Translational Molecular Approaches in Substance Abuse Research

Sasha L. Fulton and Ian Maze

Contents

1 Introduction 32
2 The Neurobiology of Substance Use Disorder 33
3 Substance Use Disorder Heritability 34
4 Genetic Components of SUD 35
4.1 Consideration of SUD Phenotypes in Human Clinical Populations 35
4.2 Genome-Wide Associations and Functional Validation Studies Reveal Genetic Susceptibility and Neurobiological Mechanisms of Substance Use Disorder 36
4.3 GWAS Identified a nAChR Subunit Risk Variant Associated with Nicotine Dependence 37
4.4 Molecular and Pharmacological Approaches Have Defined Roles for Genetic Polymorphisms in Substance Use Disorder Physiology 41
4.5 Animals: Behavioral Models of Substance Use Disorder 42
4.6 Use of Animal Models to Explore Genetic Polymorphisms Associated with Substance Use Disorder 43
4.7 Transcriptomics and Substance Use Disorder 45
4.8 Cell-Type Specificity: Single-Cell and FACS-Based Approaches 46
5 Epigenetic Components of SUD 47
5.1 Histone Modifications 47
5.2 DNA Methylation 51
5.3 Chromatin Structure 52

S. L. Fulton
Department of Neuroscience, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

I. Maze (✉)
Department of Neuroscience, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Department of Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA
e-mail: ian.maze@mssm.edu

M. A. Nader, Y. L. Hurd (eds.), *Substance Use Disorders*,
Handbook of Experimental Pharmacology 258, https://doi.org/10.1007/164_2019_259

6 Conclusions and Future Directions 53
References 54

Abstract

Excessive abuse of psychoactive substances is one of the leading contributors to morbidity and mortality worldwide. In this book chapter, we review translational research strategies that are applied in the pursuit of new and more effective therapeutics for substance use disorder (SUD). The complex, multidimensional nature of psychiatric disorders like SUD presents difficult challenges to investigators. While animal models are critical for outlining the mechanistic relationships between defined behaviors and genetic and/or molecular changes, the heterogeneous pathophysiology of brain diseases is uniquely human, necessitating the use of human studies and translational research schemes. Translational research describes a cross-species approach in which findings from human patient-based data can be used to guide molecular genetic investigations in preclinical animal models in order to delineate the mechanisms of reward circuitry changes in the addicted state. Results from animal studies can then inform clinical investigations toward the development of novel treatments for SUD. Here we describe the strategies that are used to identify and functionally validate genetic variants in the human genome which may contribute to increased risk for SUD, starting from early candidate gene approaches to more recent genome-wide association studies. We will next examine studies aimed at understanding how transcriptional and epigenetic dysregulation in SUD can persistently alter cellular function in the disease state. In our discussion, we then focus on examples from the literature illustrating molecular genetic methodologies that have been applied to studies of different substances of abuse – from alcohol and nicotine to stimulants and opioids – in order to exemplify how these approaches can both delineate the underlying molecular systems driving drug addiction and provide insights into the genetic basis of SUD.

Keywords

Epigenetics · Genetics · Molecular approaches · Substance use disorder · Translational research

1 Introduction

Excessive abuse of psychoactive substances is one of the leading contributors to morbidity and mortality worldwide, affecting a population of nearly half a billion (Degenhardt and Hall 2012). In the United States, 11.3% of Americans smoked cigarettes daily, 5.9% abused alcohol, and 2.9% had an illicit drug use disorder in the past year – an estimated 30.2 million people in total (Abuse and Administration 2016). Effective treatments for substance use disorder (SUD) are lacking, and recovery rates are often very low – for example, while 68% of US smokers stated

they wanted to quit, only 7% have ceased using tobacco (Babb 2017). SUD is a chronic relapsing disease driven by neuroadaptations in the brain's reward circuitry. Persistent changes in these systems trigger compulsive drug-seeking behaviors despite negative consequences, though the precise mechanisms that underlie the progression from drug exposures to drug abuse are not well understood. Given the present shortage of treatment possibilities and prevention options, it is critical that new advances in translational research be leveraged and integrated with current methods in order to parse the neurobiological mechanisms underlying SUD.

In this book chapter, we review translational research strategies that are applied in the pursuit of new and more effective therapeutics for SUD. The complex, multidimensional nature of psychiatric disorders presents difficult challenges to investigators. While animal models are critical for outlining the mechanistic relationships between defined behaviors and genetic and/or molecular changes, the heterogeneous pathophysiology of brain diseases is uniquely human. Many of the cortical brain regions involved in psychiatric disorders have weak conservation with nonhuman species, including other primates (Konopka et al. 2012), necessitating the use of human studies and translational research schemes. Translational research describes a cross-species approach in which findings from human patient-based data can be used to guide molecular genetic investigations in preclinical animal models in order to delineate the mechanisms of reward circuitry changes in the addicted state. Results from animal studies in turn can inform clinical trials for the development of novel treatments for substance abuse.

In the following sections, we will begin by describing strategies that are used to identify and functionally validate genetic variants in the human genome which may contribute to increased risk for SUD, starting from early candidate gene approaches to more recent genome-wide association studies. We will next examine studies aimed at understanding how transcriptional and epigenetic dysregulation in SUD can persistently alter cellular function in the disease state. In our discussion, we then focus on examples from the literature illustrating molecular genetic methodologies that have been applied to studies of different substances of abuse – from alcohol and nicotine to stimulants and opioids – in order to exemplify how these approaches can both delineate the underlying molecular systems driving drug addiction and provide insights into the genetic basis of SUD. Our emphasis will be on developments that have markedly advanced our mechanistic understanding of SUD, as well as those that have identified novel biomarkers and promising new therapeutic targets for improved pharmacogenomics-based treatments.

2 The Neurobiology of Substance Use Disorder

SUD is a chronic relapsing disorder characterized by aberrant plasticity in reward and learning-related processing systems. The three major stages of SUD have been conceptualized in a heuristic framework that is defined by disturbances in three major neurocircuits (Koob and Volkow 2016). In the initial binge/intoxication stage, the acutely reinforcing use of psychoactive substances works through their primary

sites of action to influence dopamine and opioid signaling in the basal ganglia, including the ventral tegmental area (VTA) and nucleus accumbens (NAc), which integrates dopaminergic and glutamatergic input from the midbrain and cortex to modulate emotion, motivation, reward, and goal-directed behavior (Nestler 2005; Lüscher and Malenka 2011). Maladaptations in these regions can cause drug use to escalate to compulsive use and dependence. During the withdrawal/negative affective stage, there is a marked increase in anxiety, depression, amotivational symptoms, and physiological states that involves a reduction in dopaminergic signaling and concomitant increases in stress-related neurotransmitters in the extended amygdala. Such negative withdrawal symptoms in turn precipitate craving and cognitive deficits in the preoccupation/anticipation stage, which recruits prefrontal cortex and insular afferents back to the basal ganglia and amygdala, often leading to relapse (D'Souza 2015; Scofield et al. 2016). This recurring pattern can repeat in cycles and is associated with enormous medical, social, and occupational consequences. The ultimate aim of addiction research is to identify and characterize both the environmental and genetic molecular drivers of these functional alterations in reward circuitries in order to better leverage for targeted SUD therapeutics.

3 Substance Use Disorder Heritability

SUD is a highly complex, multifactorial psychiatric disorder driven by both genetic and environmental influences. Importantly, not all people who use addictive substances develop SUD; individual genetic differences influence susceptibility to the disease. Establishing evidence for heritable vulnerability to substance abuse across specific drug classes has emerged based on large-scale family-based genetic studies, including family pedigree analyses, adoption, and twin linkage studies (Uhl et al. 1995; Merikangas et al. 1998; Kendler et al. 2000). Early family-based studies examined risks for SUD in first-degree relatives of individuals with and without the disorder. For example, in a study of individuals with alcohol dependence and their siblings, it was found that, relative to controls, siblings of alcohol-dependent cases had increased rates of alcohol dependence themselves – up to 50% for men and 25% for women (Bierut et al. 1998). In a similar study surveying first-degree relatives of addicted individuals, an eightfold increase in risk was reported for developing SUD for a range of addictive substances (including opioids, cocaine, cannabis, and alcohol) (Merikangas et al. 1998), implicating familial influences as a non-specific risk factor for drug dependence. While these types of family studies revealed that SUD clusters in related individuals, pedigree-based designs cannot separate the specific contributions of genetics vs. environment to a given disease.

In adoption studies, concordance between offspring and biological parents indicates genetic influences on behavior, while similarity between offspring and adoptive parents suggests environmental influences. This type of research scheme is based on comparing the correlation between addiction status of offspring and the characteristics of both biological and adoptive parents. By isolating the influence of environmental exposures from potential genetic confounds on risks for addiction,

Cadoret et al. determined that alcohol dependence in biological parents predicted increased drug abuse in adopted individuals, which held across both males and females (Cadoret et al. 1986, 1996). Limitations of adoption studies include the fact that adoptive children and their biological parents are not necessarily a representative sample of the population as a whole; biological parents of adopted children are more likely to have higher rates of drug addiction, while adoptive parents are less likely. In addition, prenatal environmental influences, including drug exposure in utero, cannot be ruled out.

Classical twin studies, on the other hand, have used data from monozygotic (MZ) and dizygotic (DZ) twin pairs, raised together, in order to deconstruct roles of genetic vs. environmental influences on variation measured within a given phenotype. Twin studies yield insights into the mode of inheritance for a given disease state. A polygenic disease is determined by the combination of many genetic variants, all of which individually contribute to a small percentage of genetic vulnerability. When inherited together, however, these variants can drive expression of a disease phenotype. MZ/DZ twin concordance ratios provide some insights into this issue, since MZ twins share 100% of genetic variants, where DZ twins do not. A high MZ/DZ ratio for a disease (as in, e.g., schizophrenia) indicates that a disease may be polygenic. For SUD, the MZ/DZ twin concordance ratios hover between 2:1 for hallucinogens and 4:1 for cocaine, indicating moderate polygenic effects (Swan et al. 1997). Other factors that are not captured in twin studies include epigenetic modifications and stochastic DNA changes that may occur in one twin and not in the other. For alcohol, opioids, cocaine, and cannabis, multiple groups have reported that a genetic contribution to drug disorders constitutes increased risk ranging from 0.2–0.3 (for hallucinogens and cannabis) to around 0.6–0.8 (for opiates and cocaine) and varies depending on the specific substance examined (Tsuang et al. 2001; Agrawal and Lynskey 2008). Again, these studies indicate that some risk factors for SUD genetically segregate across different substances, while others are substance-specific (Goldman and Bergen 1998).

