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Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction

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Genetic variation may partially underlie complex personality and physiological traits—such as impulsivity, risk taking and stress responsivity—as well as a substantial proportion of vulnerability to addictive diseases. Furthermore, personality and physiological traits themselves may differentially affect the various stages of addiction, defined chronologically as initiation of drug use, regular drug use, addiction/dependence and potentially relapse. Here we focus on recent approaches to the study of genetic variation in these personality and physiological traits, and their influence on and interaction with addictive diseases.

Vulnerability to develop a drug addiction is influenced by a combination of genetic and environmental factors. Both factors couple with direct drug-induced effects to influence the progression from intermittent to regular drug use, the transition from abuse to addiction, and the propensity for repeated relapse after achievement of a drug-free state[1,2].

Chronic exposure to drugs of abuse causes persistent changes in the brain, including changes in expression of genes or their protein products, in protein-protein interactions, in neural networks, and in neurogenesis and synaptogenesis, all of which ultimately affect behavior. In rodents, there are inbred strains and selectively bred lines that readily self-administer drugs of abuse (implying genetic vulnerability) as well as strains that do not readily self-administer drugs (implying genetic resistance). Different strains show differences in the cellular and molecular response to drugs[3]. Genetic factors may also be involved in direct drug-induced effects, including alteration of pharmacodynamics (a drug's effects at a receptor, including the physiological consequences of receptor activity) or pharmacokinetics (a drug's absorption, distribution, metabolism and excretion) of a drug of abuse or of a treatment agent.

Many medical disorders have some genetic component, but most, including cancer, obesity and heart disease, involve complex genetic contributions based on multiple variants of multiple genes and different combinations of these variants in different people. For some of the most studied diseases, such as certain cancers, the specific genetic contributions and genetic variants have been identified and verified by multiple studies. However, the identified variants, in their entirety, comprise only a small proportion of the estimated genetic contribution. Studying the genetics of complex psychiatric or behavioral disorders such as addiction poses additional challenges. These include precise phenotypic characterization of individuals and the characterization of ethnic/cultural backgrounds (as different backgrounds yield differences in allelic frequencies). These challenges also must be faced in the study of other complex genetic disorders.

Despite the complexity of the problem, the costs to society of drug and alcohol addiction are too enormous to ignore. Addiction has some of the highest overall medical health costs of any medical disorder, once comorbid disorders such as HIV/AIDS, hepatitis C and lung cancer are factored in. Loss of productivity, interdiction and the criminal justice system incur additional economic costs. It is therefore imperative that all components contributing to addiction be studied, including genetics, with the goal of improving primary prevention, early intervention and chronic treatment.

Family and twin epidemiological studies show that genes contribute to the vulnerability to addictive disease, with estimates of heritability of 30–60%. Addiction heritability was first demonstrated with alcoholism, which is influenced by distinct genetic factors such as the aldehyde dehydrogenase 2 genotype. Predisposition to addiction may be due both to genetic variants that are common to all addictions and to those specific to a particular addiction. For example, a genetic variance shared by multiple classes of drugs of abuse is demonstrated in twin studies[4,5]. However, some genetic variance is specific to drug class, as is particularly well documented for opiate addiction[4]. Moreover, there are different influences of environment versus genetic factors on the transitions from initiation of drug use, to regular drug use, to drug addiction/dependence and then potentially to relapse[6] (**Fig. 1**).

The genetics of addiction encompasses heritable factors that influence the different stages in the trajectory of initiation and progression to drug addiction, including severity of dependence or withdrawal and risk of relapse. Variation in personality dimensions, such as impulsivity, risk taking and novelty seeking, may contribute to the initiation of drug use as well as the transitions from initial use to regular use to addiction (**Fig. 1**). Each of these personality dimensions may have, in part, its own genetic basis.

