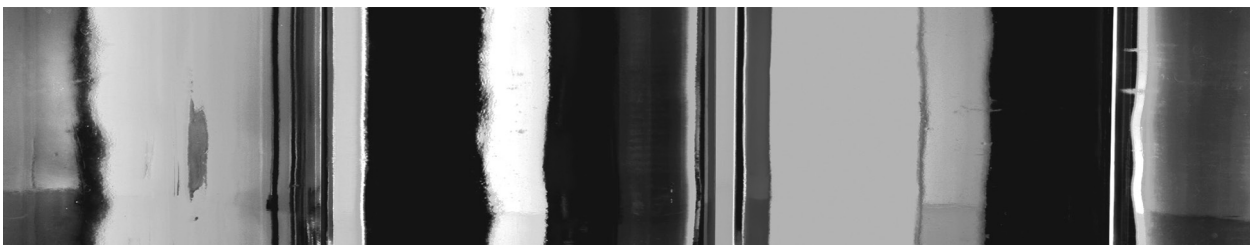
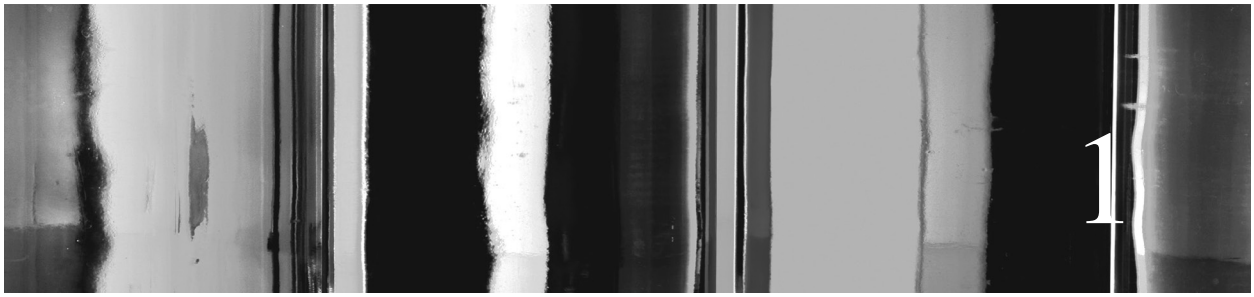
PART I

Determination of Substance Misuse





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Genetics and Genomics in Addiction Research

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INTRODUCTION

Substance use disorders are complex, multifactorial disorders that are typically characterized by the repeated use of a psychoactive substance that continues despite harmful consequences or varying degrees of impairment in function (DSM-5, 2013). Internet addiction, gambling disorders and sexual addiction are examples of a broader concept of addictions that is not simply limited to psychoactive substance use. The current approach towards studying genetic influences in such addictions involves much more than looking at the dichotomy of a disorder being present or absent. Agrawal *et al.* (2012) highlighted the importance of looking at addiction in stages, with early stages being less affected by genetic factors than the later stages. Many factors mediate the transition from initial exposure to a substance to abuse or dependence, and these include those that are specific to the individual (e.g. family history of addiction or mental illness, gender, psychiatric comorbidity, etc.) or environmental (such as access to or availability of drugs, and quality of the social network). Key questions of both research and clinical importance include to what extent the various substance use conditions and behavioural addictions have shared versus distinct genetic and epigenetic mechanisms.

Phenotypes for genetic studies of addictions

Genetic studies of addiction investigate associations of clinical phenotypes with genotypes. Such a method of investigation works best for clearly defined and observable phenotypes,for example, cardiomyopathy, "never forgetting that hundreds if not thousands of polymorphic codes may underly one observable phenotype. Unfortunately, we are far from the stage where we can definitively

related a specific genetic change to addiction.". However, in psychiatry it is sometimes difficult to achieve diagnostic concordance between clinicians looking at the same patient. The methods to diagnose substance use disorders vary in the available genetic studies and they include physician interviews using a DSM checklist (Bart et al., 2004) or structured interviews such as the Structured Clinical Interview for DSM disorders (SCID) (Clarke et al., 2013) and Mini International Neuropsychiatric Interview (MINI) (Benyamina et al., 2009). In addition, changes to diagnostic criteria have an impact on what studies will define as a diagnostic phenotype. For example, the fifth version of the DSM (APA, 2013), released in 2013, combined the DSM-IV categories of abuse and dependence in a single diagnosis. Many genetic studies have instead used addiction behavioural phenotypes, such as the number of cigarettes (Rice et al., 2012), time to first cigarette (Haberstick et al., 2007) and number of sexual partners (Cherkas et al., 2004) to study association. Similarly, animal studies that research genetic influences involve different behavioural phenotypes. Therefore, there can be a lot of variation in the phenotypic traits that the genetic studies attempt to measure, and this should be taken into consideration when comparing studies. It has been suggested that without a major move towards defining phenotypes, the progress in genomic medicine will be very slow (MacRae, 2015). We shall now go on to have a closer look at the different methods used to research genetic influences in addiction and review the relevant literature.

HERITABILITY OF SUBSTANCE USE DISORDERS

Initial evidence of the role of heritable influences in the risk of developing these conditions came from twin, adoption and family studies. The twin study methodology is used to estimate the degree to which genes may be contributing towards a particular phenotype. The difference in prevalence of that phenotype between monozygotic and dizygotic twins can then point towards a genetic contribution to the phenotype by estimating its heritability and separating the effects of shared versus unique environments. Drawbacks of twin studies include that they may be neither genetically nor environmentally representative of the general population (e.g. owing to the differential likelihood of risk of exposure to relevant factors), which leads to difficulties in generalizability. Adoption studies look at individuals adopted into families other than their family of origin; this design is also useful for examining genetic versus environmental influences. Family studies examine the risk of relatives developing a phenotype of interest.

Twin, adoption and family study findings in addictions

Addictions have a relatively high heritability, with estimates ranging from 39% to 72%; cocaine and opiate addictions are the most heritable (Goldman et al., 2005). Merikangas *et al.* (1998) found that having family history of drug abuse increased the risk of having a drug disorder by eight times. Tsuang *et al.* 1996 (Vietnam Era Twin Registry study on drug use and dependence) found a higher concordance among monozygotic twins than dizygotic twins. They also concluded that 34% of the variance of the risk of developing a drug use disorder was explained by genetic factors (Tsuang et al., 1996). *et al.* (2000) studied heavy substance use, abuse and dependence in 1198 male twins and found a higher heritability, of up to 60–80% for most substances (Kendler et al., 2000).

Carmelli *et al.* (1990) published one of the earliest large male twin studies in nicotine addiction from the National Academy of Sciences-National Research Council (NAS-NRC) twin registry; they found that heritable

influences explained up to 53% of the variation in risk of addiction. The contribution of genetic influences to the risk of nicotine addiction varies from 11% to 75% in other studies (Haberstick et al., 2007; Han et al., 1999; Kendler et al., 1999; True et al., 1997). Twin studies of nicotine addiction have demonstrated that not just dependence but also other aspects of the addiction, such as withdrawal and failed smoking cessation, are also heritable (Xian et al., 2003). Adult adoption studies have found a correlation between adoptees' smoking behaviour and smoking behaviour of biological relatives in the same generation (Osler et al., 2001).

The estimates of heritable influences on alcoholism range from 45% to 65% in twin and family studies (Pickens et al., 1991; Prescott and Kendler, 1999). Bierut *et al.* (1998) reported that rates of alcohol dependence in males and females were 50% and 25% respectively in the siblings of alcohol-dependent subjects. The risk of alcoholism in adoptees is more similar to that of their biological parents than that of their adoptive parents (Sigvardsson et al., 1996).

Twin studies have confirmed that genetic influences also play a role in cannabis dependence as well (Agrawal et al., 2007; Lynskey et al., 2012). Initial estimates of heritability for cannabis dependence varied from 30% to 80% (Agrawal and Lynskey, 2009). A meta-analysis of twin studies of the initiation of cannabis use reported heritability estimates of 45% in males and 39% in females (Verweij et al., 2010). For cannabis use disorders, the study reported heritability estimates of 50–60%.

Kendler *et al.* (1999) found that opiate addiction was heritable in a population based female twin study and replicated the finding a year later in a male twin study (Kendler et al., 2000).

Heritability estimates for cocaine use, abuse and dependence were 39%, 79% and 65% in a telephone interview study of female twins (Kendler and Prescott, 1998). A twin and sibling Swedish study of cannabis, cocaine, other stimulants and sedatives found heritability ranged from 64% to 70% for these substances (Kendler et al., 2015). Other twin studies of cocaine use have found evidence of a genetic contribution to the risk of cocaine use, abuse or dependence (Kendler et al., 2006).

Small family studies of probands with gambling disorder (Black et al., 2006), kleptomania (Grant, 2003) or compulsive buying (Black et al., 1998) each found that first-degree relatives of those probands had significantly higher lifetime rates of alcohol and other substance use disorders. Heritability estimates for problematic or compulsive internet use are about 48% in both genders (Vink et al., 2015). Another twin study found the heritability estimates for internet addiction to be even higher at 58–66%.

Compulsive sexual behaviour that is not part of another psychiatric disorder such as bipolar disorder is another example of a behavioural addiction that has been difficult to categorize in mental health, due to issues such as phenotypic heterogeneity (Derbyshire and Grant, 2015). It can be conceptualized as repeated and intrusive thoughts and/or actions that are of a sexual nature but cause distress and impairment in an individual's life. Hypersexual behaviour has been researched and its inclusion in DSM-5 been discussed (Kor et al., 2013). Family studies of patients with Tourette's syndrome or attention deficit hyperactivity disorder found that a variety of sexual behaviours in relatives of the patients were correlated with the degree of genetic loading, suggesting a genetic contribution to each (Comings, 1994). Familial influences on sexual behaviour such as age at first intercourse have been seen in twin and family studies (Carlson et al., 2014; Donahue et al., 2013; Guo and Tong, 2006; Harden and Mendle, 2011; Rodgers et al., 2008; Waldron et al., 2008). Siblings and daughters of teenage mothers have an elevated risk of teenage pregnancy (East and Jacobson, 2001; Meade et al., 2008).

CANDIDATE GENE STUDIES

Candidate gene studies look at gene variants or polymorphisms within candidate genes, or in regions adjacent or in linkage disequilibrium, using *a priori* hypotheses about their relationship with the addiction phenotype. Subjects with an addiction phenotype are compared with those that do not have the addiction. Limitations of candidate gene studies in addiction include the requirement of prior knowledge of the gene and its function(s) and the fact that many addictions are polygenic in nature. In the following sections, the risk alleles are shown in square brackets after each single nucleotide polymorphism (SNP) rs number.

Nicotine

Candidate gene studies investigating the gene cluster *CHRNA5-CHRNA3-CHRNB4* located on 15q25.1 that encodes for subunits of the nicotinic acetylcholine receptors (nAChRs) and smoking have found significant associations consistent between European (Bierut et al., 2008), African (Li et al., 2010) and Asian (Li et al., 2010) ancestry populations. Saccone *et al.* (2007) were the first group to report an association between the *CHRNA5* non-synonymous SNP rs16969968[A] and nicotine dependence, although in addition to the signals from the *CHRNA5-CHRNA3-CHRNB4* gene cluster, they found that *CHRNB3* polymorphisms were also significant. Within the dense *CHRNA5-CHRNA3-CHRNB4* locus, the *CHRNA5* SNP rs16969968[A] and *CHRNA3* SNP rs578776[G] may represent two groups of risk variants (Wen et al., 2014). Gene variants in the *CHRNA5-A3-B4* gene cluster are important for the treatment of nicotine dependence too. The number of cigarettes smoked per day and response to treatment with smoking cessation therapy, such as bupropion and transdermal nicotine patches, can be predicted by SNPs rs8192475[T], rs680244[A] and rs12914008[A] in the *CHRNA5-CHRNA3-CHRNB4* gene cluster (Sarginson et al., 2011). Neuronal signaling pathway genes such as neuregulin 1 (*NRG1*) and Erb-B2 receptor tyrosine kinase 4 (*ERBB4*) that have been associated with psychosis (Bakker et al., 2004; Bramon et al., 2008; Douet et al., 2014; Wang et al., 2009a) have also been investigated in nicotine dependence. Animal studies provide support for the role of neuregulin 3 (*NRG3*) in nicotine dependence and a clinical trial demonstrated that a SNP rs1896505[A] in this gene might play a role in smoking cessation (Turner et al., 2014). Within the category of genes expressing metabolizing enzymes for nicotine, specific *CYP2A6* alleles (*CYP2A6*9*, *CYP2A6*12*, *CYP2A6*2* and *CYP2A6*4*) provide some protection against nicotine addiction and increase chances of smoking cessation (Gold and Lerman, 2012; Iwahashi et al., 2004; Mwenifumbo et al., 2007).