Although these early findings support a strong heritable component associated with vulnerability to SUD, these designs are not able to identify the specific genes that drive susceptibility to the disease. In order to classify the particular genes involved in SUD risk and progression, researchers within recent years utilize genome-wide sequencing methods and molecular profiling techniques, as discussed below.

4 Genetic Components of SUD

4.1 Consideration of SUD Phenotypes in Human Clinical Populations

A forward genetic approach begins with a phenotype of interest and aims to reveal genetic variants or genotypes that may contribute to that phenotype. Precise phenotypic definitions of case vs. control individuals (e.g., subjects exposed vs. unexposed

to psychoactive substances) is a central issue in the analysis of complex traits and is an essential component of forward genetics. Quantitative phenotypes or endophenotypes may afford greater reliability and reproducibility compared to an overt addiction diagnosis by providing researchers with a more clinically or biologically homogenous case population. For example, for nicotine use, the Fagerstrom Test of Nicotine Dependence (FTND), a validated, expert-recommended, low-burden questionnaire of six items used to assess severity of physiological nicotine dependence symptoms among cigarette smokers (Breslau and Johnson 2000; Thorgeirsson et al. 2010), is the most widely used measure of nicotine dependence. Other examples of quantitative phenotypes for nicotine use include number of cigarettes per day and urine levels of nicotine metabolites or other equivalents, such as cotinine, which act as specific biomarkers of nicotine update and tobacco exposure (Scherer et al. 2007; Wang et al. 2011).

While quantitative phenotypes help researchers standardize domains of a diagnosed disease, endophenotypes define phenotypes that may emerge before a disease is clinically diagnosed. Endophenotypes are biological or psychological phenomena of a disorder that may be intermediates in the causal chain between genetic contributions to a disorder and diagnosable symptoms of a psychopathology. For SUD, examples include novelty seeking, reward sensitivity, and risk taking. When considering results from forward genetic approaches, careful consideration should be taken into how these measures map onto phenotypic outcomes of drug addiction (e.g., DSM-IV vs. DSM-V criteria or other validated and heritable outcome measures).

4.2 Genome-Wide Associations and Functional Validation Studies Reveal Genetic Susceptibility and Neurobiological Mechanisms of Substance Use Disorder

Given the high heritability of SUD, enormous efforts have been taken to resolve genetic variations that may cause vulnerability to the disease. In addition, it has been shown that treatment response is highly dependent on genetic variation in genes that regulate the synthesis, metabolism, and transport of major neurotransmitters involved in reward behaviors and drug use. Exploring the mechanisms of the strong link between treatment responsiveness and genetic profile may thereby improve the efficacy of pharmacotherapies for SUD (Heilig et al. 2011).

Genome-wide association studies (GWAS) measure and analyze DNA sequence variation across the entire genome to identify genetic risk factors for complex diseases. In large genetic association studies, several important factors determine the power of this approach to detect important risk variants. The number of subjects examined, number of genetic markers compared between subjects, the specificity and definition of case vs. control subjects, etc. are all critical to the impact of these studies. Ultimately, the goal of GWAS is to exploit such results to improve predictions about individuals and populations at higher risk for developing a given disease. From GWAS datasets, researchers can also establish which molecular

systems are critical to disease susceptibility and progression. These data in turn can be used in the development of prevention and treatment strategies – including those based on tailoring specific treatments to individuals based upon genotype. Several classes of genes have been elucidated as being important to the genetic component of SUD through GWAS (Table 1). Overall, genes identified in GWAS as being related to SUD tend to cluster around components of drug mechanisms of action, as well as factors associated with neuroplasticity: gene components of extracellular proteins, cytoskeleton/cell adhesion, cell signaling, and gene expression regulation. In the next section, we will examine an example of a genetic risk variant for SUD identified with GWAS and the series of studies that applied molecular genetic techniques to validate and characterize this polymorphism in vitro and in animal models.

4.3 GWAS Identified a nAChR Subunit Risk Variant Associated with Nicotine Dependence

The first addiction GWAS reports focused on nicotine dependence (Bierut et al. 2006; Saccone et al. 2006). Comparing 1,050 cases defined by FTND scores for nicotine dependence vs. 879 controls, these studies identified significant associations for variants in a region on human chromosome 15 encoding the α3, α5, and β4 subunits of the nicotinic acetylcholine receptor (nAChR). Nicotine acts as a potent agonist for these nAChR ligand-gated ion channels, which are distributed in specific reward-related brain regions in varying combinations of subunits, each with diverse functional characteristics. The risk polymorphisms in these genes were found to increase probabilities for nicotine dependence (Bierut et al. 2006; Sherva et al. 2008). One of the identified variants, the non-synonymous SNP rs16969968 found in exon 5 of the α5 gene (α5SNP), causes an amino acid change (D398N). This SNP is fairly frequent in the general population, found in approximately 35% of Europeans and 50% of Middle Eastern populations (Saccone et al. 2006). In meta-analyses of human clinical populations, it has been associated with an increased risk for nicotine dependence, lung cancer, lower aversive experience to smoking, and increased cognitive enhancement after nicotine exposure (Saccone et al. 2006; Chen et al. 2015). Moreover, the haplotype carrying the rs16969968 risk allele has implications for cessation treatment success, with smokers at highest nicotine dependence risk being less likely to quit smoking overall but responding most effectively to pharmacologic treatments (Chen et al. 2015). These findings highlight the potential for personalized cessation treatments based upon genetic risk variants for nicotine dependence. However, the exact structural changes and resulting functional differences caused by this SNP could not be determined from simple genome associations. In the years following the discovery of this SNP, the connection between nicotine dependence and genetic variation at this locus has become the most widely replicated GWAS finding in the psychiatric disease literature, providing a particularly illustrative example of the variety of molecular genetic techniques that are applied to investigate genetic variants identified by human GWAS.

Table 1 Summary table of genetic loci identified at genome-wide statistical significance ($P < 5 \times 10^{-8}$) in GWAS of SUD-related phenotypes/biomarkers

Drug	Associated gene	Chromosome	SNP	Annotation	Phenotype/biomarker
Nicotine	*UGT2B10-UGT2A3*	4q13	rs115765562	Upstream	Cotinine glucuronidation (Patel et al. 2015)
			rs114612145	Downstream	Cotinine levels (Ware et al. 2016)
	PDE1C	7p14	rs215605	Intronic	CPD (Thorgeirsson et al. 2010), heavy vs. never (Wain et al. 2015)
	CHRNB3-CHRNA6	8p11	rs6474412	Upstream	CPD (Thorgeirsson et al. 2010)
			rs1451240	Upstream	Nicotine dependence (Rice et al. 2012)
	DBH	9q34	rs3025343	Intronic	Current vs. former (Furberg et al. 2010), current vs. former (Siedlinski et al. 2011), current vs. former (David et al. 2012), FTND score (Yang et al. 2015), heavy vs. never (Wain et al. 2015)
	BDNF	11p14	rs6265	Missense	Ever vs. never (Furberg et al. 2010), heavy vs. never (Wain et al. 2015)
	CHRNA5-CHRNA3-CHRNB4	15q25	rs16969968	Missense	Nicotine dependence (Bierut et al. 2006; Saccone et al. 2006), CPD (Furberg et al. 2010; Thorgeirsson et al. 2010; David et al. 2012), CPD, years of smoking, pack-years, current vs. former (Gabrielsen et al. 2013), CPD (Munafò et al. 2012), CPD (Richmond-Rakerd et al. 2016)
			rs1051730	Synonymous	CPD (Furberg et al. 2010; Thorgeirsson et al. 2010; David et al. 2012), heavy vs. light (Wain et al. 2015), adherence to NRT, NRT dose (Ware et al. 2015)
			rs2036527	Intergenic	CPD (David et al. 2012), Nicotine dependence (Broms et al. 2012), abstinence (Zhu et al. 2014)
			rs34684276	Intronic	Nicotine dependence (Hancock et al. 2015)
	EGLN2	19q13	rs3733829	Intronic	CPD (Furberg et al. 2010), CPD, exhaled CO (Bloom et al. 2013)
	CYP2A6-CYP2B6	19q13	rs4105144	Upstream	CPD (Thorgeirsson et al. 2010), CPD (Siedlinski et al. 2011), cotinine levels (Timofeeva et al. 2011)
			rs8102683	Upstream	CPD (Kumasaka et al. 2012)
			rs56113850	Intronic	NMR (Loukola et al. 2015)
	DNMT3B	20q11	rs910083	Intronic	Nicotine dependence (Hancock et al. 2015), heavy vs. never (Hancock et al. 2015; Wain et al. 2015)
	NOL4L	20q11	rs57342388	Intronic	Heavy vs. never (Wain et al. 2015), nicotine dependence (Hancock et al. 2015)