A number of inventories have been developed for the description and classification of personality dimensions and to tease out the influence of genetics on personality. Four instruments often used in genetics

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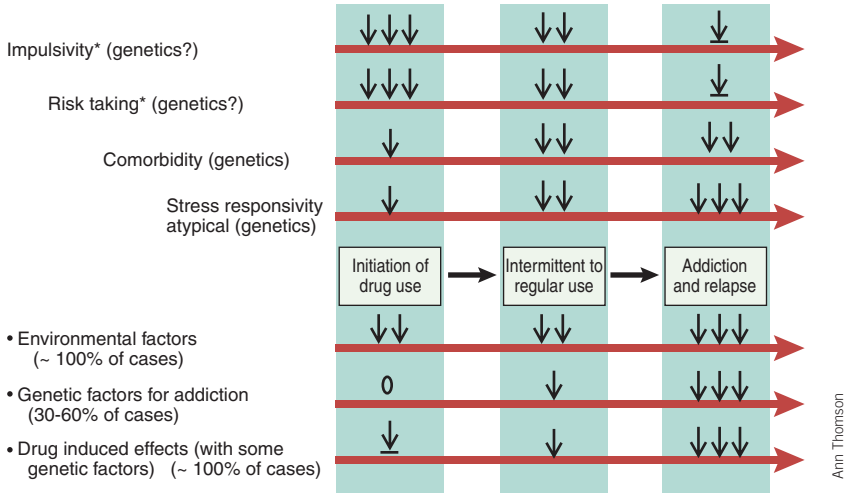

Figure 1 Diverse contribution of genetic influences to initial drug use, abuse and addiction. We suggest that impulsivity and risk taking contribute most to the initiation of drug use and the progression to regular drug use. We expect that these personality factors contribute less to addiction and relapse after substantial changes to the brain, effected by chronic exposure to the drug of abuse. These two personality factors, comorbidity and stress responsivity (top) and the three domains (bottom) interact to influence the progression to addiction, as depicted. *Lifelong or identified in early childhood. ↓↓↓, greatest relative influence; ↓↓, medium relative influence; ↓, small relative influence; ⇓, minor relative influence; 0, no influence. These ratings reflect our estimates and opinion based on current information.

research to quantify personality dimensions are the Tridimensional Personality Questionnaire (TPQ) or the more complete version, the Temperament and Character Inventory (TCI; which measures novelty seeking, harm avoidance, reward dependence and persistence), the NEO Personality Inventory-Revised (NEO-PI-R; which measures neuroticism, extroversion, openness, agreeableness and conscientiousness) and the Barratt Impulsiveness Scale. Some of these questionnaires and variables are based on the concept of factor analysis, in which a large number of individual questions contribute to a smaller number of underlying traits. Of the traits measured by these tests, some addiction research has focused on impulsivity, with or without aggression or suicidality, and risk taking, which is often associated with novelty seeking. In addition, addictions can be defined with scales such as the KMSK, which measure duration and magnitude of drug use.

The TPQ, TCI, NEO-PI-R provide a broad and more time-intensive characterization of personality traits. By contrast, the Barratt and KMSK scales provide a relatively rapid evaluation of a particular phenotype (impulsiveness and degree of exposure to a drug of abuse, respectively). Use of common questionnaires does facilitate the comparative interpretation of different studies. However, optimization of more focused instruments may also be advantageous for the study of more refined phenotypes relevant to a particular clinical situation.

Identifying genetic factors in personality traits and addiction

Until recently, family-based linkage studies have been most widely used. Linkage studies investigate the transmission of genetic markers on specific genomic regions of interest and phenotypes in pedigrees consisting of, preferably, two or more generations, including studies of affected sibling pairs (more powerful when both siblings and parents are included). The alternative is association studies, which ask whether a particular gene allele is more prevalent in patients, compared with control subjects, than would be expected by chance. This is increasingly the experimental approach of choice for identifying genes responsible for complex traits. Association studies are able to detect linked variants involved in a disease (i) if they are within 40,000–80,000 nucleotides of the genotyped variant, (ii) if linkage disequilibrium (which occurs when there is a non-random distribution of allele combinations; for example, in a haplotype) is relatively high, and (iii) if the effect sizes are moderate to high. This is a much smaller distance than is possible with family-based linkage studies. Although family-based studies may be feasible for personality traits such as impulsivity, risk taking and stress responsivity, family studies in illicit drug addiction are difficult to conduct because of the enormous stigma of addiction, the disruption of involved families and the difficulty in ascertaining family members. However, outstanding family studies have been done in the field of alcoholism, notably the Collaborative Study on the Genetics of Alcoholism sponsored by United States National Institute on Alcoholism and Alcohol Abuse[7]. Strong evidence has been provided by these studies for the involvement of several genes, including the GABA receptor subunit A2 (*GABRA2*) and muscarinic acetylcholine receptor M2 (*CHRM2*), in alcohol dependence[8,9].