Alcohol

Alcohol addiction has been studied in much more detail than other addictions. Genetic studies of alcohol addiction were the first amongst such studies in addiction and early candidate gene studies of alcoholism focused on genes that express enzymes involved in the metabolism of ethanol. The *ALDH* genes for the aldehyde dehydrogenase enzymes that catabolize acetaldehyde to acetic acid have been studied extensively in alcohol addiction. Variants in *ALDH2* have been well known to confer a protective effect against alcohol dependence in northeast Asians and this finding is highly replicated (Higuchi, 1994; Samochowiec et al., 2014; Whitfield, 1994). Individuals homozygous for the *ALDH2*2* variant will experience severe nausea and vomiting with small amounts of alcohol intake and a have lower risk of developing alcohol dependence. This relates to the finding that individuals who are homozygous

for the *ALDH2*2* variant have a flushing syndrome (Thomasson et al., 1993) and experience a greater degree of flushing than heterozygotes. Alcohol dehydrogenase (*ADH*) polymorphisms contribute towards the risk of developing alcoholism (Higuchi, 1994; Thomasson et al., 1991). In a Chinese study, the *ADH1*B* and *ADH1*C* alleles were found to protect against alcohol dependence, but this effect is smaller than that of *ALDH2* variants (Thomasson et al., 1991). Similarly, in British and Irish populations, a SNP rs12229984[G] in the *ADH1B* gene conferred protection against alcohol dependence (Way et al., 2015). Individuals who have alcohol addiction also tend to use nicotine, and there is some evidence linking the aforementioned nicotinic receptor gene cluster (*CHRNA5-CHRNA3-CHRNB4,* SNPs rs1979906A/G, rs3841324L/S, rs601079A/T, rs680244A/G, rs621849A/G, rs692780C/G, rs6495307C/T, rs1051730C/T) to alcohol dependence (Edenberg and Foroud, 2014; Wang et al., 2009b). Among its many effects, alcohol affects gamma-aminobutyric acid (GABA). The chromosomal region of 4p12 has a cluster of four genes that encode for GABA-A receptors and chromosome 5q contains another cluster of GABA-A receptor genes, and both are relevant to alcohol problems (Grzywacz et al., 2012). *GABRA2* encodes for the GABA-A alpha2 receptor. Edenberg *et al.* (2004) found multiple SNPs in the *GABRA2* gene were associated with alcohol dependence and the beta frequency of the electroencephalogram in patients who had alcohol dependence and their relatives (Edenberg et al., 2004). In this study, the region from intron 3 up to past the 3′ end of GABRA2 gene, with a three-SNP haplotype, had a strong correlation with alcohol dependence. Covault et al. (2008) extended the markers studied by Edenberg et al. 2004 by genotyping into the 5′ region of *GABRAG1* and found that these variations better explained the association with alcohol dependence (Covault et al., 2008; Villafuerte et al., 2012). There are many other genes that have been linked to alcohol dependence such as *ACN9* (Hill et al., 2015), X-ray repair complementing defective repair in Chinese hamster cells 5 (*XRCC5*) (Juraeva et al., 2015), dopamine receptor type 2 (*DRD2*) (Buhler et al., 2015), ankyrin repeat and kinase domain containing 1 (*ANKK1*) that is 10 Kb upstream of the *DRD2* in the complementary strand (Buhler et al., 2015), serine incorporator 2 (*SERINC2*) (Zuo et al., 2013a), *KIAA0040* (Hill et al., 2013; Wang et al., 2011) and *NRD1* (Wang et al., 2011). The chromosome 7q region is of interest in alcoholism as evidenced in a genome-wide linkage study by Hill *et al.* (2004) (Hill et al., 2004). Six SNPs (three upstream of the gene, two within intron 1 and one in exon 4) in the *ACN9* gene (involved in gluconeogenesis) located on chromosome 7q were associated with alcohol dependence in a family-based association study (Hill et al., 2015). Gene variation in *XRCC5* can affect the maximum blood alcohol concentration in an allele-dose-dependent manner (Juraeva et al., 2015). The Taq1A polymorphism located downstream of the DRD2 gene in *ANKK1* on the complementary strand has been associated with alcohol dependence in a large-scale meta-analysis (Wang et al., 2013) and suicidal behaviour in alcohol dependence may also be associated with haplotypes in the *ANKK1* and *DRD2* genes (Jasiewicz et al., 2014). Among these two genes, the *ANKK1* gene may have a stronger association with alcohol dependence than *DRD2* (Ma et al., 2015). Zuo et al. (2013) found that a rare variant constellation was *NKAIN1-SERINC2* was correlated with alcohol dependence in a European-American population (Zuo et al., 2013a). Six SNPs in the *KIAA00040* gene were significantly associated with alcohol dependence in a family-based association analysis (Hill et al., 2013). Gene variants such as the *COMT*Val158Met substitution (rs4680) may moderate the effect of adverse childhood experiences on the risk of having alcohol dependence (Schellekens et al., 2013), with Met carriers being more at risk

than Val/Val homozygotes. In a study of genomic losses in copy number (CNV) in alcohol dependence, an excess of losses was found at 16q12.2, which would affect the genes *CES1p1* and *CES1*, involved in the generation of alcohol from chemicals including esters (Ulloa et al., 2014).

Cannabis

A few candidate genes have been investigated for association with cannabis dependence. SNPs rs806368[C] and rs806380[A] in the cannabis receptor 1 gene (*CNR1*) were associated with cannabis dependence in a study that consisted largely of alcohol-dependent subjects (Agrawal and Lynskey, 2009; Agrawal et al., 2009). The *CNR1* SNP rs2023239[G] mediated the effect of heavy cannabis use on reduced hippocampal volume (Schacht et al., 2012). The *NRG1* SNP rs17664708[T] was associated with cannabis dependence in patients of African-American ethnicity (Han et al., 2012). Met/Met or Met/Val genotypes of the *COMT* Val158Met polymorphism have been associated with cannabis dependence (Baransel Isir et al., 2008). Other genes implicated in cannabis use disorders are ATP-binding cassette, sub-family B (MDR/TAP), member 1 (*ABCB1*) (Benyamina et al., 2009) and monoglyceride lipase (*MGLL*) (Agrawal and Lynskey, 2009). In addition, genotypic and haplotypic variations at or near the *GABRA2* gene are associated with vulnerability to cannabis, alcohol and nicotine dependence (Philibert et al., 2009). Genotypes can influence withdrawal from cannabis too, and evidence to support this includes the fact that the *CNR1* SNP rs2023239[G] exerts an influence on cannabis withdrawal and the fatty acid amide hydrolase(*FAAH*) SNP rs324420[C] affects craving during abstinence (Haughey et al., 2008). These SNPs may have an additive effect on cannabis withdrawal.

The *COMT* Val158Met polymorphism has been investigated for moderating the effect of cannabis use on the development of psychotic symptoms, and it has been found that the Val/Val genotype has a significant association in many studies (Caspi et al., 2005; Ermis et al., 2015; Estrada et al., 2011; Henquet et al., 2006, 2009) but not all (Zammit et al., 2011). Van Winkel *et al.* (2008) observed that the Met allele of *COMT*Val158Met was an important mediator of the effect of stress on psychotic symptoms (van Winkel et al., 2008). *COMT*Val158Met variants can mediate more complex relationships such as those between adverse childhood experiences, cannabis use and psychosis, with Vinkers *et al.* (2013) finding that childhood trauma moderated the effect of cannabis use on psychotic experiences in Val carriers. Later, this was confirmed by Alemany *et al.* (2014), who in addition noted that having the Met allele affected psychotic experiences in individuals who suffered childhood abuse but did not use cannabis.

Opioids

The opioid receptors mu, kappa and delta are intricately involved in the pharmacodynamic effects of opioids. The mu receptor gene (*OPRM1*) SNP rs1799971 (118A/G, Asn40Asp) has been associated with opioid dependence in Indian (Kapur et al., 2007) and European Caucasian (Bart et al., 2004; Drakenberg et al., 2006) study populations. Prior to this, Bond *et al.* (1998) had sequenced DNA from opioid addicts to identify five SNPs in the same gene and the 118A/G was the most prevalent SNP (Bloom et al., 2014; Bond et al., 1998). Endorphin binds the 118A/G receptor three times more tightly than the Asn (i.e. aspargine) form of the receptor (Bond et al., 1998). Postmortem brain analysis of 118G heroin users has shown significant alterations in the opioid neuropeptide system, such as reduced preproenkephalin transcription (Drakenberg et al., 2006). However, the 118A/G SNP association with opioid addiction was not

replicated in Han Chinese (Glatt et al., 2007). Yuferov *et al.* (2004) reported that the 36G>T variation in the kappa receptor gene (*OPRK1*) was associated with opioid addiction in a Hispanic population (Yuferov et al., 2004) and this finding was replicated in a West European Caucasian study sample (Gerra et al., 2007). G alleles of the SNP rs6265 and rs13306221 in the gene encoding brain-derived neurotropic factor (*BDNF*) are more frequently found in subjects with heroin addiction (Jia et al., 2011). Melanocortin receptor type 2 (*MC2R*) gene polymorphisms are also associated with heroin addiction (Proudnikov et al., 2008). Specifically, rs2186944[A] may protect against and rs4797824[T] may increase the risk of developing heroin addiction in Hispanics. Haplotype analysis in the same group found the haplotype GACT (rs2186944, -179A>G, rs28926182 and rs4797824) to be a risk factor for heroin addiction, while the AACT haplotype from the same variants was protective against heroin addiction.

Methadone is a treatment for opiate addiction. However, this result would depend greatly on the chirality of the methadone formulation being given. The *ABCB1* gene SNP rs1128503[C>T] differentiated between patients who required high and low dose methadone maintenance treatment (Levran et al., 2008). In the same study, patients with the three-locus genotype pattern TT-TT-TT (rs1045642, rs2032582 and rs 1128503 in the *ABCB1* gene) were five times more likely to require high methadone maintenance dose and those who were heterozygous for the SNPs were three times as likely to require a low methadone maintenance dose.

Amphetamines

Genetic polymorphisms in the opioid system genes have been assessed for a role in amphetamine addiction (Ide et al., 2004; Levran et al., 2012). Within these genes, the *OPRM1* SNP rs2075572 in intron 2 has been associated with methamphetamine dependence, and methamphetamine-induced psychosis (Ide et al., 2006). Methamphetamine-induced euphoria was moderated by intronic SNPs rs510769 (A/A genotype) and rs2281617 (C/C genotype), a two-SNP (AA) haplotype of rs1799971 and rs510769 and a three-SNP haplotype (ATA) of rs1918760, rs2281617 and rs1998220 (Dlugos et al., 2011). *OPRD1* variants were not a risk factor for methamphetamine dependence and induced psychosis (Kobayashi et al., 2006). Methamphetamines promote the release of dopamine in the synaptic cleft, and genetic variants rs509707[C] and rs4709426[C] and haplotypes of these in the monoamine transporter *SLC22A3* gene may have a role in the development of polysubstance use in patients with methamphetamine dependence (Aoyama et al., 2006). Jugurnauth *et al.* (2011) observed an association of a *COMT* gene haplotype, including A alleles of rs4680 and rs165599, with methamphetamine abuse. Hosak *et al.* (2006) found that having the Met allele compared to Val of the *COMT Val158Met* polymorphism was related to novelty seeking in a Czech methamphetamine use population but not to methamphetamine dependence (Hosak et al., 2011). Other variants that are associated with methamphetamine addiction are prokineticin 2 receptor gene (*PROKR2)* SNPs rs6085086(G>A), rs3746682(G>C) and rs4815787(G>A) (Kishi et al., 2010), ghrelin signaling system gene polymorphisms (*GHRL* SNP rs4684677[T] and *GHSR* SNP rs2948694[G]) (Suchankova et al., 2013), V-Akt murine thymoma viral oncogene homolog 1 (AKT1) SNP rs3730358 (C>T) (Ikeda et al., 2006) and the adenosine receptor gene (*ADORA2A*) SNP rs5751876[C] (Kobayashi et al., 2010).

Cocaine

The 'dopamine hypothesis' has often been used to explain the reinforcing properties of

cocaine (Kuhar et al., 1991). Genetic polymorphisms within the dopamine system are therefore logical targets for research in cocaine addiction. Associations with certain types of cocaine use with dopamine system gene variants include the 30-bp variable number tandem repeat polymorphism in intron 8 of a dopamine transporter gene (*SLC6A3*) (Guindalini et al., 2006), minor alleles (A1 and B1) of *DRD2* polymorphisms (Noble et al., 1993), the *Msc*I/*Bal*l polymorphism of *DRD3* (Comings et al., 1999) and the Met allele of the *COMT*Val158Met polymorphism and a two-marker haplotype of this with rs737865 (Levran et al., 2015; Lohoff et al., 2008). However, other studies looking at the same genes did not find similar results (Fernandez-Castillo et al., 2010; Gelernter et al., 1999; Lohoff et al., 2010). A study in European-Americans found that a minor[A] allele of the *CHRNA5* SNP rs16969968 was associated with an increased risk of nicotine dependence and reduction in risk of cocaine dependence (Grucza et al., 2008), while a more recent investigation concluded that multiple variants in the *CHRNB3-A6* gene locus were associated with an increased risk of developing nicotine and cocaine dependence (Sadler et al., 2014). Interestingly, *CNR1* SNPs rs6454674[G] and rs806368[C] were associated with cocaine addiction (Clarke et al., 2013).

GENOME-WIDE ASSOCIATION STUDIES

The human genome project, HapMap and other collaborative efforts generating such data have improved understanding of the variability of the human genome, and advances in array technology to facilitate high-throughput multiplex genotyping have rendered genome-wide association studies (GWAS) feasible. Arrays or gene chips enable the genotyping of tens of thousands to millions of gene markers per individual. GWAS can identify common SNPs (minor allele frequencies of greater than 1%) associated with a disorder or a particular phenotype. However, the testing of up to 1 million SNPs for association with disease may generate false positives. For genome-wide significance, correction for multiple testing gives a P-value between 5×10^{-7} and 10^{-8} (660,000–1 million SNPs). Each SNP may be considered to be independent (if for example they are haplotype tagging SNPs with a recombination fraction, r^2 less than or equal to 0.5); however, as there may be functional connection between them, this approach is conservative. Although such conservative correction reduces the risk of false-positive findings, a drawback is that true association signals with small effect sizes may be overlooked. The National Institutes of Health compiled a catalog of SNP-trait associations from published GWAS, which was available online at the National Human Genome Research Institute (http://www.genome.gov/gwastudies/), and detailed findings at a significance level of $P<1 \times 10^{-5}$. The main GWAS database is http://www.gwascentral.org/. In addition, there is a specific addiction GWAS resource (http://addictiongwas.com/AAGR/), hosted by the psychiatry department of Amsterdam Academic Medical Centre (AMC). GWAS look at all markers tested without *a priori* hypotheses regarding the relationship between the markers and phenotypes.