	CHRNA4	20q13	rs2273500	Splice acceptor	Nicotine dependence, heavy vs. never (Hancock et al. 2015)
Alcohol	*SERPINC1*	1q25	rs1799876	Intronic	Maximum drinks in 24-h period (Xu et al. 2015), weekly alcohol intake (Jorgenson et al. 2017)
	GCKR	2p23	rs780094	Intronic	Daily alcohol intake (Schumann et al. 2016)
			rs4665985	Downstream	Weekly alcohol intake, drinker vs. nondrinker (Jorgenson et al. 2017)
	SGOL1	3p24	rs11128951	Upstream	Drinker vs. nondrinker (Jorgenson et al. 2017)
	TF-SRPRB	3q22	rs1799899	Missense	CDT% (Kutalik et al. 2011)
			rs3811647	Intronic	Total transferrin (Kutalik et al. 2011)
			rs1534166	Intronic	CDT concentration (Kutalik et al. 2011)
	KLB	4p14	rs11940694	Intronic	Daily alcohol intake (Schumann et al. 2016)
			rs7686419	Upstream	Drinker vs. nondrinker, weekly alcohol intake (Jorgenson et al. 2017)
	ADH1B-ADH1C	4q23	rs1789891	Intronic	DSM-IV-defined cases vs. controls (Frank et al. 2012), DSM-IV-defined cases vs. controls (Way et al. 2015)
			rs1229984	Missense	Ever vs. never (Takeuchi et al. 2011), DSM-IV-defined cases vs. controls, maximum drinks in 24-h period (Bierut et al. 2012), DSM-IV-defined cases vs. controls (Park et al. 2013), DSM-IV symptom count (Gelernter et al. 2014a), maximum drinks in a 24-h period (Xu et al. 2015), DSM-IV-defined cases vs. controls (Way et al. 2015), weekly alcohol intake, drinker vs. nondrinker (Jorgenson et al. 2017)
			rs2066702	Missense	DSM-IV symptom count (Gelernter et al. 2014a) Maximum drinks in 24-h period (Xu et al. 2015)
			rs145452708	Intronic	Weekly alcohol intake (Clarke et al. 2017)
			rs141973904	Intronic	Alcohol use disorder identification test (AUDIT) score (Sanchez-Roige et al. 2019)
	AUTS2	7q11	rs6943555	Intronic	Average consumption (grams/day/kg body weight) (Schumann et al. 2011)
	ALDH2	12q24	rs2074356	Intronic	Average consumption (grams alcohol/day) (Baik et al. 2011)
			rs671	Missense	Ever vs. never (Takeuchi et al. 2011), DSM-IV-defined cases vs. controls (Park et al. 2013), past year drinkers vs. nondrinkers (Yang et al. 2013),

(continued)

Table 1 (continued)

Drug	Associated gene	Chromosome	SNP	Annotation	Phenotype/biomarker
					maximum drinks in a 24-h period, flush response, alcohol dependence (Quillen et al. 2014), drinker vs. nondrinker, weekly alcohol intake (Jorgenson et al. 2017)
			rs11066280	5′ untranslated	Past year drinkers vs. nondrinkers, daily alcohol intake (Yang et al. 2013)
Amphetamine	*CDH13*	16	rs3784943	Intronic	Positive subjective drug response to amphetamine (Hart et al. 2012)
Cocaine	*FAM53B*	10	rs2629540	Intronic	DSM-IV symptom count for cocaine dependence (Gelernter et al. 2014c)
Opioids[a]	*KCNG2*	18	rs62103177	Intronic	Opioid dependence (Gelernter et al. 2014b)
	KCNC1	11	rs60349741	UTR_3	Opioid dependence (Gelernter et al. 2014b)
	APBB2	4	rs114070671a	Intronic	Opioid dependence (Gelernter et al. 2014b)
	CNIH3	1	rs10799590	Intronic	Opioid-dependent daily injectors ($N = 1{,}167$) with opioid misusers who never progressed to daily injection (Nelson et al. 2014)

[a]Identified SNPs that have not been independently replicated

4.4 Molecular and Pharmacological Approaches Have Defined Roles for Genetic Polymorphisms in Substance Use Disorder Physiology

Candidate loci identified by GWAS provide valuable targets for mechanistic dissections in preclinical models. Once a genetic variant has been identified through GWAS, it is critical to determine if the polymorphism has an effect on the gene product's expression or function. Once a definitive effect is identified, researchers can then use molecular genetic and pharmacological approaches to determine its specific role in the drug's mechanism of action.

In vitro studies are those performed using molecules, cells, or organisms outside of their biological context and can be powerful tools for examining the ultimate effect of a SNP on a protein's structure and function before investigations in more complex animal models. In the case of the CHRNA5 polymorphism, early in vitro studies utilized HEK cells to validate the effects of the rs16969968:G > A SNP and found that the amino acid variant confers a partial loss of function to the nAChR by reducing Ca^{2+} influx after nicotine-mediated activation, implying that the variant receptor desensitizes more quickly vs. its wild-type counterpart (Saccone et al. 2006; Kuryatov et al. 2011). While these studies provided critical validation of CHRNA5's effect on receptor function, HEK cell lines are derived from human embryonic kidney cells grown in tissue culture and cannot recapitulate the cell-type-specific genetic architectures and proteomes of differentiated human neurons.

A more biologically relevant in vitro system has been made available with recent advances in induced pluripotent stem cells (hiPSCs), which have allowed researchers to create differentiated cell types with skin fibroblasts collected from a human patient. Differentiated cells from hiPSCs have the exact genome as the donor patient and thus can be utilized as a model cellular system. Recently, Deflorio et al. generated hiPSCs from individuals with and without polymorphisms in the nAChR α5 subunit and then differentiated them into midbrain dopaminergic (DA) neurons. By measuring the functional electrophysiological properties of wild-type (WT) vs. variant nAChRs expressed in these human DA neurons, the authors discovered that with this SNP, more nicotine and/or acetylcholine chloride is necessary to obtain the same downstream calcium influx in comparison to the wild-type receptor (Deflorio et al. 2016). Moving forward, this hiPSC system can be used in drug discovery approaches to further dissect dependence-related phenotypes and screen for compounds that interact specifically with human wild-type vs. polymorphic nAChRs (Collo et al. 2018). While in vitro studies are indispensable for probing the functional consequences of genetic variation identified in GWAS, they are performed in artificial systems isolated from the complexity of a biological context. Moreover, in disease modeling, the most valuable translational insights inform how a gene or gene variant impacts behavior. For this purpose, genetically engineered rodents are one of the most important means used to tease out functional roles for specific genes.

4.5 Animals: Behavioral Models of Substance Use Disorder

Animal models are critical for use in genetic manipulation experiments aimed at mechanistic investigation, which are currently impossible to perform in human subjects. Achieving face and construct validity for an animal model of psychiatric disease is one of the primary concerns when designing behavioral paradigms of SUD. However, the models for SUD described here are some of the most reproducible and useful for studying distinct aspects of the addiction cycle. Because the vast majority of animal studies for SUD have been conducted in rodents (i.e., mice and rats), we will focus on paradigms utilizing these species. It should be noted, however, that there are examples of investigations performed in nonhuman primates to analyze more complex behaviors, as well as research that has utilized more basic models, such as *Drosophila*, to examine conserved mechanisms of drug-taking behaviors (often with alcohol or cocaine). Drugs that have positive reinforcing effects in rodents and primates mirror closely with those that have high abuse potential in humans, including alcohol, cocaine, and heroin. Below, we will briefly describe a couple of the behavioral paradigms used to investigate SUD in rodents.

Conditioned Place Preference (CPP) The basic characteristics of this task involve the association of a particular environment with drug treatment, followed by the association of a different environment with the absence of said drug (i.e., the drug's vehicle). In a simple version of the CPP paradigm, animals are first exposed to two distinct environments, each of which is paired with either a drug or nondrug state – note that in these assays, the drug is often passively administered, which should be considered in the evaluation of studies utilizing CPP. During the testing phase, the animal is then given the choice to enter and explore both environments, with the time spent in either the drug-paired vs. vehicle-paired environments (e.g., the animal's place preference) used as an index of the reinforcing value of the drug. Animals will often spend more time in a drug-associated environment and will avoid environments paired with aversive states, such as drug withdrawal, which can be applied in a variation of CPP called conditioned place aversion.

Self-Administration (SA) The intravenous drug self-administration animal model is a powerful tool for investigating the addictive cycle of rewarding drugs. An intravenous catheter is implanted in the animal, such that upon completion of a task (often a lever press or nose poke), the drug of interest is delivered directly to the bloodstream. Intravenous cocaine and heroin self-administration in rodents thus recapitulates the voluntary pattern of behavior of the human addiction cycle, including preoccupation/anticipation, drug seeking, escalation of drug taking, withdrawal, extinction, and cue-induced seeking or relapse. Experimental manipulations that increase the rate of self-administration, such as administering a drug that counteracts the effects of the drug of abuse, may be interpreted as decreasing the reinforcing potency of the drug. Once an animal is tested with a particular experimental drug, additional pharmacological manipulations can be done with standard reference compounds, using the same animals to validate the effects.

These paradigms can be carried out to study the effects of acute (or short-term) vs. chronic (long-term) drug taking, with the desired behavioral or molecular readouts assessed immediately after drug taking or after extended periods of abstinence to mimic long-term adaptations that develop during withdrawal and/or relapse.

4.6 Use of Animal Models to Explore Genetic Polymorphisms Associated with Substance Use Disorder

In the case of nicotinic receptor variants identified in human GWAS studies, manipulation of these receptor subunit genes in rodents has greatly expanded our understanding of the neurobiology of nicotine addiction. One of the earliest genetic editing studies conducted for the *CHRNA5* risk variant was the generation of a Chrna5 subunit knockout (KO) mouse, in which the gene for Chrna5 was silenced or excised from the germline. A KO mouse can give researchers clues into the overall function of a gene of interest and the effects of disrupting its protein product. A series of these studies showed that mice lacking the α5 subunit escalate their nicotine self-administration at high doses in comparison to normal control mice (Fowler et al. 2011; Morel et al. 2014), while wild-type animals appear to titrate the amount of nicotine self-administered to maintain a consistent dosage. Several brain regions involved in nicotine dependence have been evaluated for potential changes in function after disruption of the *Chrna5* gene. Fowler et al. reported increased nicotine intake in mice with a null mutation in *Chrna5*. This effect was "rescued" in knockout mice by re-expressing wild-type α5 subunits in the medial habenula (MHb), a brain region associated with inhibition of rewarding signals during an aversive experience. Interestingly, knocking down the α5 subunit in the MHb did not change reinforcing effects of nicotine but did eliminate the MHb's inhibitory brake on nicotine taking at high doses, which would otherwise be aversive.