One general approach for identifying specific genes involved in a disease is hypothesis-oriented selection. It is frequently useful to investigate specific genes involved in diseases based on a prior understanding of the diseases and/or addiction and based on specific hypotheses about these factors[1,2]. In studying drug addiction, one can initially consider genes governing direct and downstream molecular events altered by chronic exposure to a drug of abuse. For example, cocaine produces a surge in extracellular dopamine by blocking the action of the dopamine transporter. Cocaine also increases gene expression and promotes release of the κ opioid ligand dynorphin in the striatum. Variants of the preprodynorphin gene (*PDYN*) have been associated with vulnerability to develop cocaine addiction[2].

Another strategy is to use positional approaches—conducting genome-wide scans to identify chromosomal positions that may be associated with a specific disorder or addiction. Further fine mapping in identified chromosomal regions is then required. Until recently, microsatellite marker panels were used to scan the whole genome. However, over the last few years, various approaches using single nucleotide polymorphism (SNP) arrays or other panels of single SNPs have allowed the identification of more defined regions for fine mapping in a far simpler manner, a more refined approach than microsatellite marker panels. As SNP panels become more inclusive of the common variants in the human genome, it should be possible to examine the variants associated with a phenotype more quickly.

Variants in the coding region of genes may change the protein product, as in the A118G variant of the μ opioid receptor gene (*OPRM1*). Other variants may alter the amount of gene expression (for example, prodynorphin promoter region variants), and yet other variants may alter the rate of mRNA degradation (for example, a dopamine receptor D2 variant, *DRD2*), all of which can contribute to functionality[2]. Such variants can affect both normal physiology and specific aspects of addiction pathophysiology.

Table 1 Genes having one or more variants that have been reported to be associated with one or more addictions

Gene	Protein	System	Chromosomal location[a]	I	R	E	S	A	Drug	Status
OPRM1	μ opioid receptor	Opioid	6q24-q25	–	–	–	+	+	H/O; Alc	D/A[b]
OPRK1	κ opioid receptor	Opioid	8q11.2	–	–	–	–	+	H/O	D/A
PDYN	Preprodynorphin	Opioid	20pter-p12.2	–	–	–	–	+	C/S	D/A
TH	Tyrosine hydroxylase	Dopaminergic	11p15.5	–	–	+	–	+	Alc	D/A
DRD2	Dopamine receptor D2	Dopaminergic	11q23	–	–	–	–	+	Alc	D/A[b]
DRD3	Dopamine receptor D3	Dopaminergic	3q13.3	–	+	–	–	+	Alc; C/S	D/A[b]
DRD4	Dopamine receptor D4	Dopaminergic	11p15.5	+	+	–	–	+	H/O; C/S; Alc	D/A[b]
DBH	Dopamine β-hydroxylase	Dopaminergic	9q34	–	–	–	–	+	C/S	D/A
DAT (SLC6A3)	Dopamine transporter	Dopaminergic	5p15.3	+	–	–	–	+	Alc	D/A[b]
TPH1	Tryptophan hydroxylase 1	Serotonergic	11p15.3-p14	+	–	–	–	+	Alc	D/A[b]
TPH2	Tryptophan hydroxylase 2	Serotonergic	12q21.1	–	–	–	–	+	H/O; Alc	CSA; D/A[b]
HTR1B	Serotonin receptor 1B	Serotonergic	6q13	–	–	–	–	+	Alc; H/O	D/A[b]
HTR2A	Serotonin receptor 2A	Serotonergic	13q14-q21	–	–	–	–	+	Alc	CSA; D/A[b]
SERT (SLC6A4)	Serotonin transporter	Serotonergic	17q11.1-q12	+	–	+	–	+	H/O; Alc	D/A[b]
MAOA	Monoamine oxidase A	Catecholaminergic, serotonergic	Xp11.23	+	–	+	–	+	Alc	D/A
COMT	Catechol-*O*-methyl transferase	Catecholaminergic	22q11.2	+	–	–	+	+	Alc; H/O	D/A[b]
GABRA1	GABA receptor subunit α-1	GABAergic	5q34-q35	+	–	–	–	+	Alc	D/A[b]
GABRA6	GABA receptor subunit α-6	GABAergic	5q31.1-q35	+	–	–	–	+	Alc	D/A
GABRB1	GABA receptor subunit β-1	GABAergic	4p13-p12	+	–	–	–	+	Alc	D/A
CHRM2	Muscarinic acetylcholine receptor M2	Cholinergic	7q35-q36	–	–	–	–	+	Alc	D/A[b]
CNR1	Cannabinoid receptor 1	Cannabinoid	6q14-q15	–	–	–	–	+	Alc; C/S	CSA; D/A[b]
FAAH	Fatty acid amide hydrolase	Cannabinoid	1p35-34	–	–	–	–	+	Alc	CSA
NPY	Neuropeptide Y	Neuromodulatory	7p15.1	–	–	–	–	+	Alc	CSA; D/A[b]
ADH1B	Alcohol dehydrogenase 1B	Ethanol metabolism	4q22	–	–	–	–	+	Alc	D/A[b]
ADH1C	Alcohol dehydrogenase 1C	Ethanol metabolism	4q22	–	–	–	–	+	Alc	D/A[b]
ALDH2	Aldehyde dehydrogenase 2	Ethanol metabolism	12q24.2	–	–	–	–	+	Alc	D/A[b]
CYP2D6	Cytochrome CYP450	Drug metabolism	22q13.1	–	–	–	–	+	H/O	D/A
ANKK1	Ankyrin repeat and kinase domain–containing 1	Signal transduction (predicted)	11q23.2	–	+	–	–	+	Alc	D/A[b]