A limitation of GWAS is that this approach can only detect associations with variants that are relatively common in the general population; thus, rare variants with larger effect sizes will be missed. However, GWAS are designed to identify genes involved in common aetiological mechanisms for clinical conditions of interest including underlying pathways that may interact with each other and with the environment. It is hoped that this will feed into the discovery of drugs that may be relevant for many people. Currently, at least some of the gene polymorphisms that are used to predict disorder or risk of disorder

are, in fact, those with relatively large effect size that do not account for the majority of the disease (for example, breast cancer genes). It is possible that prediction of the development of polygenic disorders might become more feasible once enough common risk alleles and clinical factors including environmental interactions have been identified. By analogy with *ALDH2,* it is envisaged that genes related to metabolism may be relevant not only to response to treatment for addiction, but also to susceptibility to disorder.

GWAS in addiction

The following section describes the progress in genetic association for substance abuse disorders that has been achieved through the GWAS approach. The majority of GWAS in addiction to date have focused on drinking behaviours; the next most common phenotype studied is smoking.

GWAS have confirmed that the nAChRs genes are associated with nicotine dependence. In fact, meta-analyses of tobacco GWAS have confirmed the importance of genes encoding the nAChRs in susceptibility to nicotine addiction (Liu et al., 2010; Thorgeirsson et al., 2010; Tobacco and Genetics, 2010). The Tobacco and Genetics Consortium found SNPs in genes *CHRNA3* [rs1051730A], *EGLN2* [rs3733829G], and in the 10q25 [rs1329650G, rs1028936A] were associated with the number of cigarettes per day, and a SNP in *BDNF* [rs6265C] was associated with smoking initiation (Tobacco and Genetics, 2010). Loukola *et al.* (2014) provided tentative evidence of *ERBB4* [rs7562566G] and nicotine-dependence association in a GWAS study (Loukola et al., 2014). Nicotine-metabolizing enzyme *CYP2A6* and *CYP2B6* [rs4105144C] and *CHRNB3-CHRNA6* [rs6474412T] genes were also relevant to smoking behaviour in meta-analyses of GWAS (Thorgeirsson et al., 2010).

GWAS in other addictions to date are limited by issues such as variability of phenotype identification and sample size. Interestingly, the role of the *ADH* gene cluster in alcohol dependence was confirmed by a study that investigated associations using a polygenic risk model (Frank et al., 2012). Other GWAS of alcohol addiction found that rs2066702[T] and rs1229984[A] in *ADH1B* affect the risk of developing alcohol dependence in African-American and European-American patients respectively (Hart et al., 2015). A recent GWAS found that genes involved in signal transduction and neurogenesis are possibly involved in 'alcohol problems' in young adults (Edwards et al., 2015). Variants in *PTP4A1-PHF3-EYS* were associated with alcohol dependence in a GWAS conducted by Zuo *et al.* (2014) (Zuo et al., 2014).

GWASs of cannabis dependence (Agrawal et al., 2011; Verweij et al., 2012) and cannabis use initiation (Verweij et al., 2013) have to date not identified any associations significant at the genome-wide level (Minica et al., 2015).

A GWAS found that rs2377339[G] in the *NCK2* gene (NCK is a family of adaptor proteins) was associated with opioid dependence in men of African origin (Liu et al., 2013). Nielsen *et al.* (2010) conducted a pooled GWAS using a relatively low density array of 100,000 markers in a comparatively small sample consisting of 325 former heroin addicts (200 Caucasians, 125 African-Americans) and 250 controls (150 Caucasians, 100 African-Americans) (Nielsen et al., 2010). An apparent association was nonetheless detected in Caucasians for the variant rs10494334[A] (located at chr1q23.3), and in people of African-American ethnicity, the variant most significantly associated was rs950302[T], located in the cytosolic dual specificity phosphatase 27 gene (*DUSP27*), which may be involved in energy metabolism.

The *CDH13* (cadherin 13) gene SNP rs3784943[G] in the eighth intron was associated with response to d-amphetamine in healthy volunteers in a GWAS (Hart et al., 2012).

Table 1.1 Courtesy: National Human Genome Research Institute (https://www.genome.gov)

Reference	*Disease/Trait*	*Initial Sample Size*	*Replication Sample Size*	*Region*	*Reported Gene(s)*	*Mapped_gene*	*SNPs*	*Context*	*Risk Allele Frequency*	*Pvalue_mlog*
Liu et al. (2013)	Addiction	1,393 European ancestry females, 1,131 European ancestry males,	NA	2q12.2		NCK2	rs2377339	intron	0.0159	11
				2q12.2		NCK2	rs2377339	intron	0.0159	7.698970004
Zuo et al. (2013b)	Alcohol and nicotine co-dependence	568 African ancestry females, 535 African ancestry males	Up to 907 European ancestry cases, up to 2,830 European ancestry controls, up to 449 African American cases, up to 480 African American controls	5q12.1		ISCA1P1 - HTR1A	rs7445832	intergenic	NR	9
				2q12.2		NCK2	rs2377339	intron	0.0159	7.698970004
Zuo et al. (2013b)	Alcohol and nicotine co-dependence	818 European ancestry cases, 1,396 European ancestry controls	Up to 907 European ancestry cases, up to 2,830 European ancestry controls, up to 449 African American cases, up to 480 African American controls	5q12.1		ISCA1P1 - HTR1A	rs7445832	intergenic	NR	9
				6p24.1		HIVEP1	rs1570989	intron	NR	5.698970004
				6q25.1		PLEKHG1	rs17427389	UTR-3	NR	6
				5q12.2		ISCA1P1 - HTR1A	rs10042968	intergenic	NR	6.096910013
				2p21		CAMKMT - SIX3-AS1	rs528301	intergenic	NR	5.301029996
Zuo et al. (2012b)	Alcohol and nicotine co-dependence	818 European ancestry cases, 1,396 European ancestry controls, 449 African American cases,	NA	3p25.1	SH3BP5, NR2C2, ZFYVE20	SH3BP5; SH3BP5-AS1	rs1318937	nearGene-3;nearGene-5	NR	6.397940009
				6p24.1		HIVEP1	rs1570989	intron	NR	5.698970004
McGue et al. (2013)	Alcohol consumption	480 African American controls	NA	6q25.1		PLEKHG1	rs17427389	UTR-3	NR	6
				11q24.2		PKNOX2	rs1426153	intron	NR	5.698970004

				20q12		LOC339568	rs6028335	intron	NR	5.301029996
				7q31.31		KCND2	rs728115	intron	NR	5.397940009
		7,188 European ancestry individuals		2p12		REG3A - CTNNA2	rs2100290	intergenic	0.49	5.698970004
Kapoor et al. (2013)	Alcohol consumption			2q37.1		TRPM8	rs12472151	intron	0.048	5.698970004
		2,322 European ancestry individuals from 118 families,		1q41		ESRRG	rs7553212	intron	0.32	5.15490196
McGue et al. (2013)	Alcohol consumption		NA	2q33.1		PLCL1	rs67031482	intron	0.48	5.397940009
				3q26.32		LINC00578	rs1353899	intron	0.22	5.397940009
Kapoor et al. (2013)	Alcohol consumption			7q22.3		SLC26A4	rs2188561	intron	0.21	5.045757491
		2,593 European ancestry individuals		4q23		ADH1B	rs1229984	missense	0.03	7.698970004
				14q22.3		AP5M1 - NAA30	rs7144649	intergenic	0.23	5.397940009
				20q13.31		RBM38 - HMGB1P1	rs59972978	intergenic	0.2	5.301029996
				22q12.1		RPL15P22 - MN1	rs16985179	intergenic	0.11	5.22184875
				9p22.3		TRNAH5 - CDCA4P1	rs59677118	intergenic	0.06	6
				11p15.4		LMO1	rs4758317	intron	0.42	6.15490196
Schumann et al. 2011)	Alcohol consumption		21,185 European ancestry individuals	8q22.3		RPS20P23 - RPS26P6	rs36061340	intergenic	0.05	5.15490196
Baik et al. (2011)	Alcohol consumption	26,316 European ancestry individuals	1,113 Korean ancestry male individuals	20q13.13		RNA5SP486 - LINC00494	rs62202398	intergenic	0.06	5.045757491
Schumann et al. (2011)	Alcohol consumption		21,185 European ancestry individuals	14q22.3		EXOC5	rs11851015	UTR-3	0.15	5.045757491
				4p14		TLR10 - TLR1	rs4543123	intergenic	0.24	5.045757491
				7q11.22		AUTS2	rs6943555	intron	0.24	7.397940009
				12q24.13	C12orf51, ALDH2	HECTD4	rs2074356	intron	0.15	58.04575749
Baik et al. (2011)	Alcohol consumption	1,721 Korean ancestry male individuals	1,113 Korean ancestry male individuals	12q24.11		MYL2 - CUX2	rs12229654	intergenic	0.14	34.39794001
				12q24.12		ALDH2	rs671	missense	NR	16

(*Continues*)

Table 1.1 (Continued)

Reference	*Disease/Trait*	*Initial Sample Size*	*Replication Sample Size*	*Region*	*Reported Gene(s)*	*Mapped_gene*	*SNPs*	*Context*	*Risk Allele Frequency*	*Pvalue_mlog*
Kutalik et al. (2011)	Alcohol consumption (transferrin glycosylation)	5,181 European ancestry individuals	2,284 European ancestry individuals	1p31.3		PGM1 - ROR1	rs2749097	intergenic	0.19	8.698970004
				3q22.1		TF	rs1049296	missense	0.17	42.30103
				3q22.1		TF	rs1799899	missense	0.06	9
				3q22.1		SRPRB	rs1534166	intron	0.30	16.69897
				3q22.1		TF	rs3811647	intron	0.32	35
				6p22.2		HFE	rs1800562	missense	0.045	31.69897
Quillen et al. (2014)	Alcohol dependence	102 Han Chinese ancestry cases, 212 Han Chinese ancestry controls	NA	4q28.2		CYCSP14 - PGBD4P4	rs11933661	intergenic	NR	5.397940009
				12q24.12		ALDH2	rs671	missense	NR	7.301029996
McGue et al. (2013)	Alcohol dependence	7,188 European ancestry individuals	NA	1p36.11		CLIC4 - RUNX3	rs3131513	intergenic	0.401	5.698970004
				4q32.2		FSTL5 - MTHFD2P4	rs4440177	intergenic	0.338	5.22184875
				8p23.1		NEIL2	rs804292	UTR-3	0.234	5.698970004
				11q25		OPCML	rs1793257	intron	0.037	5.15490196
Park et al. (2013)	Alcohol dependence	117 Korean ancestry cases, 279 Korean ancestry controls	504 Korean ancestry cases, 471 Korean ancestry controls	4q23		ADH1B	rs1229984	missense	NR	20.52287875
Zuo et al. (2012a)	Alcohol dependence	1,409 European ancestry cases, 1,518 European ancestry controls	6,438 European ancestry individuals from 1,645 affected families	1p35.2		SERINC2	rs4478858	intron	NR	7.522878745
Frank et al. (2012)	Alcohol dependence	1,333 European ancestry male cases, 2,168 European ancestry male controls	NA	2q35		MREG - PECR	rs1344694	intergenic	NR	5.301029996
				3p22.3		RPSAP11 - CMTM8	rs9825310	intergenic	NR	5.096910013
				4p16.2		STX18-AS1 - MSX1	rs1000579	intergenic	NR	6.397940009
				4q23		ADH1B - ADH1C	rs1789891	intergenic	NR	8
				13q12.12		SGCG	rs4770403	intron	NR	5.22184875
				14q24.2		PCNX	rs2810114	intron	NR	5.397940009

		1,409 European ancestry cases,		3p25.1	SH3BP5	SH3BP5;SH3BP5-AS1	rs1318937	nearGene-3;nearGene-5	NR	5.522878745
		1,518 European ancestry		11q24.2		PKNOX2	rs10893366	intron	NR	5.096910013
Zuo et al. (2012a)	Alcohol dependence	controls, 681 African American cases, 508 African American controls	NA	2q22.1		AHCYP4 - MRPS18BP2	rs9636231	intergenic	NR	5.096910013
Lydall et al. (2011)	Alcohol dependence	506 European ancestry cases, 510 European ancestry controls	NA	16p12.3		ITPRIPL2 - SYT17	rs8062326	intergenic	0.02	5.397940009
Wang et al. (2011)	Alcohol dependence	1,283 European ancestry cases, 1,416 European ancestry controls	1,650 European ancestry cases and 1,684 European ancestry controls from 778 nuclear families	1q25.1		TNN - KIAA0040	rs6701037	intergenic	0.46	6.698970004
				11q24.2		PKNOX2	rs750338	intron	0.22	6
Kendler et al. (2011)	Alcohol dependence	2,357 European ancestry individuals, 812 African American individuals	NA	3p25.2		MARK2P14 - SYN2	rs6777876	intergenic	0.09	6.397940009
				1q44		VN1R16P - ZNF731P	rs3738443	intergenic	0.03	5.397940009
				13q12.13		RPS21P8 - RPS20P32	rs12020569	intergenic	0.10	5.301029996
Treutlein et al. (2009)	Alcohol dependence	476 European ancestry cases, 1,358 European ancestry controls	1,024 European ancestry cases, 996 European ancestry controls	2q35		PECR	rs7590720	intron	0.29	8
				5q15	CAST, ERAP1	ERAP1	rs13160562	intron	0.68	5.15490196
				5q32		PPP2R2B	rs1864982	intron	0.13	5.522878745
				6q25.1		ESR1	rs6902771	intron	0.51	5.096910013
				14q24.2		MAP3K9 - PCNX	rs36563	intergenic	0.15	5.301029996
				Xp22.2		CLCN4 - MID1	rs12388359	intergenic	0.11	5.397940009

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Table 1.1 (Continued)