Such KO and rescue studies yield invaluable insights into the overall function of a gene of interest and the role that it might play in producing a given phenotype. However, they cannot precisely address the consequences of constitutive expression of a specific SNP throughout development. Until recently, the available toolbox of rat genetics lacked the ability to easily introduce site-directed, heritable mutations into the genome to create KO or knock in (KI) rats. To this end, programmable molecular gene-editing systems have been developed in progressively accessible iterations, including zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR (clustered regularly interspaced short palindromic repeat)-associated protein 9 (Cas9) RNA-guided engineered nucleases (RGENs) (Kim and Kim 2014). These gene-editing machineries can be injected into single-cell rat embryos to induce sequence-specific double-strand breaks and the subsequent insertion of a transgene through homologous recombination. Recently, researchers were able to utilize the ZFN system to KO the WT nCHR5 subunit and replace it with the rs16969968:G > A SNP in rat germline, creating animals constitutively expressing the variant form of this subunit during development (Forget et al.

2018). The authors then ran WT vs. KO vs. α5SNP rats through a self-administration paradigm in order to determine the differential effects of manipulating this gene on nicotine taking behavior. Unlike the WT animals, the nCHR5 KO rats did not acquire self-administration of nicotine at the dose used in this study, which is consistent with previous reports in mice showing that KO animals require higher doses of nicotine to develop a learned response – suggesting the nCHR5 subunit is important for sensitivity to nicotine. At high nicotine doses, however, KO mice do show increased nicotine taking over WT mice. Interestingly in this study, the α5SNP rats were able to acquire nicotine taking at the lower dose of nicotine similarly to the WT rats, but unlike the WT condition, the α5SNP did not reduce their nicotine intake at high doses and were willing to exert more energy to obtain a nicotine infusion. These results demonstrate how different the resulting phenotype can be when a gene is knocked out vs. when the same gene with disease-relevant mutations is edited in.

These gene-editing strategies can be used to gain even more specific control of transgene expression by using systems like Cre recombinase in order to create cell-type-specific or inducible expression of the editing molecule in a certain brain region or cell type of interest. Cre recombinase is a protein that recognizes and mediates site-specific recombination between loxP site sequences. This unique property can be harnessed by inserting these loxP sites around a transgene of interest. When Cre recombinase is expressed in a cell containing these loxP sites, researchers can elicit gene deletion, insertion, translocation, and inversion depending on the location and orientation of the sites. The Cre/loxP recombination system has become an ideal tool for genetic manipulation in mammalian cells and genetically modified animal models. For example, Morel et al. utilized a Cre recombinase approach in order to investigate the role of the mutant nCHR5 subunit specifically in dopaminergic neurons in the mouse ventral tegmental area (VTA) (Morel et al. 2014). In this paper, the authors utilized the Cre recombinase system to re-express the α5SNP version of the subunit under control of the dopamine transporter (DAT), which resulted in a partial loss of nicotine-evoked receptor function and yielded intermediate behavioral and electrophysiological phenotypes compared with those of the α5 KO mice, suggesting that the α5 subunit has a critical role in defining the sensitivity of the VTA DA system to nicotine through its effects on raising the threshold for dopaminergic release in this brain region.

Taken together, these functional validation studies have helped to form a more complete picture of nAChRs subunit genetic variants and their role in nicotine addiction. In the case of smoking cessation, there are now three FDA-approved medications in existence: *nicotine replacement therapy*; *varenicline* – a partial agonist of the α4β2 nicotine receptors that produces less effects on dopamine release in comparison to nicotine; and *bupropion, an atypical norepinephrine-dopamine reuptake inhibitor (NDRI) antidepressant and nicotinic receptor antagonist*. Emerging evidence suggests that stratifying patients based on *CHRNA5* genetic biomarkers may improve responsiveness to nicotine replacement therapy (Chen et al. 2015). For example, Chen et al. described two randomized cessation trials in which the efficacy of nicotine replacement therapy varied with rs16969968 genotype but not for varenicline treatment. This variant also contributes to increased risk for

lung cancer and chronic obstructive pulmonary disease (Hung et al. 2008; Timofeeva et al. 2012), highlighting its association with heavier smoking and a lower likelihood of quitting. While there is equivocal evidence of an interaction between genotype and treatment efficacy, the *CHRNA5* SNP is a known biomarker for difficulty in quitting smoking, and genotyping can help to identify individuals with CHRNA5 high-risk alleles, as they typically have an increased need for pharmacological cessation aids (Chen et al. 2015).

Finally, the α5-containing nAChRs impacted by *CHRNA5* variation may represent an important target for medication development. Considering that *CHRNA5* risk variants result in hypofunction of α5 nAChRs, novel pharmacological agents that enhance the activity of a5 nAChRs may decrease nicotine use by restoring appropriate cholinergic signals that mediate the aversive properties of nicotine. Moving forward, advances in methodological approaches will allow studies to leverage our current knowledge of CHRNA5 and other variants in order to better characterize their downstream effects toward improved treatments.

4.7 Transcriptomics and Substance Use Disorder

Persistent changes in gene expression drive neuroplastic maladaptations in the reward circuitry that underlie craving, drug-seeking, and relapse during SUD progression (Lüscher and Malenka 2011). For this reason, researchers have sought to outline the coordinated alterations in transcriptional programs that may precipitate aberrant synaptic plasticity in these cells. Moreover, GWAS results can be integrated and overlaid with transcriptomic data to reveal where SNPS and CNVs might be associated with gene expression changes in key brain regions implicated in reward learning. Much of the early work interrogating gene expression in clinical SUD and in animal models of addiction utilized real-time polymerase chain reaction or in situ hybridization in a priori, hypothesis-driven approaches to measure amounts of candidate genes in controls vs. cases – studies that corroborated the idea of transcriptional dysregulation in the reward system (Nikoshkov et al. 2005; Bach et al. 2014). More recently, transcriptomic analysis of the full complement of mRNA in a given tissue with next-generation sequencing techniques, such as RNA-seq, has provided researchers with greater insights into the coordinated networks of gene expression changes that may underlie SUD progression (Wolf 2010; Robison and Nestler 2011; Egervari et al. 2017).

In order to illustrate how profiling studies can validate and extend our understanding of genetic association findings, we will use the example of the *OPRM1* gene, which encodes the human G-protein coupled mu opioid receptor (MOR). The MOR is responsible for mediating the rewarding effects of opioids. Numerous SNPs in the *ORPM*1 gene have been identified as associated with heroin addiction in candidate genome association studies (Nelson et al. 2014). Several years of work have used in vitro studies and mouse genetics to demonstrate that MOR represents the primary in vivo molecular target for both the most clinically useful (morphine) and most largely abused (heroin) opiates (Bond et al. 1998; Befort et al. 2001; Wang

et al. 2001). MOR KO in mice abolishes both heroin and morphine CPP and self-administration (Becker et al. 2000; Contet et al. 2004). Targeted expression studies have shown that one of the most common SNPs in this locus, A118G, is a functional variant with deleterious effects on both mRNA and protein levels (Zhang et al. 2005). However, the transcriptional events downstream of reduced ORPM1 expression associated with this SNP were not well characterized. Sillivan et al. thus performed transcriptional microarray analyses on NAc from human patients dependent on heroin, a brain region involved in goal-directed behavior and reward processing (Sillivan et al. 2013), whereby they revealed evidence of dysregulated MOR signaling pathways in heroin abusers compared to controls. The authors then analyzed differentially expressed genes using pathway analyses and identified the ELK1 transcription factor as an important regulator of these genes. ELK1 is a known target of the ERK signaling pathway, which itself has been widely studied in cocaine-related dopaminergic signaling. Interestingly, ELK1 expression was found to also correlate with risk variants of ORPM1 in a dose-dependent manner, suggesting a link between ELK1-associated transcriptional programs and reduced expression of ORPM1. Furthermore, ELK1 expression correlates with the severity of heroin use in both human subjects and rat self-administration models of heroin abuse. ELK1 has also been implicated in the mechanisms of drug addiction to other substances besides heroin, including synthetic opioids, THC, and cocaine (Valjent et al. 2001). Several other studies have identified ELK1-regulated genes as being differentially expressed after drug exposures. For example, after ELK1 was identified in mechanistic and transcriptional studies of cocaine administration, Besnard et al. used a cell-penetrating peptide, named TAT-DEF-Elk-1 (TDE), to specifically inhibit ELK1 phosphorylation and the subsequent induction of plasticity-related genes. In doing so, they found that such inhibition reverses cocaine-induced increases in dendritic spine density and delays CPP for cocaine (Besnard et al. 2011). Together, these findings indicate ELK1 as a potential molecular target mediating cellular phenotypes in opioid addiction and highlight transcriptomics as a powerful tool in probing the mechanisms of SUD.

4.8 Cell-Type Specificity: Single-Cell and FACS-Based Approaches

The extreme cellular heterogeneity of the brain is maintained by transcriptional and epigenetic signatures that are unique to given cell types of interest. For this reason, detecting important differences in gene expression in bulk tissue preparations may be occluded by inclusion of many different heterogeneous profiles. In addition, several lines of research have demonstrated the importance of non-neuronal cells in the etiology of SUD, including astrocytes, microglia, oligodendrocytes, and neuroimmune cells (Knapp and Hauser 1996; Slezak et al. 2013). For example, it has been well documented that heroin users have deficits in white matter integrity and myelination, processes that are mediated by oligodendrocytes (Bora et al. 2012; Li et al. 2016). Advances in microfluidics and sequencing technology have made it feasible to analyze thousands of single-cell transcriptomes in a single experiment.

Single-cell RNA sequencing (scRNA-seq) platforms have been developed (Klein et al. 2015; Zheng et al. 2017), enabling the characterization of dozens of molecularly distinct CNS cell types from multiple regions. To investigate cell-type-specific transcription response to opioid administration, Avey et al. performed single-cell RNA sequencing (scRNA-seq) of mouse NAc following acute morphine treatments, where they identified unique morphine-dependent transcriptional responses in both oligodendrocytes and astrocytes (Avey et al. 2018). While not a model of addiction per se, these types of studies allow researchers to further narrow the biological response to abused substances. Further analyses using RNA-seq of FACS-purified oligodendrocytes revealed a large gene set regulated by morphine that were highly enriched for roles in oligodendrocyte maturation and myelination, including the unfolded protein response, confirming the hypothesis that aberrant oligodendrocyte function may contribute to white matter deficits in heroin addiction. These data demonstrate that single-cell and cell-type-specific techniques can illuminate mechanistic insights into the etiology of SUD and that, going forward, it will be critical that addiction studies take into consideration cell-type-specific contributions to SUD phenotypes.