I: impulsivity, R: risk taking, E: environment, S: stress responsivity, A: addiction. H/O: heroin or opiate, Alc: alcohol, C/S: cocaine or stimulants, CSA: continued substance abuse, D/A: dependence/addiction.

[a]Gene map locus: Online Mendelian Inheritance in Man, Johns Hopkins University, Baltimore (http://www.ncbi.nlm.nih.gov/omim) as of July 2005.
[b]Association with drug addiction in two or more studies

Ultimately, however, rigorous phenotypic assessment is essential for all studies of addiction genetics because poor or inadequate phenotypic assessments lead to incorrect results. Such assessment entails the use of a diverse battery of instruments to evaluate personality traits, comorbid disorders, detailed histories of initiation of drug use, and progression to addiction. Precise phenotyping takes time, and requires highly trained personnel. Moreover, because of the time and expense, it can lead to a decrease in numbers of subjects studied. There is therefore an inherent trade-off that most geneticists have to make—whether to study large numbers of subjects, which would in turn give the study and the statistics greater validity, or to do very careful phenotyping of the subjects, without which one runs the risks of generating more false positives or negatives. Population genetics can also be influenced by additional factors—for instance, there are significant ethnic/cultural differences in allelic frequencies of variants of many specific genes. These must be controlled for or analyzed using a variety of newly developing techniques involving primarily combinations of SNPs or other variants. There are innumerable further challenges to molecular genetics studies of any complex disorder, including other diseases that are present at the same time, the specificity in diagnosis of subjects, vigilance for error of any type in the molecular work, and rigorous state-of-the-art statistical genetics analyses[10]. Statistical genetics methods involve techniques that are evolving, such as methods for statistically determining inferred haplotypes . Here we have included only a selection of studies that we consider of potential importance, primarily from established research teams using acceptable or optimal study designs, phenotypic assessments, molecular techniques and statistical genetics analyses. This perspective is not intended to be a comprehensive review.

Moreover, we must emphasize that evidence of enhanced genetic vulnerability to addiction does not imply that addiction will occur. Many factors, such as environmental influences or availability of drugs, strongly influence the development of drug abuse or addiction. Conversely, a 'genetically resistant' individual (or strain of rat) may self-administer a drug of abuse under specific environmental conditions (**Fig. 1**)[3].

Personality traits and addiction: impulsivity

Impulsivity is a personality trait characterized by behavioral disinhibition, defined as acting suddenly in an unplanned manner to satisfy a desire: for example, acting on the spur of the moment, not thinking through the potential impact before carrying out actions, or making

statements without thinking in advance about what is to be said. Acts of impulsivity may include aggression, violence and suicide. However, impulsivity, as a trait, occurs on a continuum; thus, impulsivity *per se* is not an indicator of pathology.

Early work implicated low serotonin levels and its metabolites in various forms of impulsivity. Low levels of cerebrospinal fluid 5-hydroxylindolacetic acid, a major metabolite of serotonin and an indicator of serotonin metabolism, is related to impulsivity, aggression and depression, as well as to early-onset alcoholism (reviewed in refs. 11,12). In addition, prolactin release after fenfluramine challenge, a biomarker of serotonin metabolism, demonstrates a relationship between low serotonin metabolism and impulsive behavior[13], which is also associated with an increased risk for impulsive personality traits in first-degree relatives[13].

