic influences, with up to fivefold differences, in different environments (Dick *et al.* 2001).

Social environment is a known influential factor for substance use disorders, and environmental stress has long been established as one of the main external risk factors for alcohol abuse, including binge drinking and alcohol dependence (Pohorecky 1991; Aseltine & Gore

2000; Schmidt *et al.* 2000). Maternal stress, lack of normal parental care and stressful life events as well as childhood physical maltreatment, delinquent peer groups and head injury are some examples of environmental stress (Caspi & Moffitt 2006). Environmental stress is associated with the perpetuation of alcohol abuse, relapse and aggravation of alcohol use disorder (Brady & Sonne 1999; Sinha 2001). Increased response to psychosocial stress and enhanced dampening effects of alcohol were observed in non-alcoholic sons of alcoholic fathers when compared with family history-negative subjects (Zimmermann *et al.* 2004).

7. PSYCHOSOCIAL STRESS IS AN IMPORTANT RISK FACTOR FOR SUBSTANCE USE DISORDERS AND IS MEDIATED BY GENETIC VARIATIONS IN THE CORTICOTROPHIN-RELEASING HORMONE SYSTEM

The hypothalamic–pituitary–adrenal (HPA) axis is a fundamental neuroendocrine system that facilitates and regulates stress reaction (see Clarke *et al.* 2008). Upon the activation of the HPA axis, a cascade of hormones including vasopressin, corticotrophin-releasing hormone (CRH), adrenocorticotrophic hormone and glucocorticoids are secreted (Tsigos & Chrousos 2002). In a CRH receptor 1 (*CRHR1*) knockout (KO) model, mice lacking a functional *CRHR1* receptor demonstrated diminished stress response and the effect could not be compensated by other systems or by the highly homologous *CRHR2* receptor (Timpl *et al.* 1998). The critical role of *CRHR1* in mediating G×E interaction that affects drinking behaviour was illustrated by the identification of a genetic variant in the promoter region of *CRHR1* in Marchigian-Sardinian Preferring (msP) rats, genetically selected for alcohol preference (Hansson *et al.* 2006). This polymorphism of *CRHR1* results in higher expression of *CRHR1* in msP rats when compared with Wistar control rats, and was associated with increased sensitivity to relapse into alcohol seeking induced by environmental stress. Recent human studies have also demonstrated interaction between genetic variants of *CRHR1* and environmental stress on alcohol drinking behaviour. Association of *CRHR1* with specific patterns of alcohol consumption was reported in two independent samples of alcohol-naïve adolescent and alcohol-dependent adult samples (Treutlein *et al.* 2006). Significant group differences between *CRHR1* genotypes were observed in binge drinking, lifetime prevalence of alcohol intake and drunkenness in the adolescent samples while an association of *CRHR1* with high amounts of drinking was demonstrated in the adult alcohol-dependent samples. The G×E interaction of *CRHR1* genotypes and environmental stress on alcohol consumption is further supported by a follow-up study demonstrating an association of polymorphism in *CRHR1* and stressful life events with heavy adolescent alcohol use (Blomeyer *et al.* 2008). Adolescents homozygous for the C allele of rs242938 drank higher maximum amounts of alcohol per occasion and showed greater lifetime rates of heavy drinking than individuals with the T allele in relation to negative life events. These results illustrated the

potential importance of inter-individual differences in stress response on drug-taking behaviour. Further functional analyses of HPA axis genes will aid the characterization of molecular mechanisms in HPA axis regulation and define their role for substance use disorders.