5 Epigenetic Components of SUD

Over a decade of GWAS, studies have indicated that the majority of SNPs that contribute to risk for SUD reside in noncoding regions of DNA or the regulatory sequences that determine how a gene is expressed, termed gene regulatory elements (GREs). How and when these GREs are made available for transcription factor binding is determined by chromatin-based (so-called epigenetic) influences, such as posttranslational covalent modifications to DNA or histone proteins, which ultimately function to alter the accessibility of GREs and/or gene coding loci in order to allow trans-regulatory factors to bind and increase/decrease the probability of transcription events occurring at a given locus. Dynamic restructuring of nucleosome organization in order to allow for transcription machinery access to regulatory DNA sequences is the basis of epigenetic regulation of gene expression. In the following section, we will briefly turn our attention to epigenetic profiling and validation methods (see Fig. 1 for overview of methods) – with a focus on histone and DNA modifications – that are commonly used to reveal both the stable and dynamic properties of chromatin that modify the transcriptomes in SUD.

5.1 Histone Modifications

The basic unit of transcription (i.e., the nucleosome) is comprised of a protein/DNA complex composed of ~147 base pairs of double-stranded DNA wrapped around a core histone octamer containing two copies each of the histone proteins H2A, H2B, H3, and H4. Histones are small, highly alkaline proteins containing both globular domains and more flexible N- and C-terminal "tails" that can undergo

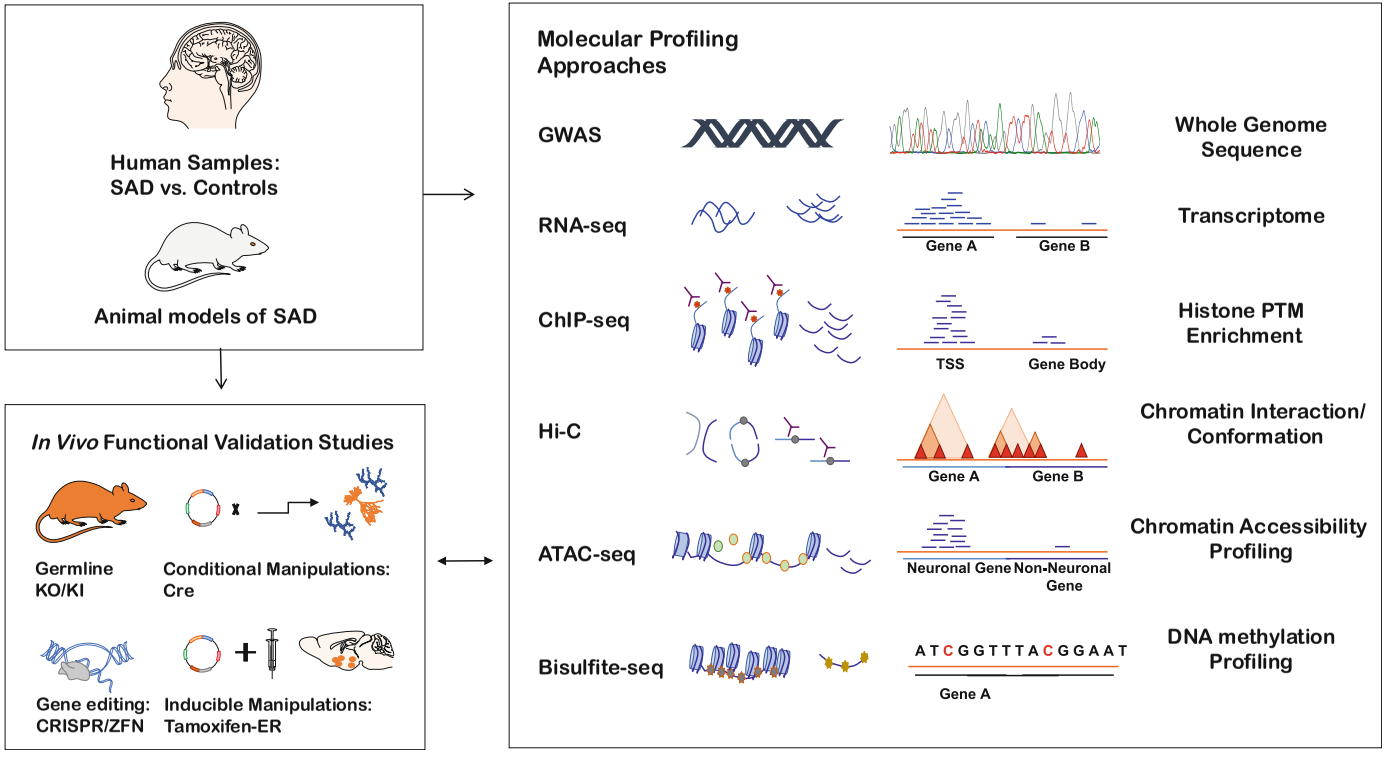

Fig. 1 Translational approaches for investigating SUD: Both clinical SUD human samples and preclinical animal models of SUD can be utilized in molecular disease profiling. *Molecular profiling approaches*: Alterations in chromatin following drug-related experience underlies the development of SUD. Measuring these changes can be achieved by using cell-tagging methods or isolation of distinct cell populations. (1) GWAS studies sequence entire genomes to find associations between genetic variants and disease phenotypes. (2) RNA-seq measures relative levels of transcripts. (3) ChIP-seq utilizes antibodies specific for

Fig. 1 (continued) histone PTMs and/or transcription factors, with associated DNA then being isolated to examine genomic enrichment. (4) Hi-C is used to discover contacts between proximal and distal genomic loci. (5) ATAC-seq uses the transposase Tn5 to cut and ligate specific adapters to open chromatin regions that can be used for downstream sequencing of chromatin accessibility. (6) Bisulfite-seq converts unmethylated cytosines into uracil, and DNA sequences are amplified to detect methylation profile. *In vivo functional validation studies*: Candidate target genes, modifications, or proteins identified in profiling studies can be manipulated in functional validation studies in rodent models using genetic approaches. (1) Germline mutations: knockout (KO) will remove a gene from all cells in an animal. Knock in (KI) will insert a transgene in all cells. (2) Conditional manipulations allow knockout/induction of transgene expression only when Cre recombinase is present. (3) Inducible manipulations allow induction of transgene expression only upon injection of a specific compound, such as tamoxifen. (4) Gene editing with CRISPR or zinc finger nucleases (ZFNs) will replace the endogenous version of a gene with a risk variant or other mutation

posttranslational modifications in order to alter their structure and change the nature of their interactions with neighboring DNA. These tails are heavily modified by the covalent addition of acetyl, methyl, phospho, ubiquitin, and other chemical groups that affect their charge and/or how they interact with DNA-binding proteins. These modifications are placed by specialized enzymes termed "writers" that have highly specific motifs that recognize a combination of DNA sequence/amino acid position/histone modifications, and they are removed by equally specific "eraser" enzymes. Complex combinatorial "histone codes" are thus hypothesized to dictate which regions of DNA will be accessible to transcription factors, enhancers, silencers, and other regulatory factors. These histone marks provide a reversible, labile substrate for the aberrant neural plasticity observed in drug addiction. As SUD is expressed as an aberrant form of neural plasticity (Hyman et al. 2006), there has been much focus on examining potential epigenetic sources of gene regulation in drug addiction (LaPlant and Nestler 2011; Walker et al. 2015).

Over the past 10 years, many studies have demonstrated alterations in global, temporally defined histone modification states in the human and rodent brain in response to cocaine administration (Kumar et al. 2005; Maze et al. 2010; LaPlant and Nestler 2011). Similar phenomena have been observed for other drugs of abuse, including methamphetamine (Schmidt et al. 2012; Jayanthi et al. 2014) and morphine (Mashayekhi et al. 2012). Global levels of histone modifications can be evaluated using chromatin immunoprecipitation-sequencing (ChIP-seq) methods. ChIP-seq begins by cross-linking the DNA from a given cell population to all associated DNA-bound proteins and then using antibodies specific to a protein of interest to immunoprecipitate and sequence the stretches of DNA associated with that protein. These types of studies in animal models of SUD suggest that the presence of distinct histone modification marks at gene promoters (e.g., on H4 vs. H3) can encode the temporal progression of drug taking in the form of chromatin structural changes from acute to chronic exposures through relapse.

Manipulation of the enzymatic machineries responsible for depositing/removing these marks can yield insights into the regulatory mechanics of histone modification changes. For example, Maze et al. identified persistent decreases in levels of classical repressive H3 lysine methylation, as well as in the expression of the G9a K-methyl transferase, 24 h after cocaine administration, events that correlated with increased synaptic plasticity and the upregulation of a subset of plasticity-related genes (Maze et al. 2010). We subsequently used a series of regional-specific conditional manipulations of G9a in the mouse NAc to selectively knock down G9a in this brain region to directly determine its role in cocaine-induced neuronal plasticity and CPP behaviors. In doing so, we found that G9a downregulation increased dendritic spine plasticity of NAc neurons and enhanced preferences for cocaine, thereby establishing a crucial role for histone methylation in the long-term actions of cocaine (Maze et al. 2010). Interestingly, the example of G9a manipulation in animal models of cocaine taking also offers a demonstration of the complexities of interpreting different drug-related behavioral paradigms. Recently, it was shown that even though artificially reducing G9a in the NAc enhances cocaine CPP and overexpression reduces cocaine CPP, overexpression of G9a actually increased

cocaine-seeking behavior in a different experimental paradigm, self-administration (Anderson et al. 2018). The authors also revealed the anxiogenic effect of G9a overexpression during the self-administration training, and overall these data highlight the importance of carefully defining the drug-related behavioral phenotype in question. These and many other studies have cemented a role of histone modifications in neural plasticity and SUD. However, many of the approaches used above measure and manipulate overall levels of a given histone modification across the genome but do not necessarily address the downstream effects of individual histone modification located at specific gene loci. Below, we discuss examples of new approaches that can be used to further examine the role of gene-specific deposition of histone modifications in the precipitation of molecular and behavioral effects caused by drug exposures.