Reference	*Disease/Trait*	*Initial Sample Size*	*Replication Sample Size*	*Region*	*Reported Gene(s)*	*Mapped_gene*	*SNPs*	*Context*	*Risk Allele Frequency*	*Pvalue_mlog*
Heath et al. (2011)	Alcoholism (12-month weekly alcohol consumption)	2,062 European ancestry alcohol dependence cases, 6,692 European ancestry controls	3,393 European ancestry individuals	7q11.23		HIP1	rs237238	cds-synon	0.07	5.15490196
				14q21.1		OR10V7P - YWHAQP1	rs2154294	intergenic	0.44	5.522878745
				2q23.3		MMADHC - TRNAE38P	rs6716455	intergenic	0.13	5.045757491
				6p21.31	ANKS1	ANKS1A	rs2140418	intron	0.22	5.397940009
				7q31.32		RPL18P4 - PNPT1P2	rs10253361	intergenic	0.38	5.22184875
	Alcoholism (alcohol dependence factor score)			13q32.1		MBNL2	rs9556711	intron	0.08	6.096910013
				15q26.2		LINC00924 - NR2F2-AS1	rs933769	intergenic	0.19	5.15490196
				21q22.3		COL6A1 - PSMA6P3	rs4293630	intergenic	0.14	5.15490196
				2q23.3		MMADHC - TRNAE38P	rs6716455	intergenic	0.13	6.15490196
				3q27.1		CHRD - EPHB3	rs3930234	intergenic	0.15	5.096910013
				5p13.1		LINC00603 - PTGER4	rs2548145	intergenic	0.47	5.698970004
				6p21.31	ANKS1	ANKS1A	rs2140418	intron	0.22	5.397940009
				13q32.1		MBNL2	rs9556711	intron	0.08	5.698970004
				18q21.2		DCC	rs768048	intron	0.12	5.096910013
	Alcoholism (heaviness of drinking)			1p13.2		TSPAN2 - NGF	rs195204	intergenic	0.24	5.045757491
				3q22.1		NPHP3-AS1 - TMEM108	rs2369955	intergenic	0.16	5.698970004
				4q13.3		MUC7	rs1109501	intron	0.24	5.301029996
				9q22.2		GADD45G - UNQ6494	rs10908907	intergenic	0.25	5.22184875
				13q12.2		RASL11A - GTF3A	rs9512637	intergenic	0.32	7
				14q22.1		DDHD1 - RPS3AP46	rs1380131	intergenic	0.07	5.045757491
				15q26.1		C15orf32	rs8040009	UTR-3	0.21	6.522878745
				21q21.1		LINC00308 - MAPK6PS2	rs2827312	intergenic	0.32	5.096910013

Agrawal et al. (2011)	Cannabis dependence	708 African American or European ancestry cases, 2,346 African American or European ancestry controls	NA	1q31.2		UCHL5	rs9427573	intron	NR	5.301029996
				2p16.1		LINC01122	rs17552189	intron	NR	5.397940009
				10p12.1		RPL21P93 - LYZL1	rs11007350	intergenic	NR	5.522878745
				3q13.12		CBLB - FCF1P3	rs12491921	intergenic	NR	6
				12q23.3		CHST11	rs12811699	intron	NR	5.096910013
				13q12.12		SGCG	rs9507041	intron	NR	5.096910013
				17q22		ANKFN1	rs1019238	intron	NR	6.22184875
				17q22		PCTP - ANKFN1	rs1431318	intergenic	NR	6.045757491
				17q22		PCTP - ANKFN1	rs8065311	intergenic	NR	5.698970004
				22q13.33		CRELD2 - PIM3	rs28372448	intergenic	NR	5.096910013
				13q14.2		GNG5P5 - NAP1L4P3	rs1417205	intergenic	0.95	6.096910013
Verweij et al. (2013)	Cannabis use (initiation)	10,091 individuals from 4,622 families	NA	17p12		ELAC2 - HS3ST3A1	rs9900808	intergenic	0.04	5.698970004
				11p15.1		RNA5SP337 - ANO5	rs1573535	intergenic	0.56	5.397940009
				17q25.2		MGAT5B - SNHG20	rs4789400	intergenic	0.08	5.301029996
				6q12	RP11-406O16.1	LOC101928280	rs10455657	intron	0.81	5.22184875
				6q25.3		SNX9 - SYNJ2	rs9458975	intergenic	0.45	5.15490196
				2q14.2		STEAP3	rs72840936	nearGene-3	NR	5.522878745
				3q11.2		MTHFD2P1 - HNRNPKP4	rs111325002	intergenic	NR	6.397940009
Gelernter et al. (2014)	Cocaine dependence	1,809 European ancestry cases, 570 European ancestry controls, 2,482 African American cases, 836 African American controls	4,498 European ancestry individuals, 2,114 African American individuals	4q34.1		MORF4 - RANP6	rs4129566	intergenic	NR	5.522878745
				4q34.1	RANP6	MORF4 - RANP6	rs11944332	intergenic	NR	5.698970004
				8q22.3	RIMS2	RIMS2	rs75686122	intron	NR	5.522878745
				17p13.3		OR3A2 - OR3A1	rs2005290	intergenic	NR	6.397940009
				10q26.13		FAM53B	rs2629540	intron	NR	7.397940009
				12q24.31		NCOR2	rs150954431	intron	NR	6.301029996
				10q21.2		CDK1	rs2456778	intron	NR	5.301029996
				1q31.3		EEF1A1P14 - KCNT2	rs6677435	intergenic	NR	5.045757491

(*Continues*)

Table 1.1 (Continued)

Reference	*Disease/Trait*	*Initial Sample Size*	*Replication Sample Size*	*Region*	*Reported Gene(s)*	*Mapped_gene*	*SNPs*	*Context*	*Risk Allele Frequency*	*Pvalue_mlog*
				4q34.1		MORF4 - RANP6	rs4129566	intergenic	NR	5.522878745
				4q34.1		MORF4 - RANP6	rs11944332	intergenic	NR	5.698970004
				17p13.3		OR3A2 - OR3A1	rs2005290	intergenic	NR	5.698970004
				10q26.13		FAM53B	rs2629540	intron	NR	6
				10q21.2		CDK1	rs2456778	intron	NR	5.522878745
Yang et al. (2013)	Drinking behavior	1,420 Han Chinese ancestry cases, 3,590 Han Chinese ancestry controls	4,896 Han Chinese ancestry cases, 13,293 Han Chinese ancestry controls	12q24.13	C12orf51, CCDC63, MYL2, ALDH2	HECTD4	rs11066280	intron	0.84	214.5228787
Takeuchi et al. (2011)	Drinking behavior	733 Japanese ancestry cases, 729 Japanese ancestry controls	2,794 Japanese ancestry drinkers, 1,521 Japanese ancestry chance drinkers, 1,351 Japanese ancestry non-drinkers	12q24.12	ALDH2, BRAP	ALDH2	rs671	missense	0.75	210.39794
				12q24.11		CCDC63	rs10774610	intron	0.78	119.0457575
				21q22.13		KCNJ6	rs2835872	intron	0.681	9.301029996
				14q32.2		DEGS2 - YY1	rs2766692	intergenic	0.691	5.698970004
Kang et al. (2012)	Electroenceph-alographic traits in alcoholism	1,560 European ancestry individuals from 117 families	NA	22q13.31	PRR5, ARHGAP8	ARHGAP8; PRR5-ARHGAP8	rs16992796	intron;intron	0.05	5.522878745
				3p11.2		PSMC1P6 - HTR1F	rs9860340	intergenic	0.749	5.397940009
				6p12.1		TRNAI25	rs9395865		0.279	5.22184875
				11q13.1		C11orf84	rs10897449	intron	0.453	5.15490196
				6p22.3		FAM65B	rs4256430	intron	0.465	5.096910013
				15q26.2		NR2F2-AS1	rs7181753	intron	0.199	5.522878745
				1p13.2		SYT6	rs529989	intron	0.443	5.22184875
				1q21.2		MRPS21	rs12403795	intron	0.142	5.301029996
				1q43		WDR64	rs10926554	intron	0.126	5.522878745
McGue et al. (2013)	Illicit drug use	7,188 European ancestry individuals	NA	3p22.2		ITGA9;ITGA9-AS1	rs11129773	intron;intron	0.061	5.096910013
				3q27.3		DGKG - CRYGS	rs1868152	intergenic	0.124	7.301029996

				4p15.31		KRT18P63 - RPL21P46	rs1503874	intergenic	0.372	5.397940009
				6q16.2		FAXC	rs17059400	intron	0.065	5.698970004
				8p23.2		CSMD1	rs13259289	intron	0.399	5.22184875
				12q24.33		TMEM132D	rs7979367	intron	0.402	5.698970004
				17q25.3		SEPT9	rs4788985	intron	0.492	5.045757491
Rice et al. 2012)	Nicotine dependence	2,267 European ancestry individuals, 99 Hispanic individuals, 999 African American individuals	835 individuals	8p11.21		SMIM19 - CHRNB3	rs1451240	intergenic	NR	15.15490196
Thorgeirsson et al. (2008)	Nicotine dependence	10,995 European ancestry individuals	4,848 European ancestry individuals	15q25.1	CHRNA3, CHRNA5, CHRNB4	CHRNA3	rs1051730	STOP-GAIN	0.35	19.22184875
Bierut et al. (2007)	Nicotine dependence	482 European ancestry cases, 466 European ancestry controls	568 European ancestry cases, 413 European ancestry controls	21q22.2		LOC102724740	rs2836823	intron	0.40	5.698970004
				10q21.3		CTNNA3	rs4142041	intron	0.34	5.22184875
McGue et al. (2013)	Nicotine use	7,188 European ancestry individuals	NA	1p32.3		DMRTB1 - GLIS1	rs1298637	intergenic	0.261	5.301029996
				2q37.1		SP140L	rs6712333	intron	0.119	5.301029996
				7q36.2		ACTR3B - DPP6	rs4285401	intergenic	0.447	5.698970004
				8p23.1		NEIL2	rs804292	UTR-3	0.234	5.522878745
				8q24.13		ZHX2	rs6470120	intron	0.331	6.15490196
				10q21.1		ZWINT - MIR3924	rs1907926	intergenic	0.110	6.698970004

(*Continues*)

Table 1.1 (Continued)

Reference	*Disease/Trait*	*Initial Sample Size*	*Replication Sample Size*	*Region*	*Reported Gene(s)*	*Mapped_gene*	*SNPs*	*Context*	*Risk Allele Frequency*	*Pvalue_mlog*
Kumasaka et al. (2012)	Smoking behavior	11,696 Japanese ancestry smokers	5,462 Japanese ancestry smokers	19q13.2		CYP2A6 - CYP2A7	rs8102683	intergenic	NR	41.39794001
				7q31.31		ANKRD7 - GTF3AP6	rs2106595	intergenic	NR	5.301029996
				19q13.2		CYP2A6	rs1801272	missense	0.012	5.301029996
				15q25.1		PSMA4 - CHRNA5	rs2036527	intergenic	0.22	7.698970004
David et al. (2012)	Smoking behavior	Up to 32,389 African American individuals	NA	1q44		C1orf100	rs3101457	intron	0.75	6.522878745
				15q12		LINC00929 - GABRB3	rs547843	intergenic	0.65	6.22184875
				15q25.1		CHRNA5	rs667282	intron	0.29	6.698970004
				10q22.1		CHST3 - SPOCK2	rs1678618	intergenic	0.74	6.096910013
				5q33.2		PPIGP1 - SGCD	rs11134474	intergenic	NR	5.096910013
Yoon et al. (2012)	Smoking behavior	8,842 Korean ancestry individuals	1,366 African American individuals, 671 European ancestry individuals	7q31.1		C7orf66 - EIF3IP1	rs1404697	intergenic	NR	5.096910013
				7q31.1		C7orf66 - EIF3IP1	rs1404697	intergenic	NR	5.301029996
				7q31.1		C7orf66 - EIF3IP1	rs848353	intergenic	NR	5.698970004
				7q31.1		C7orf66 - EIF3IP1	rs848353	intergenic	NR	6.045757491
				7q31.1		C7orf66 - EIF3IP1	rs848353	intergenic	NR	6.522878745
Liu et al. (2010)	Smoking behavior	41,150 European ancestry individuals	120,516 European ancestry individuals	15q25.1	CHRNA5, CHRNA3	CHRNA3	rs1051730	STOP-GAIN	0.66	65.69897
Tobacco and Genetics (2010)	Smoking behavior	Up to 74,035 European ancestry individuals	Up to 68,988 participants	15q25.1	CHRNA3	CHRNA3	rs1051730	STOP-GAIN	0.65	72.52287875
				10q23.32		LOC100188947	rs1329650	intron	0.28	9.22184875
				19q13.2	CYP2A6, EGLN2	EGLN2;RAB4B-EGLN2	rs3733829	intron;intron	0.36	8
				11p14.1	BDNF	BDNF;BDNF-AS	rs6265	missense;ncRNA	0.79	7.698970004
				9q34.2		FAM163B - DBH	rs3025343	intergenic	0.84	7.397940009
				15q25.1		CHRNA3	rs1051730	STOP-GAIN	0.34	68.69897
Thorgeirsson et al. (2010)	Smoking behavior	31,266 European ancestry individuals	54,731 European ancestry individuals	8p11.21		SMIM19 - CHRNB3	rs6474412	intergenic	0.78	8
				7p14.3		SNX2P2 - SLC25A5P5	rs215614	intergenic	0.36	6.698970004
				19q13.2		CYP2A6 - CYP2A7	rs4105144	intergenic	0.70	11.69897
				19q13.2		CYP2B6	rs7260329	intron	0.69	5.22184875

				3q13.12		BBX	rs6437740	intron	0.24	6.698970004
				1p35.2		PTPRU - MATN1	rs910696	intergenic	0.31	5.522878745
				19p12	ZNF505	ZNF93	rs10411195	intron	0.03	5.22184875
				Xq23		TRPC5	rs7050529	intron	0.07	5.22184875
				17p13.2		CAMKK1	rs758642	intron	0.34	5.15490196
				18q11.2		CABLES1	rs11082304	intron	0.49	5.22184875
				4q31.1		RAB33B - SETD7	rs17050782	intergenic	0.21	5.096910013
				1p22.3	AK002179	LINC01140	rs7553864	intron	0.39	5.522878745
				13q33.1		SLC10A2 - ATP6V1G1P7	rs912969	intergenic	0.07	5.096910013
				4q28.1		MIR2054 - NUPL1P1	rs950063	intergenic	0.39	5.045757491
Caporaso et al. (2009)	Smoking behavior	2,617 European ancestry smokers, 1,725 European ancestry controls	NA	3q24		RNA5SP144 - LARP7P4	rs800082	intergenic	0.42	5.522878745
				12q21.2		NAV3	rs1402279	intron	0.95	5.301029996
				5q14.3	LOC133789	ARRDC3-AS1 - RAB5CP2	rs933688	intergenic	0.17	5.22184875
				9p21.2		FAM71BP1 - CAAP1	rs1889899	intergenic	0.37	5.22184875
				9q31.1		MTND3P4 - ARL2BPP7	rs10989661	intergenic	0.74	5.22184875
				12q21.33		MRPL2P1 - LINC00615	rs1847461	intergenic	0.94	5.096910013
				16p13.3		RPS26P51 - TMEM114	rs3112740	intergenic	0.86	5.22184875
				14q24.1		ACTN1	rs2268983	intron	0.51	5.15490196
				Xp11.3		MAOA - MAOB	rs3027409	intergenic	0.95	5.15490196
				7p15.2		SNX10 - KIAA0087	rs886716	intergenic	0.69	5.096910013
				3q27.2		MIR548AQ - TRA2B	rs6444087	intergenic	0.26	5.045757491
Uhl et al. (2010)	Smoking cessation	369 European ancestry individuals	NA	14q24.2		RGS6	rs7159300	intron	NR	5.397940009
Chen et al. (2011)	Substance dependence	2,524 European ancestry individuals, 1,103 African ancestry individuals	NA	11q24.2		PKNOX2	rs12284594	intron	NR	7.15490196

Functional genomics

Assessing the functional implications of any genetic variant that has been found to be associated with a particular phenotype is key to further understanding. For some variants, such as some repeat regions and non-synonymous coding SNPs, functional biological consequences have been identified. However for the majority of the SNPs identified by GWASs, the functional correlates are, as yet, unknown. Functional genomics includes the identification of expression quantitative loci (eQTLs), *in vitro* studies, bioinformatics, the establishment of relevant databases (such as ENCODE), forward genomics, reverse genomics and convergent genomics.