As substance use disorders are complex, multifactorial disorders where a phenotype is likely to be the effect of multiple genetic variants with no main gene effect, the identification of specific genes contributing to well-defined phenotypes of substance use disorders would facilitate the elucidation of underlying genes and deconstruction of the complex genetic network for substance use disorders. A number of gene identification strategies have been used in the study of alcoholism including both linkage and association studies. In whole-genome linkage studies, the inheritance of polymorphic markers within families is used to identify chromosomal regions where susceptibility genes may reside. Linkage approaches to map chromosomal regions linked to alcohol use and dependence have been extensive with some substantial results, predominantly from the COGA. COGA is a large, family-based study whose goal is to detect and map susceptible genes for alcoholism and alcohol-related characteristics and behaviour (Dick *et al.* 2006). Increased susceptibility to alcohol use and dependence has been linked to regions on several chromosomes, including chromosomes 1, 2, 3, 7 and 8 (see Edenberg & Foroud 2006). Two regions on chromosome 4p, by contrast, showed significant and suggestive linkage to non-alcoholic sibling pairs in the COGA study, which suggested a protective factor in these two regions of chromosome 4 (Reich *et al.* 1998). Regions that showed significant and suggestive linkage to chromosome 4p were close to the *ADH* gene cluster and the gamma-aminobutyric acid receptor A (*GABA_A*) gene cluster, respectively. Linkage findings on chromosome 4p have validated the role of genetic variation of *ADH* in conferring a protective effect against alcohol dependence. Subsequent analysis on the *GABA_A* receptor gene cluster has resulted in the identification of a significant association between genetic variants of *GABRA2* with both alcohol dependence and brain oscillations in beta frequency range (Edenberg *et al.* 2004). In addition to the phenotype of alcohol dependence, several chromosomal regions have been linked to intermediate phenotypes, including a severity phenotype to chromosome 16, low level of response to alcohol to chromosomes 1, 2, 9 and 21 and MAXDRINKS, a quantitative phenotype defined as the maximum number of drinks consumed in a 24 hour period, to the *ADH* region of chromosome 4 (Foroud *et al.* 1998; Saccone *et al.* 2000; Schuckit *et al.* 2001).

Linkage analysis has been demonstrated to have limited power for the identification of common genetic variants that have modest effects on disease. A study examining the power of linkage studies in locating disease genes demonstrated that a sample size of approximately 700 affected sibling pairs (ASPs) was required for the detection of loci with high genotype relative risks ($g \geq 4$) and intermediate allele frequencies ($p = 0.05$ – 0.50). For loci with similar allele frequency ($p = 0.30$ – 0.40) but more modest relative risks ($g \leq 2$),

a large sample size of approximately 3000 ASPs would be needed for their detection. Association studies, by contrast, demonstrated adequate power in detecting genes of low relative risks in smaller sample size. Statistical evidence for loci with modest genotype relative risks ($g \leq 2$) and intermediate allele frequencies ($p = 0.10\text{--}0.70$) was provided by a sample size of approximately 700 ASPs in association study. Moreover, loci with intermediate allele frequencies ($p = 0.20\text{--}0.70$) but lower relative risks ($g \leq 1.5$) could be detected in association study by a sample size of approximately 1000 ASPs (Risch 2000). Owing to the low sensitivity of linkage analysis, association studies have been used for the identification of variants in complex polygenic disorders that show no main gene effect. In association studies, correlations between genetic variants and trait differences on a population scale are assessed (see Cardon & Bell 2001). Frequency of alleles or genotypes of a particular variant is compared between disease cases and controls. Any significant differences in allele frequencies between cases and controls are taken as evidence for the involvement of an allele in disease susceptibility. Candidate gene association studies have some advantages over linkage studies, including a more straightforward sample collection process and a greater power to detect variants of the modest effect size. However, findings from association studies are not always replicable and are prone to be the consequence of type I error.

8. FUNCTIONAL CHARACTERIZATION OF CANDIDATE GENES USING BEHAVIOURAL ANIMAL MODELS IS USEFUL FOR DISSECTING GENETIC HETEROGENEITY

Association analyses based on candidate genes derived from behavioural animal experiments, pharmacological data, neurobiological findings or previously determined region of linkage have led to the identification of several susceptible genes for substance use disorders involving different neurotransmitter systems, such as dopamine, glutamate, GABA, opioids, CRH, noradrenaline, serotonin, cannabinoids and circadian rhythm system. Detailed reviews of candidate genes for alcoholism have been published elsewhere (see Dick & Fourud 2003; Gorwood *et al.* 2006). In this article, we will focus on specific candidate genes identified from our own work as well as a genetic variation where translational relevance to clinical application has been demonstrated: a functional polymorphism, A118G (Asn40Asp), of μ -opioid receptor gene (*OPRM1*) was associated with an attributable risk to alcohol dependence and greater feelings of reward during intravenous administration of alcohol to binge drinkers (Ray & Hutchison 2004; Bart *et al.* 2005). This same polymorphism in exon 1 of *OPRM1* has been demonstrated to predict the clinical response to naltrexone in alcohol-dependent individuals (Oslin *et al.* 2003). Under treatment of naltrexone, a competitive opioid receptor antagonist, individuals with one or two copies of the Asp40 allele showed significantly lower rates of relapse and a longer time to return to heavy drinking than those homozygous for the Asn40 allele. These findings on the effect of genetic

variation of opioid receptor on alcoholism highlight the fundamental role of the opioid system in alcohol reinforcement and provide an example for clinically relevant pharmacogenetic approaches to predict response to treatment.