Serotonergic neurons project from the raphe throughout the brain to diverse regions, including the hippocampus, frontal cortex and amygdala. Loss of impulse control may be due to impaired inhibitory control resulting from drug-induced changes in the frontal cortex. Experimentation with addictive drugs and the onset of drug abuse generally occur in adolescence, with the rare exception of some reported cases of alcoholism and prescription opiate addiction, which may occur later, even in the elderly. Neurodevelopmental processes and reproductive hormone changes during adolescence and early adulthood may modulate impulse control, which may contribute to vulnerability to experimentation with drugs of abuse, with possible progression to addiction.

Behaviors characterized by a deficit in impulse control have been studied for association and linkage with candidate genes (**Table 1**) in the serotonergic system (for example, tryptophan hydroxylase 1 and 2 [*TPH1* and *TPH2*] and serotonin transporter [*SERT*]), the dopaminergic system (tyrosine hydroxylase [*TH*], dopamine receptors, and dopamine transporter [*DAT*]), the monoamine metabolism pathway (monoamine oxidase A [*MAOA*] and catechol-*O*-methyltransferase [*COMT*]), and the noradrenergic system (dopamine β-hydroxylase [*DBH*]), inhibitory system, GABAergic and nitric oxide systems, as well as other genes)[11,14,15]. Each of these genes is reportedly associated with alcoholism or some other addiction (**Table 1**). In addition, the neurotransmitter systems coded by these genes are interactively involved in the acute and chronic effects of most drugs of abuse and, thus, underlie addiction as well as the initiation of drug use.

The earliest candidate gene studies on impulsivity were conducted on *TPH1*, which codes for the rate-limiting enzyme in the production of serotonin. In impulsive violent offenders, a *TPH1* gene variant was associated with reduced CSF 5-HIAA and suicidal behavior[12,14]. *TPH1* variants are also associated with impulsivity, aggression and various forms of suicidality. Other genes such as *SERT*, *DRD3*, *MAOA*, *5-HT2A*, and dopamine receptors D3 and D4 (*DRD3* and *DRD4*) are related to impulsivity (**Table 1**)[11,14].

Although a substantial body of knowledge is accumulating from these genetic studies, the results gleaned from these investigations may not extrapolate to all people. These studies vary in their assessment instruments used, the ethnic/cultural populations studied and the statistical methods applied. Hence, the results of apparently similar studies cannot be directly compared and, for this reason, meta-analyses of these studies may be fraught with pitfalls.

Thus, several major candidate genes with variants associated with impulsivity have been reported. Most of these candidate genes code for proteins that control major neurotransmitter systems, for which a wealth of basic data is available. Furthermore, impulsivity itself is associated with specific addictive diseases. Future studies on the role of impulsivity, and its genetic variants, at specific stages of addiction could therefore shed light on neurobiological mechanisms underlying clinically defined stages in the process of addiction, relapse and recovery.

Personality traits and addiction: risk taking

Risk taking is characterized by behaviors performed under uncertainty, with or without inherent negative consequences, with or without any possible or probable harm to oneself or others, and without robust contingency planning. Risk taking may be operationally measured in tasks that require an evaluation of relative risk versus reward (for instance, in the choice of career opportunities or selection of automobiles). Pathological gamblers and addicted patients may exhibit signs of risk taking, which can be assessed by specific clinical questionnaires such as the South Oaks Gambling Screen. Novelty seeking, often defined as one aspect of risk taking, with potentially high reactivity to novel stimuli, can alternatively be considered a personality trait, detected in certain psychometric instruments (such as the Temperament and Character Inventory). It is part of a constellation of traits observed in individuals with a propensity to experiment with novel stimuli, including those produced by drugs of abuse.

Novelty seeking may be correlated with progression from abuse to addiction for several drugs. DRD4 receptors are members of the 'D2-like' family of Gi-coupled dopamine receptors. Some studies report an association between novelty seeking and DRD4 receptor variants, for example, between high Tridimensional Personality Questionnaire novelty-seeking scores and a particular allelic variant[16,17]. In human brain tissue, DRD4 binding is found in brain regions that include the prefrontal and entorhinal cortex, hippocampus, dorsomedial thalamus, lateral septal nucleus and hypothalamus[18]. Notably, no apparent DRD4 binding is detected in the nucleus accumbens, caudate or putamen, which are major sites of D2 receptor binding and mediate the direct psychostimulant and reinforcing effects of drugs of abuse. In contrast, the DRD4 receptor distribution pattern suggests roles in attentional, motivational, emotional and mnemonic processing, on the basis of some major functions thought to be mediated by these brain areas. Notably, the prefrontal cortex is a site of cognitive and executive functions and decision making.