In forward genomics, candidate genes are identified by animal studies. An example of this is gene expression and other molecular studies in alcohol-preferring and non-preferring rats that led to the identification of neuropeptide Y (NPY), alpha-synuclein and corticotrophin-releasing factor receptor 2 as being associated with the linkage signal for alcohol consumption on rat chromosome 4 (Spence et al., 2005). The role of NPY in regulating alcohol consumption and other alcohol-related behaviour has been convincingly demonstrated by the use of NPY knockout and NPY overexpressing mice (Hayes, et al. 2012; Thiele et al., 1998). Alpha-synuclein is expressed throughout the central nervous system (especially in presynaptic nerve terminals; Iwai et al., 1995; Maroteaux et al., 1988; Mori et al., 2002), and may inhibit dopamine synthesis by tyrosine hydroxylase inhibition (Perez et al., 2002). Moreoever, alpha-synuclein has been shown to reduce dopamine transporter activity *in vitro* (Wersinger and Sidhu, 2003).

In reverse genetics, attempts are made to delineate gene function by manipulating the gene in animal models (such as knockouts and knockin with transgenes). The hypothesis that the expression of networks of genes is disrupted in alcohol dependence has been tested using postmortem pre-frontal cortex RNA profiling from alcohol-dependent patients (Farris et al., 2015a, 2015b), which revealed sustained pairwise differential expression profiles related to alcohol use disorder. Convergent functional genomics, a term coined by Alexander Niculescu, approaches gene identification by looking at different lines of evidence (Niculescu et al., 2000). The aim is to create an overall ranking using Bayesian scoring based on the multiple sources of evidence (e.g. from human and animal studies). For example, a convergent functional genomics (CFG) score can be obtained from combining animal genomic, transcriptomic and proteomic data, which is then added to lines of evidence obtained from human studies (linkage, GWAS, genomic, gene expression and proteomic data). In this manner, candidate genes can be ranked according to their CFG score. The potential applicability of this methodology to addictions has been reviewed by Spanagel *et al* (2013).

The Encyclopedia of DNA Elements (ENCODE) database (The ENCODE Project Consortium, 2012; Maurano et al., 2012; Schaub et al., 2012; see also nature.com/ENCODE) aims to facilitate predictions of the functional effects of SNPs by answering the following questions: 1) is the nucleotide transcribed? 2) is the nucleotide part of a transcription factor binding site (TFBS)? 3) is the nucleotide part of a DNase I hypersensitive site (DHS)? 4) is the nucleotide part of a region with altered chromatin marks (histone modifications or DNA methylation): and 5) does the nucleotide physically interact with DNA at great distance from it on the chromosome?. The potential role of ENCODE in nicotine addiction research has been reviewed by Vandenbergh *et al.* (2014). For example, ENCODE shows that the glucocorticoid receptor (transcribed from *NR3C1*) is the transcription factor that binds to the DNA around SNP rs4105144 (relevant to CYP2A6, and associated with number of cigarettes smoked per day) (Onica et al., 2008; Vandenbergh and Schlomer, 2014).

EPIGENETICS

Epigenetics is defined as all meiotically and mitotically heritable changes in gene expression that are not coded by the DNA sequence itself (Egger et al., 2004). Such changes may be affected by environmental factors, with gene silencing being effected by mechanisms including changes in chromatin structure (Toyokawa et al., 2012). Recent data indicates that the three different types of mechanisms involved in such silencing (DNA methylation, histone modification, RNA-associated silencing) may interact with and stabilize each other (Egger et al., 2004). Disruption of one or more of these may lead to inappropriate expression as well as to silencing of genes.

Methylation has long been recognized as an epigenetic silencing mechanism of fundamental importance (Holliday and Pugh, 1975; Riggs, 1975), relevant to transcriptional repression of genes, silencing of transposable elements such as Alu repeat sequences and defence against viral sequences. In DNA methylation, a family of DNA methyltransferases are responsible for adding a methyl group to DNA at a CpG site (Egger et al., 2004), i.e. where a cytosine is linked to a guanine nucleotide by its usual phosphate bridge on a strand of DNA, with methylation occurring at the C^5 position of the cytosine, resulting in 5-methylcytosine. 5-methylcytosine is readily converted to thymine (by spontaneous deamination), resulting in loss of the CpG site. Residual CpG islands (regions of more than 500 base pairs with a GC content of greater than 55%) are conserved in areas of the genome such as promoter regions owing to relative hypomethylation of these areas. Such regions may then be subject to differential methylation, e.g. in response to early environmental insults, leading to differential susceptibility to the effects of subsequent exposures including addictive substances and behaviours.

In chromatin, DNA coiled around a core group of eight histone proteins is known as a nucleosome, with this level of structure acting as a regulatory site for subsequent higher levels of coiling and looping of DNA, which render the DNA more (in euchromatin) or less (in heterochromatin) accessible for transcription. The eight core histone proteins comprise two each of four types of histones (H2A, H2B, H3 and H4), with each nucleosome also containing one linker histone (H1). Post-translational modifications of histone proteins (acetylation, methylation, phosphorylation or ubiquitylation) play essential roles in regulating the dynamic structure of chromatin. The particular combination of histone modifications found in a cell has been termed a 'histone code, and is one epigenetic mechanism whereby the information potential of the genetic code is extended. Histone acetylation or methylation occurs at conserved lysine residues in histone amino acid tail domains, with acetylation (by histone acetyl transferase) in most cases enhancing transcription and deacetylation (by histone deacetylases, otherwise known as HDACs) being associated with inactive chromatin. Histone methylation, by contrast, can be a marker for both active and inactive regions of chromatin, with H3 lysine 9 methylation (H3-K9) occurring in gene promoters that have been 'silenced' and H3 lysine 4 (H3-K4) methylation occurring in promoters of active genes. Interactions between histone deacetylases, histone methyltransferases and methylcytosine-binding proteins may lead to the recruitment of DNA methyltransferases (Egger et al, 2004), and hence methylation of susceptible regions of DNA.

Mechanisms of RNA-associated silencing include antisense transcripts, noncoding RNAs and RNA interference (RNAi). In a case of alpha-thalassemia, it was shown that antisense transcription could lead to DNA methylation and stable silencing of a globin gene (Egger et al., 2004; Tufarelli et al., 2003). Noncoding RNAs (biologically functional RNAs that do not encode proteins) include microRNAs (miRNAs, approximately 22 nucleotides long), which bind to mRNAs resulting in post-transcriptional

silencing and may be particularly relevant to the regulation of gene expression in the brain (Miska et al., 2004).

Epigenetics and substance use

In drug addiction, epigenetics has been used to explain phenomena relevant to addictions, such as the formation of memories, drug-seeking behaviour (Malvaez et al., 2009), toxicity (Kovatsi et al., 2011) and withdrawal and other behavioural changes (Pizzimenti and Lattal, 2015). The effects on gene expression can be seen after acute administration of substances as well as with chronic exposure. An example of an acute epigenetic effect is the decrease in HDAC activity that occurs in the amygdala of rats after an acute injection of alcohol (Pandey et al., 2008). Repeated exposure to drugs of addiction can lead to persistent alterations in dendritic structure and dendritic spines, in motivation and reward-related neurons (Robinson and Kolb, 2004) that is mediated through epigenetic mechanisms. Patients with addiction have a higher incidence of adverse childhood experiences (Felitti, 2003). Such experiences lead to adaptational changes in and around the hypothalamo-pituitary-adrenal axis, including via methylation changes in genes that are related to the stress response (Brockie et al., 2013). Weaver *et al.* (2004) conducted an elegant animal study to demonstrate the effects of behaviour on the epigenome. They observed that rat pups that experienced high levels of licking, grooming or nursing had reversible differences in DNA methylation at the glucocorticoid receptor gene promoter in the hippocampus, compared to pups that experienced low levels of similar activity(Weaver et al., 2004). Epigenetic changes in response to stressful events such as adverse childhood experiences may therefore be pivotal in the predisposition to drug addiction (Brockie et al., 2013).

Methylation of two CpG islands in the monoamineoxidase-A (MAOA) gene is associated with alcohol and nicotine dependence in women (Philibert et al., 2008). Chronic alcohol consumption increases NMDA receptor *N2RB* gene expression through demethylation (Marutha Ravindran and Ticku, 2004). Alcohol-mediated anxiolysis has been associated with reduced HDAC activity, while anxiety-like behaviour in alcohol withdrawal has been associated with increased HDAC activity in the rat amygdala (Pandey et al., 2008). Elevated homocysteine levels may be seen in those who are alcohol dependent and this has been related to increased homocysteine-induced endoplasmic reticulum protein (*HERP*) gene promoter DNA hypermethylation, which has been shown to reduce *HERP* mRNA expression (Bleich et al., 2006). Hypermethylation in the promoter of the alpha synuclein (*SNCA*) gene has also been correlated with elevated homocysteine levels in alcohol dependence (Bonsch et al., 2005). Nicotine use has been found to cause differential DNA methylation at loci near the *F2EL3*, *AHPR*, *GPR12*, *IE3*, *ALPP*, *RARA*, *GNG12*, *ZNF385D*, *PRSS23*, *AVPR1B*, *PSEN2*, *LINC00299*, *RPS6KA2*, *KIAA0087* and *LRP5* genes (Tsaprouni et al., 2014). Nielsen *et al.* (2009) analysed methylation at 16 CpG sites in the *OPRM1* promoter region and found that two sites had higher methylation in patients who were former heroin addicts compared to the controls. In an animal study, methamphetamine reduced mRNA and protein levels of GluA1 and GluA2 AMPA receptor subunits through epigenetic mechanisms (Cadet and Jayanthi, 2013). An epigenome-wide association study for smoking found an association with 30 probes in 15 loci in a discovery cohort. Twenty-nine of these probes were significant in the replication cohort of this study (Tsaprouni et al., 2014), and 9 of the 15 loci had previously been found to be significantly associated with to smoking.

The Schaefer *et al.* (2010) study is a good example of how miRNA-mediated change in gene and protein expression is related to addiction. They demonstrated substantial

alterations in multiple miRNA expression after cocaine exposure, some of which affect genes related to motivation, such as bdnf, fosB (FBJ murine osteosarcoma viral oncogene homolog B) and cdk5r1 (cyclin-dependent kinase 5 activator 1). Argonaute proteins bind miRNA, and are a vital component of the multiprotein RNA-induced silencing complex (RISC) that executes miRNA functions (Hutvagner et al., 2001; Lingel et al., 2003). Schaefer *et al.* (2010) found that deficiency of the Argonaute 2 (Ago2) in mouse brain D2-receptor expressing neurons was related to a reduction in certain miRNA subtypes and a motivation to self-administer cocaine. In addition, increases in the expression of Ago2 and specific miRNA increases have been seen after cocaine exposure (Eipper-Mains et al., 2011).

GENETICS OF BEHAVIOURAL ADDICTIONS

Internet addiction and internet video game addiction

Although problematic internet use is not yet included in DSM-5, this may be of a nature and severity fitting the term behavioural addiction and may pose significant problems for an individual or those with whom they interface. The Young's Internet Addiction Scale can be used to identify problematic internet use (Young, 1999). Montag et al. (2012) linked a marker in the nicotinic receptor *CHRNA4* gene, rs1044396 SNP, to internet addiction, which appeared to be particularly relevant in females. The serotonin system also appears to be relevant, specifically the low expression (SS or short-short) variant *5-HTTLPR* has associated with excessive internet use (Lee et al., 2008). This variant has also been associated with depression in individuals exposed to childhood maltreatment (Cerda et al., 2010; Uher et al., 2011).

In regard to the dopamine system, individuals with excessive internet video game addiction had higher frequencies of the Met variant of the *COMT*Val(158)Met, and *DRD2* Taq1A1 alleles (Han et al., 2007).