Recent findings have provided evidence for the involvement of the glutamate system in acute and chronic effects of alcohol on the brain. The glutamatergic hypothesis suggests that alcohol consumption leads to enhanced glutamatergic activity in alcohol-dependent patients, and the glutamate-induced hyperexcitability is uncovered during alcohol withdrawal (Tsai & Coyle 1998; Gass & Olive 2008). The hypothesis also suggests the contribution of augmented glutamatergic activity to craving and relapse behaviour, as evidenced by the clinical use of anti-glutamatergic compounds such as acamprosate for relapse prevention (Spanagel & Kiefer 2008).

Alterations in different levels of glutamatergic neurotransmission including presynaptic, synaptic, post-synaptic and intracellular signalling have been associated with drinking behaviour in animal models and biochemical experimentation (Vengeline *et al.* 2008). Glutamate receptors are primary targets of alcohol action, and a compensatory upregulation of N-methyl-D-aspartate (NMDA) receptor subunits, mainly NR1, NR2A and NR2B, has been established in chronic alcohol exposure. This upregulation results in hyperexcitatory state in periods of acute and conditioned alcohol withdrawal (Gulya *et al.* 1991). The metabotropic glutamate receptor 5 (mGluR5) was shown to modulate alcohol self-administration and relapse behaviour in rodents (Backstrom *et al.* 2004; Cowen *et al.* 2005). Initiation of an intracellular signalling pathway involving calmodulin-dependent kinase IV (CamKIV) and the transcription factor cAMP-response element-binding protein 1 (CREB) upon activation of NMDA receptors has been implicated in alcohol withdrawal and self-administration in alcohol-preferring rats (Pandey *et al.* 2001, 2005). Glutamate-induced activation of CREB also occurs through a parallel pathway where mGluR5 and NMDA receptor signalling converges on phosphatidylinositol 3 kinase (PI3K; Paul & Skolnick 2003). Alcohol sensitivity and self-administration in KO models have been associated with neuronal nitric oxide synthase (nNOS) and GMP-kinase2, which are activated by PI3K (Spanagel *et al.* 2002; Werner *et al.* 2004; Crabbe *et al.* 2006). Synaptic concentration of glutamate is regulated by glutamate transporters such as glutamate transporter-1 (GLT-1) and glutamate aspartate transporter (GLAST), to name a few (Tanaka 2000). Elevated synaptic glutamate concentration and increased amount of alcohol intake have been associated with decreased expression levels of GLAST in a recent animal study (Spanagel *et al.* 2005).

The contribution of glutamatergic transmission genes to alcohol-drinking behaviours is supported by a human genetic study that investigates the association between variations of glutamatergic neurotransmission genes and alcohol dependence (Schumann *et al.* in press). Ten glutamatergic neurotransmission genes were selected for their known alteration of alcohol-drinking behaviour in animal models. These genes

include GLAST, NMDA receptor subunits NR1, NR2A and NR2B, mGluR5, nNOS, cGMP-kinase II, CamKIV, the regulatory subunit of PI3K and CREB. Haplotype SNPs tagging functional domains were genotyped in two large independent samples of alcohol-dependent patients and one sample of adolescents. NR2A showed the highest relevance to human alcohol dependence (OR=2.18), in particular in patients with a positive family history, early onset of alcoholism and high maximum number of drinks in adults. NR2A was also associated with harmful drinking patterns in adolescents, suggesting a role of this gene, both in initiation and in maintenance of alcohol dependence.

Phosphorylation of NR2A receptor is dependent on an src-like protein tyrosine kinase (PTK) fyn (Masood *et al.* 1994; Fink & Gothert 1996). Interestingly, mice with a deleted fyn gene show enhanced alcohol sensitivity and lack of tolerance to the effects of alcohol (Miyakawa *et al.* 1997). The role of PTK fyn in human drinking behaviour was established by a study showing an association of a genetic variation of PTK fyn gene with alcohol dependence in two independent samples (Schumann *et al.* 2003).