Recent advances in gene targeting have allowed a more specific dissection of the role of histone modifications related to given phenotypes using engineered transcription factors, zinc finger proteins (ZFPs) (Snowden et al. 2002), and/or transcription activator-like effectors (TALEs) (Sanjana et al. 2012) that can be designed to recognize and bind to specific loci throughout the genome in order to deliver histone modifications directly to genes of interest in vivo. This gene-targeted approach makes it possible to directly examine the behavioral and biochemical consequences of *various* epigenetic marks in the context of drug exposures (Heller et al. 2014).

Recently, Heller et al. utilized this technique to investigate the role of a transcriptionally permissive modification, histone H3 lysine 9/14 acetylation (H3K9/14 ac), vs. a repressive mark, histone H3 lysine 9 dimethylation (H3K9me2), targeted specifically to the *Cdk5* locus in NAc, a gene implicated in reward-related behaviors in this brain region. In doing so, they demonstrated increased cocaine-induced locomotor behaviors, as well as resilience to social stress, following activation of *Cdk5* (Heller et al. 2016). Conversely, *Cdk5* repression by H3K9me2 was found to attenuate both cocaine-induced locomotor behaviors and conditioned place preference (Heller et al. 2016). These data are especially compelling given that previous work has identified different behavioral responses upon *Cdk5* overexpression vs. knockdown, demonstrating the importance of targeted epigenetic remodeling tools in studies of tunable molecular changes occurring in disease states.

5.2 DNA Methylation

Another important epigenetic modification that contributes to chromatin structure and accessibility is DNA methylation, which occurs when a methyl group is covalently added to a cytosine nucleotide (Jaenisch and Bird 2003). This modification is catalyzed by a family of enzymes called DNA methyltransferases (DNMTs). DNA methylation is often associated with targeted genomic silencing and closed heterochromatic genome regions. Methylation patterns are established and modified throughout development in tissue- and cell-type-specific configurations. In the past decade, accumulating evidence has implicated DNA methylation in learning,

cognition, and neural plasticity in response to environmental cues, making it a key mechanism of interest in epigenetic regulation of SUD-related plasticity.

A range of studies have investigated levels of DNMTs in self-administration animal models of SUD, finding global changes in DNMT expression over temporally defined stages of self-administration. For example, immediately following the last session of cocaine taking, Dnmt3a was upregulated at an early time point of withdrawal (4 h after the last cocaine dose), followed by downregulation after 24 h (LaPlant et al. 2010). However, after 28 days of withdrawal following either cocaine IP injections or cocaine self-administration, Dnmt3a was upregulated in NAc, demonstrating long-lasting inductions of Dmnt3a expression and regulation of genes downstream of Dnmt3a activity. Furthermore, artificially manipulating levels of Dnmts – and/or associated methyl-binding proteins – via knockdown or overexpression in key reward-related brain regions have been found to affect addiction-related behaviors in rodent models. For example, MeCP2 is a reader protein for DNA methylation and is thought to act primarily as a transcriptional repressor through recruitment of histone deacetylases to methylated DNA (Bird 2002). MeCP2 is broadly implicated in addiction, as extended cocaine self-administration increases its expression in the dorsal striatum and other limbic regions, and genetic manipulations of (Im et al. 2010) MeCP2 levels alter addiction-related behaviors in rodents. Given that the machinery involved in placing and recognizing DNA methylation seems to be involved in the molecular changes related to addiction behaviors, efforts have been undertaken to use genome-wide approaches to examine genetic loci where DNA methylation is gained or lost during chronic exposures to psychoactive substances.

Recently, Kozlenkov et al. performed genome-wide bisulfite sequencing on orbitofrontal cortex (OFC) of heroin addicts who died of overdose. Importantly, this profiling study was performed on FACS-purified neuronal nuclei separated from glial cells, allowing the authors to determine cell-type-specific effects of heroin abuse on methylation (Kozlenkov et al. 2017). Using this approach, they identified hypermethylated regions in exons of synaptic plasticity genes enriched in glutamatergic, but not GABAergic, neuronal subtypes. Hypomethylated regions were preferentially found in promoter and enhancer regions of genes related to transcription factor activity and gene expression regulation. Altogether, these results concur with previous reports of reduced glutamatergic transmission in the frontal cortex observed in rodent models following drug exposures, and they highlight that DNA methylation changes in neurons are specific to targeted gene regions. These observations also suggest that DNA methylation may be recruited to different genic features or regulatory domains in the context of heroin use to influence aspects of transcription.

5.3 Chromatin Structure

Chromatin conformation is the ultimate determinant of DNA accessibility, which regulates gene expression. Histone modifications and DNA methylation converge on

the regulation of chromatin structure. Both nicotine and cocaine induce global nucleosome repositioning, suggesting that chromatin accessibility represents an initial dynamic genome-wide alteration of the transcriptional landscape preceding more selective downstream transcriptional reprogramming, which characterizes cell- and tissue-specific responses to drugs of abuse (Brown et al. 2015). Advances in sequencing technology have led to new methods that allow chromatin accessibility to be analyzed using whole genome approaches, such as Hi-C, which allows for capture of the three-dimensional interactions between chromatin structure, and the *A*ssay for *T*ransposase-*A*ccessible *C*hromatin-Sequencing (ATAC-seq), which makes use of transposase enzymes to label and amplify open chromatin regions for subsequent sequencing. Egervari et al. recently used ATAC-seq in an integrated transcriptomic and epigenetic approach to investigate molecular changes in the striatum of human heroin abusers and rat SA models (Egervari et al. 2017). The authors performed microarray analyses and ATAC-seq on case vs. controls and identified striatal transcriptional dysregulation for genes related to glutamatergic neurotransmission. Moreover, at key striatal glutamatergic gene loci, both human heroin addicts and heroin SA rats displayed increased levels of a specific histone modification, H3K27ac that mapped precisely onto regions of increased chromatin accessibility, suggesting a mechanistic link between this mark and chromatin remodeling. Most interestingly, the authors found that by administering a pharmacological agent that specifically targets the enzymatic machinery that reads H3K27ac, JQ1 – an inhibitor of select bromodomain containing acetyl reader proteins – in a rodent SA model, they could alter cocaine- and fear-associated memories. This study demonstrates the power of translational approaches that combine chromatin-based genome-wide sequencing and targeted epigenetic investigations in human and rodent models.

6 Conclusions and Future Directions

Together, the studies described in this chapter exemplify the basic scheme of a translational research approach to investigating SUD mechanisms involving both genetic and epigenetic contributors to the disease. Extended focus should be given to promising new areas of genetic and epigenetic SUD research not reviewed here, such as miRNAs, alternative splicing mechanisms, exosome signaling, and immune-response pathologies. Moving forward, an emergent theme in SUD research will be the importance of integrating large-scale studies and genome-wide datasets across experimental modalities, drugs of abuse, and species of interest in order to optimize identification of important genes, pathways, and regulatory mechanisms in SUD. Toward this aim, it is critical that researchers continue to expand currently available large-scale datasets in the study of clinical and nonhuman models of SUD with methods like ATAC-seq and Hi-C to probe chromatin structures, Chip-seq and bisulfite sequencing to examine epigenetic modifications, screening for peripheral biomarkers, and improved GWAS designs to probe genetic vulnerability – strategies that have already been successfully applied in other diseases and neuropsychiatric

disorders. In addition, future mechanistic investigations into gene targets using model systems should take into consideration environmental contributors to the molecular neurobiology of SUD – stress, enriched environment, and early life experiences (aberrant or otherwise). Given the vast diversity and heterogeneity of the neural systems involved, targeted approaches will need to pay special attention to the spatial specificity of manipulations using recently developed tools like FACS and the cell-type-specific expression of genetic constructs and gene-editing systems that allow for precise targeting of brain regions, circuits, and cell types of interest. Finer temporal specificity can also be achieved using inducible manipulations that allow researchers to turn on or off a transgene of interest at a specific time point in the development of addictive-like states to investigate risk factors that may precipitate SUD or during the addiction cycle to further delineate the progression of SUD (including withdrawal periods to examine long-term changes after drug use and/or during treatment). Together, these approaches promise to accelerate our understanding of SUD neurobiology and will aid in the search for more effective therapeutics for addiction.

Acknowledgments We thank the National Institutes of Health (DP1DA042078, R01MH116900, R21DA044767 to I.M.) for their gracious support of this work.