Gambling

Approximately 50% (range 43–60%) of the variance in gambling behaviour (e.g. buying a lottery ticket, time or funds spent gambling, etc.) is attributable to genetic factors (Eisen et al., 1998; Lobo and Kennedy, 2009; Slutske et al., 2009) with problem gambling showing some common genetic loading with alcohol dependence. The first GWAS of disordered gambling was conducted on 1312 twins from 894 Australian families (Lind et al., 2013). Although no single genetic marker reached genome-wide significance, this may be owing to the moderate heritability of the trait, more than 2 million markers being used, and phenotypic variation. Suggestive evidence of association was found for *MT1X* (metallothionein), *ATXN1* (ataxin1) and *VLDLR* (encoding the very low density lipoprotein receptor), the latter confirmed in secondary case–control analyses as being associated with pathological gambling. The *VLDLR* is a receptor for reelin, and the reelin-VLDLR/ApoER2 signalling pathway controls cortical neuronal migration in early development and modulates synaptic plasticity, memory and learning in the adult brain (Herz and Chen, 2006). This signalling pathway has also been implicated in a variety of mental illnesses, including depression, bipolar disorder, and schizophrenia (Barr et al., 2007; Suzuki et al., 2008). Other pathways that have previously been associated with addictions (including dopamine agonist-induced problem gambling in the context of Parkinson's disease) also appeared to be over-represented in terms of marker associations. A study conducted in Alberta and Ontario combined information from rat models and gamblers. In this study, tagSNPs in the genes *DRD3* (rs167771) and CAMK2D (rs3815072) were associated with disordered

gambling (Lobo et al., 2015). Further genetic research in gambling is warranted, paying attention to the particular gambling phenotypes being measured and comorbidity.

Sexual addiction

Repeat regions in genes include variable number tandem repeats (VNTRs) and short tandem repeats (STRs). Sexual addiction phenotypes may have an association with these types of repeats in genes such as those encoding the dopamine-4 receptor (*DRD4*), the dopamine transporter (*SLC6A3*), the arginine vasopressin 1A receptor (*AVPR1A*), the oxytocin receptor (*OXTR*) and the serotonin transporter (*HTT* or *SLC6A4).* In addition, some SNPs in the above genes have been associated with relevant phenotypes, together with the above markers in haplotypic association analysis.

Ben Zion *et al.* (2006) reported such an association between a 5-marker *DRD4* haplotype comprising the functional exon 3 VNTR and promoter SNPs with self-reported measures of human sexual behaviour in a group of university students. Possession of at least one *DRD4* exon 3 'long' allele (defined as 7–11 repeats) was found to be associated with early sexual onset in an adverse environment when compared to *DRD4* 'short' allele (defined as 6 or fewer repeats) homozygosity in African-American youths (Kogan et al., 2014). Guo and Tong (2006) found that the exon 3 *DRD4* polymorphism was associated with age at first intercourse. Men with least one 10-repeat (10R) of 40-bp in the dopamine transporter gene *DAT1* have an 80–100% increase in number of partners compared to those with two 9-repeat alleles (Guo et al., 2007), while the Taq1A SNP polymorphism in the same gene has also been associated with relevant phenotypes. In *Drosophila*, a subset of dopamine neurons regulates age-associated male courtship activity (Kuo et al., 2015). Prichard *et al.* (2007) reported an association between *AVPR1A* and *OXTR* gene polymorphisms and behavioural phenotypes in sexual and reproductive domains. Walum *et al.* (2008) subsequently reported an association between the polymorphic repeat in the 5′ flanking region of the *AVPR1A* (RS3 variant) and traits reflecting pair-bonding behaviour in men, including partner bonding, perceived marital problems and marital quality as perceived by spouses, and marital status. Kogan *et al.* (2010) found that 5-*HTTLPR* moderated the effect of early adolescent substance use and risky sexual behaviour in African-American youths 2 years later, with the 'at risk' group being those with at least one copy of the short ('s') allele.

FUTURE DIRECTIONS

Many of the associations summarized above have not yet been replicated. The first step forward must be replication and validation. Next comes understanding of the functional, or biological consequences, of any genetic or epigenetic markers. Subsequently, and, in some cases appropriately in parallel, translation to clinical application may occur. This may range from identification of those at above average risk of developing a disorder, to those more likely to respond better or worse to specific treatment (psychological or pharmacological). Where a robust functional association has been identified with a single biomarker, this is readily translated to an assay that may be efficiently deployed. With larger sets of biomarkers, technologies such as high density microarrays render large-scale testing feasible.

REFERENCES

Agrawal A and Lynskey MT. (2009) Candidate genes for cannabis use disorders: findings, challenges and directions. *Addiction* 104: 518–532.

Agrawal A, Lynskey MT, Bucholz KK, et al. (2007) Contrasting models of genetic comorbidity for cannabis and other illicit drugs in adult Australian twins. *Psychol Med* 37: 49–60.

Agrawal A, Lynskey MT, Hinrichs A, et al. (2011) A genome-wide association study of DSM-IV cannabis dependence. *Addict Biol* 16: 514–518.

Agrawal A, Wetherill L, Dick DM, et al. (2009) Evidence for association between polymorphisms in the cannabinoid receptor 1 (CNR1) gene and cannabis dependence. *Am J Med Genet B Neuropsychiatr Genet* 150B: 736–740.

Alemany S, Arias B, Fatjo-Vilas M, et al. (2014) Psychosis-inducing effects of cannabis are related to both childhood abuse and COMT genotypes. *Acta Psychiatr Scand* 129: 54–62.

Aoyama N, Takahashi N, Kitaichi K, et al. (2006) Association between gene polymorphisms of SLC22A3 and methamphetamine use disorder. *Alcohol Clin Exp Res* 30: 1644–1649.

APA (American Psychiatric Association). (2013) *Diagnostic and statistical manual of mental disorders: DSM-5.,* Washington, DC: American Psychiatric Association.

Baik I, Cho NH, Kim SH, et al. (2011) Genome-wide association studies identify genetic loci related to alcohol consumption in Korean men. *Am J Clin Nutr* 93: 809–816.

Bakker SC, Hoogendoorn ML, Selten JP, et al. (2004) Neuregulin 1: genetic support for schizophrenia subtypes. *Mol Psychiatry* 9: 1061–1063.

Baransel Isir AB, Oguzkan S, Nacak M, et al. (2008) The catechol-O-methyl transferase Val158Met polymorphism and susceptibility to cannabis dependence. *Am J Forensic Med Pathol* 29: 320–322.

Barr AM, Fish KN and Markou A. (2007) The reelin receptors VLDLR and ApoER2 regulate sensorimotor gating in mice. *Neuropharmacology* 52: 1114–1123.

Bart G, Heilig M, LaForge KS, et al. (2004) Substantial attributable risk related to a functional mu-opioid receptor gene polymorphism in association with heroin addiction in central Sweden. *Mol Psychiatry* 9: 547–549.

Benyamina A, Bonhomme-Faivre L, Picard V, et al. (2009) Association between ABCB1 C3435T polymorphism and increased risk of cannabis dependence. *Prog Neuropsychopharmacol Biol Psychiatry* 33: 1270–1274.

Bierut LJ, Madden PA, Breslau N, et al. (2007) Novel genes identified in a high-density genome wide association study for nicotine dependence. *Hum Mol Genet* 16: 24–35.

Bierut LJ, Stitzel JA, Wang JC, et al. (2008) Variants in nicotinic receptors and risk for nicotine dependence. *Am J Psychiatry* 165: 1163–1171.

Bleich S, Lenz B, Ziegenbein M, et al. (2006) Epigenetic DNA hypermethylation of the HERP gene promoter induces down-regulation of its mRNA expression in patients with alcohol dependence. *Alcohol Clin Exp Res* 30: 587–591.

Bloom AJ, Hartz SM, Baker TB, et al. (2014) Beyond cigarettes per day. A genome-wide association study of the biomarker carbon monoxide. *Ann Am Thorac Soc* 11: 1003–1010.

Bond C, LaForge KS, Tian M, et al. (1998) Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. *Proc Natl Acad Sci U S A* 95: 9608–9613.

Bonsch D, Lenz B, Kornhuber J, et al. (2005) DNA hypermethylation of the alpha synuclein promoter in patients with alcoholism. *Neuroreport* 16: 167–170.

Bramon E, Dempster E, Frangou S, et al. (2008) Neuregulin-1 and the P300 waveform–a preliminary association study using a psychosis endophenotype. *Schizophr Res* 103: 178–185.

Brockie TN, Heinzelmann M and Gill J. (2013) A Framework to Examine the Role of Epigenetics in Health Disparities among Native Americans. *Nurs Res Pract* 2013: 410395.

Buhler KM, Gine E, Echeverry-Alzate V, et al. (2015) Common single nucleotide variants underlying drug addiction: more than a decade of research. *Addict Biol*.

Cadet JL and Jayanthi S. (2013) Epigenetics of methamphetamine-induced changes in glutamate function. *Neuropsychopharmacology* 38: 248–249.

Caporaso N, Gu F, Chatterjee N, et al. (2009) Genome-wide and candidate gene association study of cigarette smoking behaviors. *PLoS One* 4: e4653.

Carlson MD, Mendle J and Harden KP. (2014) Early adverse environments and genetic influences on age at first sex: evidence for gene x environment interaction. *Dev Psychol* 50: 1532–1542.

Caspi A, Moffitt TE, Cannon M, et al. (2005) Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biol Psychiatry* 57: 1117–1127.

Cerda M, Sagdeo A, Johnson J, et al. (2010) Genetic and environmental influences on psychiatric comorbidity: a systematic review. *J Affect Disord* 126: 14–38.

Chen X, Cho K, Singer BH, et al. (2011) The nuclear transcription factor PKNOX2 is a candidate gene for substance dependence in European-origin women. *PLoS One* 6: e16002.

Cherkas LF, Oelsner EC, Mak YT, et al. (2004) Genetic influences on female infidelity and number of sexual partners in humans: a linkage and association study of the role of the vasopressin receptor gene (AVPR1A). *Twin Res* 7: 649–658.

Clarke TK, Bloch PJ, Ambrose-Lanci LM, et al. (2013) Further evidence for association of polymorphisms in the CNR1 gene with cocaine addiction: confirmation in an independent sample and meta-analysis. *Addict Biol* 18: 702–708.

Comings DE, Gonzalez N, Wu S, et al. (1999) Homozygosity at the dopamine DRD3 receptor gene in cocaine dependence. *Mol Psychiatry* 4: 484–487.

Covault J, Gelernter J, Jensen K, et al. (2008) Markers in the 5'-region of GABRG1 associate to alcohol dependence and are in linkage disequilibrium with markers in the adjacent GABRA2 gene. *Neuropsychopharmacology* 33: 837–848.

David SP, Hamidovic A, Chen GK, et al. (2012) Genome-wide meta-analyses of smoking behaviors in African Americans. *Transl Psychiatry* 2: e119.

Dlugos AM, Hamidovic A, Hodgkinson C, et al. (2011) OPRM1 gene variants modulate amphetamine-induced euphoria in humans. *Genes Brain Behav* 10: 199–209.

Donahue KL, Lichtenstein P, Lundstrom S, et al. (2013) Childhood behavior problems and adolescent sexual risk behavior: familial confounding in the child and adolescent twin study in Sweden (CATSS). *J Adolesc Health* 52: 606–612.

Douet V, Chang L, Pritchett A, et al. (2014) Schizophrenia-risk variant rs6994992 in the neuregulin-1 gene on brain developmental trajectories in typically developing children. *Transl Psychiatry* 4: e392.

Drakenberg K, Nikoshkov A, Horvath MC, et al. (2006) Mu opioid receptor A118G polymorphism in association with striatal opioid neuropeptide gene expression in heroin abusers. *Proc Natl Acad Sci U S A* 103: 7883–7888.

East PL and Jacobson LJ. (2001) The younger siblings of teenage mothers: a follow-up of their pregnancy risk. *Dev Psychol* 37: 254–264.

Edenberg HJ, Dick DM, Xuei X, et al. (2004) Variations in GABRA2, encoding the alpha 2 subunit of the GABA(A) receptor, are associated with alcohol dependence and with brain oscillations. *Am J Hum Genet* 74: 705–714.

Edenberg HJ and Foroud T. (2014) Genetics of alcoholism. *Handb Clin Neurol* 125: 561–571.

Edwards AC, Aliev F, Wolen AR, et al. (2015) Genomic influences on alcohol problems in a population-based sample of young adults. *Addiction* 110: 461–470.

Egger G, Liang G, Aparicio A, et al. (2004) Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 429: 457–463.

Eipper-Mains JE, Kiraly DD, Palakodeti D, et al. (2011) microRNA-Seq reveals cocaine-regulated expression of striatal microRNAs. *RNA* 17: 1529–1543.

Eisen SA, Lin N, Lyons MJ, et al. (1998) Familial influences on gambling behavior: an analysis of 3359 twin pairs. *Addiction* 93: 1375–1384.

Ermis A, Erkiran M, Dasdemir S, et al. (2015) The relationship between catechol-O-methyltransferase gene Val158Met (COMT) polymorphism and premorbid cannabis use in Turkish male patients with schizophrenia. *In Vivo* 29: 129–132.

Estrada G, Fatjo-Vilas M, Munoz MJ, et al. (2011) Cannabis use and age at onset of

psychosis: further evidence of interaction with COMT Val158Met polymorphism. *Acta Psychiatr Scand* 123: 485–492.

Farris SP, Arasappan D, Hunicke-Smith S, et al. (2015a) Transcriptome organization for chronic alcohol abuse in human brain. *Mol Psychiatry* 20: 1438–1447.

Farris SP, Pietrzykowski AZ, Miles MF, et al. (2015b) Applying the new genomics to alcohol dependence. *Alcohol*.

Felitti VJ. (2003) [Origins of addictive behavior: evidence from a study of stressful chilhood experiences]. *Prax Kinderpsychol Kinderpsychiatr* 52: 547–559.

Fernandez-Castillo N, Ribases M, Roncero C, et al. (2010) Association study between the DAT1, DBH and DRD2 genes and cocaine dependence in a Spanish sample. *Psychiatr Genet* 20: 317–320.

Frank J, Cichon S, Treutlein J, et al. (2012) Genome-wide significant association between alcohol dependence and a variant in the ADH gene cluster. *Addict Biol* 17: 171–180.