Alcohol dependence has long been associated with disruptions of circadian rhythmicity, including sleep patterns, body temperature, blood pressure and hormone secretion (Reppert & Weaver 2002). The regulation of circadian rhythm is dependent on the intertwined positive and negative regulatory loops involving the *Period* (*Per*), *Cryptochrome* (*Cry*), *Bmal1*, *Clock* and *Rev-Erba* genes (Leloup & Goldbeter 2003). Association of genetic variations in the clock gene *Per2* with alcohol-taking behaviour has been demonstrated by a recent study where amount of alcohol consumption was shown to be associated with a haplotype in the *Per2* gene (Spanagel *et al.* 2005). This finding was complemented by an animal study by the same group that showed a significant enhanced alcohol consumption and preference in *Per2*^{Brdm1} mutant mice. The enhanced incentive motivation to consume more alcohol than control animals was explained by a hyperglutamatergic state in the brain reinforcement system of *Per2*^{Brdm1} mutant mice due to a downregulation of the glutamate transporter GLAST. Phenotypes of mutant mice were rescued by treatment of acamprosate, a drug that is thought to act primarily by dampening a hyperglutamatergic state and is used clinically for craving and relapse prevention in alcoholic patients. In this study, glutamate was shown to be an effector of *Per2*, thus providing a mechanistic hypothesis as to how circadian rhythm genes can influence addictive behaviour. The ascertainment of glutamate as a link between the dysfunction of the *Per2* gene and the enhanced alcohol intake further bolsters the fundamental role of the glutamatergic system in alcohol-drinking behaviour.

The role of circadian rhythm system in drug addiction was first demonstrated in the fruit fly, *Drosophila melanogaster*. In flies mutant for circadian genes including *period*, *clock*, *cycle* and *doubletime*, sensitization to repeated cocaine exposure, a phenomenon possibly related to drug craving (see article by Robinson & Berridge 2008), was eliminated (Andretic

et al. 1999). In mice, a hypersensitized response was observed in *Per2*^{Brdm1} mice after a course of repeated cocaine administration, whereas behavioural sensitization is absent in *Per1*^{Brdm1} mice (Abarca *et al.* 2002). These findings accentuate the contribution of circadian rhythm system in substance-taking behaviour.

9. WHOLE-GENOME ASSOCIATION ANALYSES ALLOW SIMULTANEOUS IDENTIFICATION OF GENES CONTRIBUTING TO POLYGENICITY OF SUBSTANCE USE DISORDERS

While single candidate gene studies based on functional characterization of genes in animal models may effectively address genetic heterogeneity, successful identification of a candidate gene represents only a fraction of genetic risk factors in polygenic complex disorders such as alcoholism (see Hirschhorn & Daly 2005). With the limitations seen with linkage and candidate gene association studies, the potential advantage of genome-wide association (GWA) studies was forecast by Risch & Merikangas (1996). A GWA approach is defined as an association study that surveys most of the genome for causal genetic variants, without the requirement of prior pathophysiological knowledge about the disease or any prediction of candidate genes. With the advancement of molecular genetic technologies, systematic association study of the whole genome can now be conducted using DNA chips or arrays. Application of the GWA approach in genetic studies of multifactorial complex disorders such as myocardial infarction, age-related macular degeneration, breast cancer, Crohn's disease, types 1 and 2 diabetes and bipolar disorder, has been extensive (see Kingsmore *et al.* 2007). The systematic approach of GWA allows the identification of novel genes for traits of interest. Promising results have been demonstrated by a recent joint GWA study from the Wellcome Trust Case Control Consortium (WTCCC), which investigated seven major common disorders including bipolar disorder, coronary artery disease, hypertension, Crohn's disease, rheumatoid arthritis, type 1 diabetes and type 2 diabetes (The Wellcome Trust Case Control Consortium 2007). Regarding addiction, some GWA studies have been performed for various substance use disorders, including dependency for alcohol, nicotine, methamphetamine and heroin, with suggestion of some novel genes (Uhl *et al.* 2008). However, findings from these GWA studies should be treated with extreme caution as sample sizes for some of these studies are small and no replication study has yet been conducted. Nevertheless, future well-characterized GWA studies with a large sample size will identify novel genes for substance use disorder, and thus provide a better understanding of the underlying mechanism for drug-taking behaviour.

10. CONCLUSION

In this article, we have described strategies for addressing the genetic structure of substance use disorders, which is characterized by phenotypic heterogeneity, including gene × environment interactions, genetic heterogeneity and polygenicity. Endophenotypic characterization using functional neuroimaging,

gene identification using both candidate and whole-genome association analysis and functional characterization of genes using behavioural animals are examples of strategies that have been employed thus far. While these approaches have demonstrated some success in the elucidation of substance use disorders, future research that implements a coordinated approach involving the integration of the various strategies described would offer great potential to better our understanding in the neurobiological basis of substance use disorders and behavioural traits associated with an increased susceptibility for addictions. Advancement of understanding in the genetics and neurobiological basis of addiction will result in the development of better and more effective interventions for addictive behaviour and substance use disorders.

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