References

Abuse S, M. H. S. Administration (2016) 2015 National survey on drug use and health. Substance Abuse and Mental Health Services Administration, Rockville

Agrawal A, Lynskey MT (2008) Are there genetic influences on addiction: evidence from family, adoption and twin studies. Addiction 103:1069–1081

Anderson EM, Larson EB, Guzman D, Wissman AM, Neve RL et al (2018) Overexpression of the histone dimethyltransferase G9a in nucleus accumbens shell increases cocaine self-administration, stress-induced reinstatement, and anxiety. J Neurosci 38:803–813

Avey D, Sankararaman S, Yim AK, Barve R, Milbrandt J et al (2018) Single-cell RNA-seq uncovers a robust transcriptional response to morphine by glia. Cell Rep 24:3619–3629. e3614

Babb S (2017) Quitting smoking among adults—United States, 2000–2015. MMWR Morb Mortal Wkly Rep 65:1457–1464

Bach H, Arango V, Kassir SA, Tsaava T, Dwork AJ et al (2014) Alcoholics have more tryptophan hydroxylase 2 mRNA and protein in the dorsal and median raphe nuclei. Alcohol Clin Exp Res 38:1894–1901

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

Becker A, Grecksch G, Brödemann R, Kraus J, Peters B et al (2000) Morphine self-administration in μ-opioid receptor-deficient mice. Naunyn Schmiedebergs Arch Pharmacol 361:584–589

Befort K, Filliol D, Décaillot FM, Gavériaux-Ruff C, Hoehe MR et al (2001) A single nucleotide polymorphic mutation in the human μ-opioid receptor severely impairs receptor signaling. J Biol Chem 276:3130–3137

Besnard A, Bouveyron N, Kappes V, Pascoli V, Pagès C et al (2011) Alterations of molecular and behavioral responses to cocaine by selective inhibition of Elk-1 phosphorylation. J Neurosci 31:14296–14307

Bierut LJ, Dinwiddie SH, Begleiter H, Crowe RR, Hesselbrock V et al (1998) Familial transmission of substance dependence: alcohol, marijuana, cocaine, and habitual smoking: a report from the Collaborative Study on the Genetics of Alcoholism. Arch Gen Psychiatry 55:982–988

Bierut LJ, Madden PA, Breslau N, Johnson EO, Hatsukami D et al (2006) Novel genes identified in a high-density genome wide association study for nicotine dependence. Hum Mol Genet 16:24–35

Bierut LJ, Goate AM, Breslau N, Johnson EO, Bertelsen S et al (2012) ADH1B is associated with alcohol dependence and alcohol consumption in populations of European and African ancestry. Mol Psychiatry 17:445

Bird A (2002) DNA methylation patterns and epigenetic memory. Genes Dev 16:6–21

Bloom AJ, Baker TB, Chen L-S, Breslau N, Hatsukami D et al (2013) Variants in two adjacent genes, EGLN2 and CYP2A6, influence smoking behavior related to disease risk via different mechanisms. Hum Mol Genet 23:555–561

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

Bora E, Yücel M, Fornito A, Pantelis C, Harrison BJ et al (2012) White matter microstructure in opiate addiction. Addict Biol 17:141–148

Breslau N, Johnson EO (2000) Predicting smoking cessation and major depression in nicotine-dependent smokers. Am J Public Health 90:1122

Broms U, Wedenoja J, Largeau MR, Korhonen T, Pitkäniemi J et al (2012) Analysis of detailed phenotype profiles reveals CHRNA5-CHRNA3-CHRNB4 gene cluster association with several nicotine dependence traits. Nicotine Tob Res 14:720–733

Brown AN, Vied C, Dennis JH, Bhide PG (2015) Nucleosome repositioning: a novel mechanism for nicotine-and cocaine-induced epigenetic changes. PLoS One 10:e0139103

Cadoret RJ, Troughton E, O'Gorman TW, Heywood E (1986) An adoption study of genetic and environmental factors in drug abuse. Arch Gen Psychiatry 43:1131–1136

Cadoret RJ, Yates WR, Troughton E, Woodworth G, Stewart MA (1996) An adoption study of drug abuse/dependency in females. Compr Psychiatry 37:88–94

Chen L-S, Hung RJ, Baker T, Horton A, Culverhouse R et al (2015) CHRNA5 risk variant predicts delayed smoking cessation and earlier lung cancer diagnosis—a meta-analysis. J Natl Cancer Inst 107:djv100

Clarke T-K, Adams MJ, Davies G, Howard DM, Hall LS et al (2017) Genome-wide association study of alcohol consumption and genetic overlap with other health-related traits in UK Biobank (N=112 117). Mol Psychiatry 22:1376

Collo G, Cavalleri L, Zoli M, Maskos U, Merlo Pich E (2018) Alpha6-containing nicotinic acetylcholine receptors mediate nicotine-induced structural plasticity in mouse and human iPSC-derived dopaminergic neurons. Front Pharmacol 9:572

Contet C, Kieffer BL, Befort K (2004) Mu opioid receptor: a gateway to drug addiction. Curr Opin Neurobiol 14:370–378

David S, Hamidovic A, Chen G, Bergen A, Wessel J et al (2012) Genome-wide meta-analyses of smoking behaviors in African Americans. Transl Psychiatry 2:e119

Deflorio C, Blanchard S, Carla Carisì M, Bohl D, Maskos U (2016) Human polymorphisms in nicotinic receptors: a functional analysis in iPS-derived dopaminergic neurons. FASEB J 31:828–839

Degenhardt L, Hall W (2012) Extent of illicit drug use and dependence, and their contribution to the global burden of disease. Lancet 379:55–70

D'Souza MS (2015) Glutamatergic transmission in drug reward: implications for drug addiction. Front Neurosci 9:404

Egervari G, Landry J, Callens J, Fullard JF, Roussos P et al (2017) Striatal H3K27 acetylation linked to glutamatergic gene dysregulation in human heroin abusers holds promise as therapeutic target. Biol Psychiatry 81:585–594

Forget B, Scholze P, Langa F, Morel C, Pons S et al (2018) A human polymorphism in CHRNA5 is linked to relapse to nicotine seeking in transgenic rats. Curr Biol 28:3244–3253. e3247

Fowler CD, Lu Q, Johnson PM, Marks MJ, Kenny PJ (2011) Habenular α5 nicotinic receptor subunit signalling controls nicotine intake. Nature 471:597

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

Furberg H, Kim Y, Dackor J, Boerwinkle E, Franceschini N et al (2010) Genome-wide meta-analyses identify multiple loci associated with smoking behavior. Nat Genet 42:441

Gabrielsen ME, Romundstad P, Langhammer A, Krokan HE, Skorpen F (2013) Association between a 15q25 gene variant, nicotine-related habits, lung cancer and COPD among 56 307 individuals from the HUNT study in Norway. Eur J Hum Genet 21:1293

Gelernter J, Kranzler H, Sherva R, Almasy L, Koesterer R et al (2014a) Genome-wide association study of alcohol dependence: significant findings in African-and European-Americans including novel risk loci. Mol Psychiatry 19:41

Gelernter J, Kranzler HR, Sherva R, Koesterer R, Almasy L et al (2014b) Genome-wide association study of opioid dependence: multiple associations mapped to calcium and potassium pathways. Biol Psychiatry 76:66–74

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

Goldman D, Bergen A (1998) General and specific inheritance of substance abuse and alcoholism. Arch Gen Psychiatry 55:964–965

Hancock D, Reginsson G, Gaddis N, Chen X, Saccone N et al (2015) Genome-wide meta-analysis reveals common splice site acceptor variant in CHRNA4 associated with nicotine dependence. Transl Psychiatry 5:e651

Hart AB, Engelhardt BE, Wardle MC, Sokoloff G, Stephens M 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

Heilig M, Goldman D, Berrettini W, O'brien CP (2011) Pharmacogenetic approaches to the treatment of alcohol addiction. Nat Rev Neurosci 12:670

Heller EA, Cates HM, Peña CJ, Sun H, Shao N et al (2014) Locus-specific epigenetic remodeling controls addiction-and depression-related behaviors. Nat Neurosci 17:1720

Heller EA, Hamilton PJ, Burek DD, Lombroso SI, Peña CJ et al (2016) Targeted epigenetic remodeling of the Cdk5 gene in nucleus accumbens regulates cocaine-and stress-evoked behavior. J Neurosci 36:4690–4697

Hung RJ, McKay JD, Gaborieau V, Boffetta P, Hashibe M et al (2008) A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. Nature 452:633

Hyman SE, Malenka RC, Nestler EJ (2006) Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci 29:565–598

Im H-I, Hollander JA, Bali P, Kenny PJ (2010) MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. Nat Neurosci 13:1120

Jaenisch R, Bird A (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 33:245

Jayanthi S, McCoy MT, Chen B, Britt JP, Kourrich S et al (2014) Methamphetamine downregulates striatal glutamate receptors via diverse epigenetic mechanisms. Biol Psychiatry 76:47–56

Jorgenson E, Thai KK, Hoffmann TJ, Sakoda LC, Kvale MN et al (2017) Genetic contributors to variation in alcohol consumption vary by race/ethnicity in a large multi-ethnic genome-wide association study. Mol Psychiatry 22:1359

Kendler KS, Karkowski LM, Neale MC, Prescott CA (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

Kim H, Kim J-S (2014) A guide to genome engineering with programmable nucleases. Nat Rev Genet 15:321

Klein AM, Mazutis L, Akartuna I, Tallapragada N, Veres A et al (2015) Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. Cell 161:1187–1201

Knapp PE, Hauser KF (1996) μ-Opioid receptor activation enhances DNA synthesis in immature oligodendrocytes. Brain Res 743:341–345

Konopka G, Friedrich T, Davis-Turak J, Winden K, Oldham MC et al (2012) Human-specific transcriptional networks in the brain. Neuron 75:601–617

Koob GF, Volkow ND (2016) Neurobiology of addiction: a neurocircuitry analysis. Lancet Psychiatry 3:760–773

Kozlenkov A, Jaffe AE, Timashpolsky A, Apontes P, Rudchenko S et al (2017) DNA methylation profiling of human prefrontal cortex neurons in heroin users shows significant difference between genomic contexts of hyper-and hypomethylation and a younger epigenetic age. Genes 8:152

Kumar A, Choi K-H, Renthal W, Tsankova NM, Theobald DE et al (2005) Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. Neuron 48:303–314

Kumasaka N, Aoki M, Okada Y, Takahashi A, Ozaki K 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

Kuryatov A, Berrettini W, Lindstrom J (2011) Acetylcholine receptor (AChR) α5 subunit variant associated with risk for nicotine dependence and lung cancer reduces (α4β2) 2α5 AChR function. Mol Pharmacol 79:119–125

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

LaPlant Q, Nestler EJ (2011) CRACKing the histone code: cocaine's effects on chromatin structure and function. Horm Behav 59:321–330

LaPlant Q, Vialou V, Covington HE III, Dumitriu D, Feng J et al (2010) Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens. Nat Neurosci 13:1137

Li W, Zhu J, Li Q, Ye J, Chen J et al (2016) Brain white matter integrity in heroin addicts during methadone maintenance treatment is related to relapse propensity. Brain Behav 6:e00436

Loukola A, Buchwald J, Gupta R, Palviainen T, Hällfors J et al (2015) A genome-wide association study of a biomarker of nicotine metabolism. PLoS Genet 11:e1005498

Lüscher C, Malenka RC (2011) Drug-evoked synaptic plasticity in addiction: from molecular changes to circuit remodeling. Neuron 69:650–663