Gelernter J, Kranzler H and Satel SL. (1999) No association between D2 dopamine receptor (DRD2) alleles or haplotypes and cocaine dependence or severity of cocaine dependence in European- and African-Americans. *Biol Psychiatry* 45: 340–345.

Gelernter J, Sherva R, Koesterer R, et al. (2014) Genome-wide association study of cocaine dependence and related traits: FAM53B identified as a risk gene. *Mol Psychiatry* 19: 717–723.

Gerra G, Leonardi C, Cortese E, et al. (2007) Human kappa opioid receptor gene (OPRK1) polymorphism is associated with opiate addiction. *Am J Med Genet B Neuropsychiatr Genet* 144B: 771–775.

Glatt SJ, Bousman C, Wang RS, et al. (2007) Evaluation of OPRM1 variants in heroin dependence by family-based association testing and meta-analysis. *Drug Alcohol Depend* 90: 159–165.

Gold AB and Lerman C. (2012) Pharmacogenetics of smoking cessation: role of nicotine target and metabolism genes. *Hum Genet*.

Grucza RA, Wang JC, Stitzel JA, et al. (2008) A risk allele for nicotine dependence in CHRNA5 is a protective allele for cocaine dependence. *Biol Psychiatry* 64: 922–929.

Grzywacz A, Malecka I, Korostynski M, et al. (2012) GABA-A receptor genes do not play a role in genetics of Lesch's typology in Caucasian subjects. *Arch Med Sci* 8: 357–361.

Guindalini C, Howard M, Haddley K, et al. (2006) A dopamine transporter gene functional variant associated with cocaine abuse in a Brazilian sample. *Proc Natl Acad Sci U S A* 103: 4552–4557.

Guo G and Tong Y. (2006) Age at first sexual intercourse, genes, and social context: evidence from twins and the dopamine D4 receptor gene. *Demography* 43: 747–769.

Haberstick BC, Timberlake D, Ehringer MA, et al. (2007) Genes, time to first cigarette and nicotine dependence in a general population sample of young adults. *Addiction* 102: 655–665.

Hall FS, Drgonova J, Jain S, et al. (2013) Implications of genome wide association studies for addiction: are our a priori assumptions all wrong? *Pharmacol Ther* 140: 267–279.

Han DH, Lee YS, Yang KC, et al. (2007) Dopamine genes and reward dependence in adolescents with excessive internet video game play. *J Addict Med* 1: 133–138.

Han S, Yang BZ, Kranzler HR, et al. (2012) Linkage analysis followed by association show NRG1 associated with cannabis dependence in African Americans. *Biol Psychiatry* 72: 637–644.

Harden KP and Mendle J. (2011) Why don't smart teens have sex? A behavioral genetic approach. *Child Dev* 82: 1327–1344.

Hart AB, Engelhardt BE, Wardle MC, et al. (2012) Genome-wide association study of d-amphetamine response in healthy volunteers identifies putative associations, including cadherin 13 (CDH13). *PLoS One* 7: e42646.

Hart AB, Lynch KG, Farrer L, et al. (2015) Which alcohol use disorder criteria contribute to the association of ADH1B with alcohol dependence? *Addict Biol*.

Haughey HM, Marshall E, Schacht JP, et al. (2008) Marijuana withdrawal and craving: influence of the cannabinoid receptor 1 (CNR1) and fatty acid amide hydrolase (FAAH) genes. *Addiction* 103: 1678–1686.

Heath AC, Whitfield JB, Martin NG, et al. (2011) A quantitative-trait genome-wide association study of alcoholism risk in the

community: findings and implications. *Biol Psychiatry* 70: 513–518.

Henquet C, Rosa A, Delespaul P, et al. (2009) COMT ValMet moderation of cannabis-induced psychosis: a momentary assessment study of 'switching on' hallucinations in the flow of daily life. *Acta Psychiatr Scand* 119: 156–160.

Henquet C, Rosa A, Krabbendam L, et al. (2006) An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. *Neuropsychopharmacology* 31: 2748–2757.

Herz J and Chen Y. (2006) Reelin, lipoprotein receptors and synaptic plasticity. *Nat Rev Neurosci* 7: 850–859.

Higuchi S. (1994) Polymorphisms of ethanol metabolizing enzyme genes and alcoholism. *Alcohol Alcohol Suppl* 2: 29–34.

Hill SY, Jones BL, Zezza N, et al. (2013) Family-based association analysis of alcohol dependence implicates KIAA0040 on Chromosome 1q in multiplex alcohol dependence families. *Open J Genet* 3: 243–252.

Hill SY, Jones BL, Zezza N, et al. (2015) ACN9 and alcohol dependence: Family-based association analysis in multiplex alcohol dependence families. *Am J Med Genet B Neuropsychiatr Genet* 168: 179–187.

Hill SY, Shen S, Zezza N, et al. (2004) A genome wide search for alcoholism susceptibility genes. *Am J Med Genet B Neuropsychiatr Genet* 128B: 102–113.

Holliday R and Pugh JE. (1975) DNA modification mechanisms and gene activity during development. *Science* 187: 226–232.

Hosak L, Libiger J, Cizek J, et al. (2006) The COMT Val158Met polymorphism is associated with novelty seeking in Czech methamphetamine abusers: preliminary results. *Neuro Endocrinol Lett* 27: 799–802.

Hosak L, Sery O, Beranek M, et al. (2011) Lack of association between the Val158Met catechol-O-methyltransferase gene polymorphism and methamphetamine dependence. *Neuro Endocrinol Lett* 32: 469–474.

Hutvagner G, McLachlan J, Pasquinelli AE, et al. (2001) A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science* 293: 834–838.

Ide S, Kobayashi H, Tanaka K, et al. (2004) Gene polymorphisms of the mu opioid receptor in methamphetamine abusers. *Ann N Y Acad Sci* 1025: 316–324.

Ide S, Kobayashi H, Ujike H, et al. (2006) Linkage disequilibrium and association with methamphetamine dependence/psychosis of mu-opioid receptor gene polymorphisms. *Pharmacogenomics J* 6: 179–188.

Ikeda M, Iwata N, Suzuki T, et al. (2006) Positive association of AKT1 haplotype to Japanese methamphetamine use disorder. *Int J Neuropsychopharmacol* 9: 77–81.

Iwahashi K, Waga C and Takimoto T. (2004) Whole deletion of CYP2A6 gene (CYP2A6AST;4C) and smoking behavior. *Neuropsychobiology* 49: 101–104.

Jasiewicz A, Samochowiec A, Samochowiec J, et al. (2014) Suicidal behavior and haplotypes of the dopamine receptor gene (DRD2) and ANKK1 gene polymorphisms in patients with alcohol dependence–preliminary report. *PLoS One* 9: e111798.

Jia W, Shi JG, Wu B, et al. (2011) Polymorphisms of brain-derived neurotrophic factor associated with heroin dependence. *Neurosci Lett* 495: 221–224.

Jugurnauth SK, Chen CK, Barnes MR, et al. (2011) A COMT gene haplotype associated with methamphetamine abuse. *Pharmacogenet Genomics* 21: 731–740.

Juraeva D, Treutlein J, Scholz H, et al. (2015) XRCC5 as a risk gene for alcohol dependence: evidence from a genome-wide gene-set-based analysis and follow-up studies in Drosophila and humans. *Neuropsychopharmacology* 40: 361–371.

Kang SJ, Rangaswamy M, Manz N, et al. (2012) Family-based genome-wide association study of frontal theta oscillations identifies potassium channel gene KCNJ6. *Genes Brain Behav* 11: 712–719.

Kapoor M, Wang JC, Wetherill L, et al. (2013) A meta-analysis of two genome-wide association studies to identify novel loci for maximum number of alcoholic drinks. *Hum Genet* 132: 1141–1151.

Kapur S, Sharad S, Singh RA, et al. (2007) A118g polymorphism in mu opioid receptor gene (oprm1): association with opiate addiction in subjects of Indian origin. *J Integr Neurosci* 6: 511–522.

Kendler KS, Kalsi G, Holmans PA, et al. (2011) Genomewide association analysis of symptoms of alcohol dependence in the molecular genetics of schizophrenia (MGS2) control sample. *Alcohol Clin Exp Res* 35: 963–975.

Kendler KS, Karkowski L and Prescott CA. (1999) Hallucinogen, opiate, sedative and stimulant use and abuse in a population-based sample of female twins. *Acta Psychiatr Scand* 99: 368–376.

Kendler KS, Karkowski LM, Neale MC, et al. (2000) Illicit psychoactive substance use, heavy use, abuse, and dependence in a US population-based sample of male twins. *Arch Gen Psychiatry* 57: 261–269.

Kishi T, Kitajima T, Tsunoka T, et al. (2010) PROKR2 is associated with methamphetamine dependence in the Japanese population. *Prog Neuropsychopharmacol Biol Psychiatry* 34: 1033–1036.

Kobayashi H, Hata H, Ujike H, et al. (2006) Association analysis of delta-opioid receptor gene polymorphisms in methamphetamine dependence/psychosis. *Am J Med Genet B Neuropsychiatr Genet* 141B: 482–486.

Kobayashi H, Ujike H, Iwata N, et al. (2010) The adenosine A2A receptor is associated with methamphetamine dependence/psychosis in the Japanese population. *Behav Brain Funct* 6: 50.

Kovatsi L, Fragou D, Samanidou V, et al. (2011) Drugs of abuse: epigenetic mechanisms in toxicity and addiction. *Curr Med Chem* 18: 1765–1774.

Kuhar MJ, Ritz MC and Boja JW. (1991) The dopamine hypothesis of the reinforcing properties of cocaine. *Trends Neurosci* 14: 299–302.

Kumasaka N, Aoki M, Okada Y, et al. (2012) Haplotypes with copy number and single nucleotide polymorphisms in CYP2A6 locus are associated with smoking quantity in a Japanese population. *PLoS One* 7: e44507.

Kutalik Z, Benyamin B, Bergmann S, et al. (2011) Genome-wide association study identifies two loci strongly affecting transferrin glycosylation. *Hum Mol Genet* 20: 3710–3717.

Lee YS, Han DH, Yang KC, et al. (2008) Depression like characteristics of 5HTTLPR polymorphism and temperament in excessive internet users. *J Affect Disord* 109: 165–169.

Levran O, O'Hara K, Peles E, et al. (2008) ABCB1 (MDR1) genetic variants are associated with methadone doses required for effective treatment of heroin dependence. *Hum Mol Genet* 17: 2219–2227.

Levran O, Randesi M, da Rosa JC, et al. (2015) Overlapping dopaminergic pathway genetic susceptibility to heroin and cocaine addictions in African Americans. *Ann Hum Genet* 79: 188–198.

Levran O, Yuferov V and Kreek MJ. (2012) The genetics of the opioid system and specific drug addictions. *Hum Genet* 131: 823–842.

Li MD, Xu Q, Lou XY, et al. (2010) Association and interaction analysis of variants in CHRNA5/CHRNA3/CHRNB4 gene cluster with nicotine dependence in African and European Americans. *Am J Med Genet B Neuropsychiatr Genet* 153B: 745–756.

Lind PA, Zhu G, Montgomery GW, et al. (2013) Genome-wide association study of a quantitative disordered gambling trait. *Addict Biol* 18: 511–522.

Lingel A, Simon B, Izaurralde E, et al. (2003) Structure and nucleic-acid binding of the Drosophila Argonaute 2 PAZ domain. *Nature* 426: 465–469.

Liu JZ, Tozzi F, Waterworth DM, et al. (2010) Meta-analysis and imputation refines the association of 15q25 with smoking quantity. *Nat Genet* 42: 436–440.

Liu Z, Guo X, Jiang Y, et al. (2013) NCK2 is significantly associated with opiates addiction in African-origin men. *ScientificWorldJournal* 2013: 748979.

Lobo DS, Aleksandrova L, Knight J, et al. (2015) Addiction-related genes in gambling disorders: new insights from parallel human and pre-clinical models. *Mol Psychiatry* 20: 1002–1010.

Lobo DS and Kennedy JL. (2009) Genetic aspects of pathological gambling: a complex disorder with shared genetic vulnerabilities. *Addiction* 104: 1454–1465.

Lohoff FW, Bloch PJ, Hodge R, et al. (2010) Association analysis between polymorphisms in the dopamine D2 receptor (DRD2) and dopamine transporter (DAT1) genes with cocaine dependence. *Neurosci Lett* 473: 87–91.

Lohoff FW, Weller AE, Bloch PJ, et al. (2008) Association between the catechol-O-methyltransferase Val158Met polymorphism and

cocaine dependence. *Neuropsychopharmacology* 33: 3078–3084.

Loukola A, Wedenoja J, Keskitalo-Vuokko K, et al. (2014) Genome-wide association study on detailed profiles of smoking behavior and nicotine dependence in a twin sample. *Mol Psychiatry* 19: 615–624.

Lydall GJ, Bass NJ, McQuillin A, et al. (2011) Confirmation of prior evidence of genetic susceptibility to alcoholism in a genome-wide association study of comorbid alcoholism and bipolar disorder. *Psychiatr Genet* 21: 294–306.

Lynskey MT, Agrawal A, Henders A, et al. (2012) An Australian twin study of cannabis and other illicit drug use and misuse, and other psychopathology. *Twin Res Hum Genet* 15: 631–641.

Ma Y, Yuan W, Jiang X, et al. (2015) Updated findings of the association and functional studies of DRD2/ANKK1 variants with addictions. *Mol Neurobiol* 51: 281–299.

MacRae CA. (2015) A Critical Need for Clinical Context in the Genomic Era. *Circulation* 132: 992–993.

Malvaez M, Barrett RM, Wood MA, et al. (2009) Epigenetic mechanisms underlying extinction of memory and drug-seeking behavior. *Mamm Genome* 20: 612–623.

Marutha Ravindran CR and Ticku MK. (2004) Changes in methylation pattern of NMDA receptor NR2B gene in cortical neurons after chronic ethanol treatment in mice. *Brain Res Mol Brain Res* 121: 19–27.

McGue M, Zhang Y, Miller MB, et al. (2013) A genome-wide association study of behavioral disinhibition. *Behav Genet* 43: 363–373.

Meade CS, Kershaw TS and Ickovics JR. (2008) The intergenerational cycle of teenage motherhood: an ecological approach. *Health Psychol* 27: 419–429.

Minica CC, Dolan CV, Hottenga JJ, et al. (2015) Heritability, SNP- and Gene-Based Analyses of Cannabis Use Initiation and Age at Onset. *Behav Genet*.

Miska EA, Alvarez-Saavedra E, Townsend M, et al. (2004) Microarray analysis of microRNA expression in the developing mammalian brain. *Genome Biol* 5: R68.

Mwenifumbo JC, Sellers EM and Tyndale RF. (2007) Nicotine metabolism and CYP2A6 activity in a population of black African descent: impact of gender and light smoking. *Drug Alcohol Depend* 89: 24–33.

Niculescu AB, 3rd, Segal DS, Kuczenski R, et al. (2000) Identifying a series of candidate genes for mania and psychosis: a convergent functional genomics approach. *Physiol Genomics* 4: 83–91.

Nielsen DA, Ji F, Yuferov V, et al. (2010) Genome-wide association study identifies genes that may contribute to risk for developing heroin addiction. *Psychiatr Genet* 20: 207–214.

Nielsen DA, Yuferov V, Hamon S, et al. (2009) Increased OPRM1 DNA methylation in lymphocytes of methadone-maintained former heroin addicts. *Neuropsychopharmacology* 34: 867–873.

Noble EP, Blum K, Khalsa ME, et al. (1993) Allelic association of the D2 dopamine receptor gene with cocaine dependence. *Drug Alcohol Depend* 33: 271–285.

Onica T, Nichols K, Larin M, et al. (2008) Dexamethasone-mediated up-regulation of human CYP2A6 involves the glucocorticoid receptor and increased binding of hepatic nuclear factor 4 alpha to the proximal promoter. *Mol Pharmacol* 73: 451–460.

Pandey SC, Ugale R, Zhang H, et al. (2008) Brain chromatin remodeling: a novel mechanism of alcoholism. *J Neurosci* 28: 3729–3737.

Park BL, Kim JW, Cheong HS, et al. (2013) Extended genetic effects of ADH cluster genes on the risk of alcohol dependence: from GWAS to replication. *Hum Genet* 132: 657–668.

Philibert RA, Gunter TD, Beach SR, et al. (2009) Role of GABRA2 on risk for alcohol, nicotine, and cannabis dependence in the Iowa Adoption Studies. *Psychiatr Genet* 19: 91–98.

Philibert RA, Gunter TD, Beach SR, et al. (2008) MAOA methylation is associated with nicotine and alcohol dependence in women. *Am J Med Genet B Neuropsychiatr Genet* 147B: 565–570.

Pizzimenti CL and Lattal KM. (2015) Epigenetics and memory: causes, consequences and treatments for post-traumatic stress disorder and addiction. *Genes Brain Behav* 14: 73–84.

Proudnikov D, Hamon S, Ott J, et al. (2008) Association of polymorphisms in the

melanocortin receptor type 2 (MC2R, ACTH receptor) gene with heroin addiction. *Neurosci Lett* 435: 234–239.

Quillen EE, Chen XD, Almasy L, et al. (2014) ALDH2 is associated to alcohol dependence and is the major genetic determinant of "daily maximum drinks" in a GWAS study of an isolated rural Chinese sample. *Am J Med Genet B Neuropsychiatr Genet* 165B: 103–110.

Rice JP, Hartz SM, Agrawal A, et al. (2012) CHRNB3 is more strongly associated with Fagerstrom test for cigarette dependence-based nicotine dependence than cigarettes per day: phenotype definition changes genome-wide association studies results. *Addiction* 107: 2019–2028.

Riggs AD. (1975) X inactivation, differentiation, and DNA methylation. *Cytogenet Cell Genet* 14: 9–25.

Robinson TE and Kolb B. (2004) Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology* 47 Suppl 1: 33–46.

Rodgers JL, Bard DE, Johnson A, et al. (2008) The cross-generational mother-daughter-aunt-niece design: establishing validity of the MDAN design with NLSY fertility variables. *Behav Genet* 38: 567–578.

Sadler B, Haller G, Agrawal A, et al. (2014) Variants near CHRNB3-CHRNA6 are associated with DSM-5 cocaine use disorder: evidence for pleiotropy. *Sci Rep* 4: 4497.

Samochowiec J, Samochowiec A, Puls I, et al. (2014) Genetics of alcohol dependence: a review of clinical studies. *Neuropsychobiology* 70: 77–94.

Schacht JP, Hutchison KE and Filbey FM. (2012) Associations between cannabinoid receptor-1 (CNR1) variation and hippocampus and amygdala volumes in heavy cannabis users. *Neuropsychopharmacology* 37: 2368–2376.

Schaefer A, Im HI, Veno MT, et al. (2010) Argonaute 2 in dopamine 2 receptor-expressing neurons regulates cocaine addiction. *J Exp Med* 207: 1843–1851.

Schellekens AF, Franke B, Ellenbroek B, et al. (2013) COMT Val158Met modulates the effect of childhood adverse experiences on the risk of alcohol dependence. *Addict Biol* 18: 344–356.

Schumann G, Coin LJ, Lourdusamy A, et al. (2011) Genome-wide association and genetic functional studies identify autism susceptibility candidate 2 gene (AUTS2) in the regulation of alcohol consumption. *Proc Natl Acad Sci U S A* 108: 7119–7124.

Slutske WS, Meier MH, Zhu G, et al. (2009) The Australian Twin Study of Gambling (OZ-GAM): rationale, sample description, predictors of participation, and a first look at sources of individual differences in gambling involvement. *Twin Res Hum Genet* 12: 63–78.

Spence J, Liang T, Foroud T, et al. (2005) Expression profiling and QTL analysis: a powerful complementary strategy in drug abuse research. *Addict Biol* 10: 47–51.

Suchankova P, Jerlhag E, Jayaram-Lindstrom N, et al. (2013) Genetic variation of the ghrelin signalling system in individuals with amphetamine dependence. *PLoS One* 8: e61242.

Suzuki K, Nakamura K, Iwata Y, et al. (2008) Decreased expression of reelin receptor VLDLR in peripheral lymphocytes of drug-naive schizophrenic patients. *Schizophr Res* 98: 148–156.

Takeuchi F, Isono M, Nabika T, et al. (2011) Confirmation of ALDH2 as a Major locus of drinking behavior and of its variants regulating multiple metabolic phenotypes in a Japanese population. *Circ J* 75: 911–918.

Thomasson HR, Crabb DW, Edenberg HJ, et al. (1993) Alcohol and aldehyde dehydrogenase polymorphisms and alcoholism. *Behav Genet* 23: 131–136.

Thomasson HR, Edenberg HJ, Crabb DW, et al. (1991) Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. *Am J Hum Genet* 48: 677–681.

Thorgeirsson TE, Geller F, Sulem P, et al. (2008) A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. *Nature* 452: 638–642.

Thorgeirsson TE, Gudbjartsson DF, Surakka I, et al. (2010) Sequence variants at CHRNB3-CHRNA6 and CYP2A6 affect smoking behavior. *Nat Genet* 42: 448–453.

Tobacco and Genetics C. (2010) Genome-wide meta-analyses identify multiple loci associated with smoking behavior. *Nat Genet* 42: 441–447.

Toyokawa S, Uddin M, Koenen KC, et al. (2012) How does the social environment 'get into the mind'? Epigenetics at the intersection of social and psychiatric epidemiology. *Soc Sci Med* 74: 67–74.

Treutlein J, Cichon S, Ridinger M, et al. (2009) Genome-wide association study of alcohol dependence. *Arch Gen Psychiatry* 66: 773–784.

Tsaprouni LG, Yang TP, Bell J, et al. (2014) Cigarette smoking reduces DNA methylation levels at multiple genomic loci but the effect is partially reversible upon cessation. *Epigenetics* 9: 1382–1396.

Tufarelli C, Stanley JA, Garrick D, et al. (2003) Transcription of antisense RNA leading to gene silencing and methylation as a novel cause of human genetic disease. *Nat Genet* 34: 157–165.

Uher R, Caspi A, Houts R, et al. (2011) Serotonin transporter gene moderates childhood maltreatment's effects on persistent but not single-episode depression: replications and implications for resolving inconsistent results. *J Affect Disord* 135: 56–65.

Uhl GR, Drgon T, Johnson C, et al. (2010) Genome-wide association for smoking cessation success in a trial of precessation nicotine replacement. *Mol Med* 16: 513–526.

Ulloa AE, Chen J, Vergara VM, et al. (2014) Association between copy number variation losses and alcohol dependence across African American and European American ethnic groups. *Alcohol Clin Exp Res* 38: 1266–1274.

van Winkel R, Henquet C, Rosa A, et al. (2008) Evidence that the COMT(Val158Met) polymorphism moderates sensitivity to stress in psychosis: an experience-sampling study. *Am J Med Genet B Neuropsychiatr Genet* 147B: 10–17.

Vandenbergh DJ and Schlomer GL. (2014) Finding genomic function for genetic associations in nicotine addiction research: the ENCODE project's role in future pharmacogenomic analysis. *Pharmacol Biochem Behav* 123: 34–44.

Verweij KJ, Vinkhuyzen AA, Benyamin B, et al. (2013) The genetic aetiology of cannabis use initiation: a meta-analysis of genome-wide association studies and a SNP-based heritability estimation. *Addict Biol* 18: 846–850.

Verweij KJ, Zietsch BP, Liu JZ, et al. (2012) No association of candidate genes with cannabis use in a large sample of Australian twin families. *Addict Biol* 17: 687–690.

Verweij KJ, Zietsch BP, Lynskey MT, et al. (2010) Genetic and environmental influences on cannabis use initiation and problematic use: a meta-analysis of twin studies. *Addiction* 105: 417–430.

Villafuerte S, Heitzeg MM, Foley S, et al. (2012) Impulsiveness and insula activation during reward anticipation are associated with genetic variants in GABRA2 in a family sample enriched for alcoholism. *Mol Psychiatry* 17: 511–519.

Vink JM, van Beijsterveldt TC, Huppertz C, et al. (2015) Heritability of compulsive Internet use in adolescents. *Addict Biol*.

Vinkers CH, Van Gastel WA, Schubart CD, et al. (2013) The effect of childhood maltreatment and cannabis use on adult psychotic symptoms is modified by the COMT Val(1)(5)(8)Met polymorphism. *Schizophr Res* 150: 303–311.

Waldron M, Heath AC, Turkheimer EN, et al. (2008) Childhood sexual abuse moderates genetic influences on age at first consensual sexual intercourse in women. *Behav Genet* 38: 1–10.

Wang F, Jiang T, Sun Z, et al. (2009a) Neuregulin 1 genetic variation and anterior cingulum integrity in patients with schizophrenia and healthy controls. *J Psychiatry Neurosci* 34: 181–186.

Wang F, Simen A, Arias A, et al. (2013) A large-scale meta-analysis of the association between the ANKK1/DRD2 Taq1A polymorphism and alcohol dependence. *Hum Genet* 132: 347–358.

Wang JC, Grucza R, Cruchaga C, et al. (2009b) Genetic variation in the CHRNA5 gene affects mRNA levels and is associated with risk for alcohol dependence. *Mol Psychiatry* 14: 501–510.

Wang KS, Liu X, Zhang Q, et al. (2011) A meta-analysis of two genome-wide association studies identifies 3 new loci for alcohol dependence. *J Psychiatr Res* 45: 1419–1425.

Way M, McQuillin A, Saini J, et al. (2015) Genetic variants in or near ADH1B and ADH1C affect susceptibility to alcohol dependence in a British and Irish population. *Addict Biol* 20: 594–604.

Weaver IC, Cervoni N, Champagne FA, et al. (2004) Epigenetic programming by maternal behavior. *Nat Neurosci* 7: 847–854.

Whitfield JB. (1994) ADH and ALDH genotypes in relation to alcohol metabolic rate and sensitivity. *Alcohol Alcohol Suppl* 2: 59–65.

Yang X, Lu X, Wang L, et al. (2013) Common variants at 12q24 are associated with drinking behavior in Han Chinese. *Am J Clin Nutr* 97: 545–551.

Yoon D, Kim YJ, Cui WY, et al. (2012) Large-scale genome-wide association study of Asian population reveals genetic factors in FRMD4A and other loci influencing smoking initiation and nicotine dependence. *Hum Genet* 131: 1009–1021.

Young KS. (1999) Internet addiction: symptoms, evaluation and treatment. *Innovations in clinical practice: a source book*. Sarasota, FL: Professional Resource press: 19–31.

Yuferov V, Fussell D, LaForge KS, et al. (2004) Redefinition of the human kappa opioid receptor gene (OPRK1) structure and association of haplotypes with opiate addiction. *Pharmacogenetics* 14: 793–804.

Zammit S, Owen MJ, Evans J, et al. (2011) Cannabis, COMT and psychotic experiences. *Br J Psychiatry* 199: 380–385.

Zuo L, Gelernter J, Zhang CK, et al. (2012a) Genome-wide association study of alcohol dependence implicates KIAA0040 on chromosome 1q. *Neuropsychopharmacology* 37: 557–566.

Zuo L, Wang K, Wang G, et al. (2014) Common PTP4A1-PHF3-EYS variants are specific for alcohol dependence. *Am J Addict* 23: 411–414.

Zuo L, Wang KS, Zhang XY, et al. (2013a) Rare SERINC2 variants are specific for alcohol dependence in individuals of European descent. *Pharmacogenet Genomics* 23: 395–402.

Zuo L, Zhang F, Zhang H, et al. (2012b) Genome-wide search for replicable risk gene regions in alcohol and nicotine co-dependence. *Am J Med Genet B Neuropsychiatr Genet* 159B: 437–444.

Zuo L, Zhang XY, Wang F, et al. (2013b) Genome-wide significant association signals in IPO11-HTR1A region specific for alcohol and nicotine codependence. *Alcohol Clin Exp Res* 37: 730–739.