Mashayekhi FJ, Rasti M, Rahvar M, Mokarram P, Namavar MR et al (2012) Expression levels of the BDNF gene and histone modifications around its promoters in the ventral tegmental area and locus ceruleus of rats during forced abstinence from morphine. Neurochem Res 37:1517–1523

Maze I, Covington HE, Dietz DM, LaPlant Q, Renthal W et al (2010) Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. Science 327:213–216

Merikangas KR, Stolar M, Stevens DE, Goulet J, Preisig MA et al (1998) Familial transmission of substance use disorders. Arch Gen Psychiatry 55:973–979

Morel C, Fattore L, Pons S, Hay Y, Marti F et al (2014) Nicotine consumption is regulated by a human polymorphism in dopamine neurons. Mol Psychiatry 19:930

Munafò MR, Timofeeva MN, Morris RW, Prieto-Merino D, Sattar N et al (2012) Association between genetic variants on chromosome 15q25 locus and objective measures of tobacco exposure. J Natl Cancer Inst 104:740–748

Nelson EC, Lynskey MT, Heath AC, Wray N, Agrawal A et al (2014) Association of OPRD1 polymorphisms with heroin dependence in a large case-control series. Addict Biol 19:111–121

Nestler EJ (2005) Is there a common molecular pathway for addiction? Nat Neurosci 8:1445

Nikoshkov A, Hurd YL, Yakovleva T, Bazov I, Marinova Z et al (2005) Prodynorphin transcripts and proteins differentially expressed and regulated in the adult human brain. FASEB J 19:1543–1545

Park BL, Kim JW, Cheong HS, Kim LH, Lee BC 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

Patel YM, Stram DO, Wilkens LR, Park S-SL, Henderson BE et al (2015) The contribution of common genetic variation to nicotine and cotinine glucuronidation in multiple ethnic/racial populations. Cancer Epidemiol Biomark Prev 24:119–127

Quillen EE, Chen XD, Almasy L, Yang F, He H 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 Part B: Neuropsychiatr Genet 165:103–110

Rice JP, Hartz SM, Agrawal A, Almasy L, Bennett S et al (2012) CHRNB3 is more strongly associated with Fagerström Test for Cigarette Dependence-based nicotine dependence than cigarettes per day: phenotype definition changes genome-wide association studies results. Addiction 107:2019–2028

Richmond-Rakerd LS, Otto JM, Slutske WS, Ehlers CL, Wilhelmsen KC et al (2016) A novel tobacco use phenotype suggests the 15q25 and 19q13 loci may be differentially associated with cigarettes per day and tobacco-related problems. Nicotine Tob Res 19:426–434

Robison AJ, Nestler EJ (2011) Transcriptional and epigenetic mechanisms of addiction. Nat Rev Neurosci 12:623

Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K et al (2006) Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. Hum Mol Genet 16:36–49

Sanchez-Roige S, Fontanillas P, Elson SL, a. R. Team, Gray JC et al (2019) Genome-wide association study of alcohol use disorder identification test (AUDIT) scores in 20 328 research participants of European ancestry. Addict Biol 24:121–131

Sanjana NE, Cong L, Zhou Y, Cunniff MM, Feng G et al (2012) A transcription activator-like effector toolbox for genome engineering. Nat Protoc 7:171

Scherer G, Engl J, Urban M, Gilch G, Janket D et al (2007) Relationship between machine-derived smoke yields and biomarkers in cigarette smokers in Germany. Regul Toxicol Pharmacol 47:171–183

Schmidt HD, Sangrey GR, Darnell SB, Schassburger RL, Cha JHJ et al (2012) Increased brain-derived neurotrophic factor (BDNF) expression in the ventral tegmental area during cocaine abstinence is associated with increased histone acetylation at BDNF exon I-containing promoters. J Neurochem 120:202–209

Schumann G, Coin LJ, Lourdusamy A, Charoen P, Berger KH 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 108:7119–7124

Schumann G, Liu C, O'Reilly P, Gao H, Song P et al (2016) KLB is associated with alcohol drinking, and its gene product β-Klotho is necessary for FGF21 regulation of alcohol preference. Proc Natl Acad Sci 113:14372–14377

Scofield M, Heinsbroek J, Gipson C, Kupchik Y, Spencer S et al (2016) The nucleus accumbens: mechanisms of addiction across drug classes reflect the importance of glutamate homeostasis. Pharmacol Rev 68:816–871

Sherva R, Wilhelmsen K, Pomerleau CS, Chasse SA, Rice JP et al (2008) Association of a single nucleotide polymorphism in neuronal acetylcholine receptor subunit alpha 5 (CHRNA5) with smoking status and with 'pleasurable buzz'during early experimentation with smoking. Addiction 103:1544–1552

Siedlinski M, Cho MH, Bakke P, Gulsvik A, Lomas DA et al (2011) Genome-wide association study of smoking behaviours in patients with COPD. Thorax 66(10):894–902

Sillivan SE, Whittard JD, Jacobs MM, Ren Y, Mazloom AR et al (2013) ELK1 transcription factor linked to dysregulated striatal mu opioid receptor signaling network and OPRM1 polymorphism in human heroin abusers. Biol Psychiatry 74:511–519

Slezak M, Korostynski M, Gieryk A, Golda S, Dzbek J et al (2013) Astrocytes are a neural target of morphine action via glucocorticoid receptor-dependent signaling. Glia 61:623–635
Snowden AW, Gregory PD, Case CC, Pabo CO (2002) Gene-specific targeting of H3K9 methylation is sufficient for initiating repression in vivo. Curr Biol 12:2159–2166
Swan GE, Carmelli D, Cardon LR (1997) Heavy consumption of cigarettes, alcohol and coffee in male twins. J Stud Alcohol 58:182–190
Takeuchi F, Isono M, Nabika T, Katsuya T, Sugiyama 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
Thorgeirsson TE, Gudbjartsson DF, Surakka I, Vink JM, Amin N et al (2010) Sequence variants at CHRNB3–CHRNA6 and CYP2A6 affect smoking behavior. Nat Genet 42:448
Timofeeva MN, McKay JD, Davey SG, Johansson M, Byrnes GB et al (2011) Genetic polymorphisms in 15q25 and 19q13 loci, cotinine levels, and risk of lung cancer in EPIC. Cancer Epidemiol Biomark Prev 20:2250–2261
Timofeeva MN, Hung RJ, Rafnar T, Christiani DC, Field JK et al (2012) Influence of common genetic variation on lung cancer risk: meta-analysis of 14 900 cases and 29 485 controls. Hum Mol Genet 21:4980–4995
Tsuang MT, Bar JL, Harley RM, Lyons MJ (2001) The Harvard twin study of substance abuse: what we have learned. Harv Rev Psychiatry 9:267–279
Uhl G, Elmer G, Labuda M, Pickens R (1995) Genetic influences in drug abuse. In: Psychopharmacology: The fourth generation of progress. Raven Press, New York, pp 1793–1806
Valjent E, Pagès C, Rogard M, Besson MJ, Maldonado R et al (2001) Δ9-tetrahydrocannabinol-induced MAPK/ERK and Elk-1 activation in vivo depends on dopaminergic transmission. Eur J Neurosci 14:342–352
Wain LV, Shrine N, Miller S, Jackson VE, Ntalla I et al (2015) Novel insights into the genetics of smoking behaviour, lung function, and chronic obstructive pulmonary disease (UK BiLEVE): a genetic association study in UK Biobank. Lancet Respir Med 3:769–781
Walker DM, Cates HM, Heller EA, Nestler EJ (2015) Regulation of chromatin states by drugs of abuse. Curr Opin Neurobiol 30:112–121
Wang D, Quillan JM, Winans K, Lucas JL, Sadée W (2001) Single nucleotide polymorphisms in the human μ opioid receptor gene alter basal G protein coupling and calmodulin binding. J Biol Chem 276:34624–34630
Wang J, Liang Q, Mendes P, Sarkar M (2011) Is 24h nicotine equivalents a surrogate for smoke exposure based on its relationship with other biomarkers of exposure? Biomarkers 16:144–154
Ware JJ, Aveyard P, Broderick P, Houlston RS, Eisen T et al (2015) The association of rs1051730 genotype on adherence to and consumption of prescribed nicotine replacement therapy dose during a smoking cessation attempt. Drug Alcohol Depend 151:236–240
Ware JJ, Chen X, Vink J, Loukola A, Minica C et al (2016) Genome-wide meta-analysis of cotinine levels in cigarette smokers identifies locus at 4q13.2. Sci Rep 6:20092
Way M, McQuillin A, Saini J, Ruparelia K, Lydall GJ et al (2015) Genetic variants in or near ADH 1 B and ADH 1 C affect susceptibility to alcohol dependence in a British and Irish population. Addict Biol 20:594–604
Wolf ME (2010) The Bermuda Triangle of cocaine-induced neuroadaptations. Trends Neurosci 33:391–398
Xu K, Kranzler HR, Sherva R, Sartor CE, Almasy L et al (2015) Genomewide association study for maximum number of alcoholic drinks in European Americans and African Americans. Alcohol Clin Exp Res 39:1137–1147
Yang X, Lu X, Wang L, Chen S, Li J et al (2013) Common variants at 12q24 are associated with drinking behavior in Han Chinese. Am J Clin Nutr 97:545–551
Yang J, Wang S, Yang Z, Hodgkinson CA, Iarikova P et al (2015) The contribution of rare and common variants in 30 genes to risk nicotine dependence. Mol Psychiatry 20:1467
Zhang Y, Wang D, Johnson AD, Papp AC, Sadée W (2005) Allelic expression imbalance of human mu opioid receptor (OPRM1) caused by variant A118G. J Biol Chem 280(38):32618–32624

Zheng GX, Terry JM, Belgrader P, Ryvkin P, Bent ZW et al (2017) Massively parallel digital transcriptional profiling of single cells. Nat Commun 8:14049

Zhu AZ, Zhou Q, Cox LS, David SP, Ahluwalia JS et al (2014) Association of CHRNA5-A3-B4 SNP rs2036527 with smoking cessation therapy response in African-American smokers. Clin Pharmacol Ther 96:256–265