Although several studies have identified associations of different *DRD4* polymorphisms with novelty seeking, these findings have not been consistently replicated[16,17]. These heterogeneous findings may result from differences in age of subjects, phenotyping instruments used and ethnic composition of patient populations, among other issues, in different studies[16].

Other molecular targets involved in monoaminergic function have been related to novelty seeking and drug abuse (**Table 1**). The *DRD2* Taq1A polymorphism is widely studied and reported in the scientific literature and popular press for its association with alcoholism and various psychiatric disorders. However, this association has yet to be solidly documented, with conflicting meta-analyses from different groups[19,20]. Interestingly, the Taq1A variant is located approximately 10,000 nucleotides downstream (3′) from the *DRD2* gene and has recently been reported to reside in the neighboring *ANKK1* gene, which codes for a serine/threonine kinase[21]. Hence, results reported for the Taq1A variant may be potentially ascribed to the action of the *ANKK1* gene product.

Comorbid disorders

For many addicts, substance abuse does not occur as an isolated disorder. Four psychiatric conditions (depression, anxiety, antisocial personality disorder and attention deficit/hyperactivity disorder) are commonly present in and probably are involved in psychopathology or physiology of addiction to opiates and alcohol[22]. The most common comorbid conditions are depression and anxiety, with unipolar depression being the most common. In epidemiological studies, 20% to over 50% of people with alcoholism, cocaine and other stimulant addiction, or opiate addiction have depressive and/or anxiety disor-

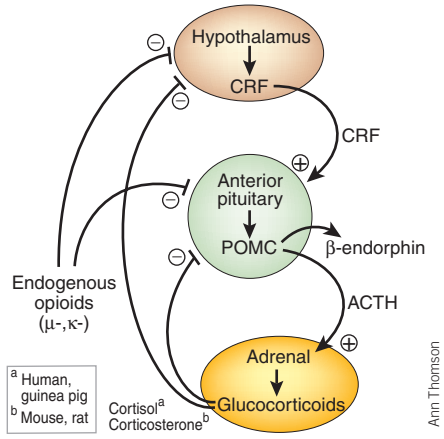

Figure 2 Stress causes increased mRNA synthesis and release of hypothalamic corticotropin releasing factor (CRF) into the portal circulation, which acts on CRFR1 receptors in the anterior pituitary. This induces synthesis of proopiomelanocortin (POMC) mRNA and peptide in the anterior pituitary and release into the circulation of β-endorphin and adrenocorticotropic hormone (ACTH), which are derived from processing of POMC. ACTH acts on ACTH receptors in the adrenal cortex and induces release of the stress hormone cortisol (in humans and guinea pigs) or corticosterone (in rats and mice), which are primary mediators of the stress response. Cortisol or corticosterone exert negative feedback regulation at both the hypothalamus and the pituitary to inhibit the synthesis of POMC and release of ACTH and β-endorphin. In addition to this classical circadian negative feedback regulation by glucocorticoids, the endogenous opioid system, including both μ and κ opioid receptors, tonically inhibits this axis.

ders[22]. However, the prevalence of comorbidity in people when they first try a drug of abuse is not well defined. Certainly, many people who are already addicted to illicit drugs potentially could be classified as having antisocial personality disorder because of their criminal activity pertaining to the acquisition and use of illicit drugs. Attention deficit/hyperactivity disorder in the childhood or adult form is common, especially in people who are dependent on cocaine and other stimulants (reviewed in ref. 23). For each of the comorbid disorders, it is important to use more refined psychiatric diagnostic tools to determine if the psychiatric disorder preceded or followed the development of the addictive disorder. It has been established that genetics are somewhat involved in each of the psychiatric disorders just as in the addictive diseases discussed here. Thus, in the presence of comorbidity, it is difficult to determine which of the gene variants contribute to the psychiatric disease, to the addictive disease, or to both.

The role of comorbidity in the genetics of addiction remains an area of controversy. For example, comorbidity may be mechanistically important in the vulnerability to or severity of addiction, requiring focused studies of its influence. Conversely, studies are needed in addictive disease populations, taking comorbidity into account as an independent variable, thus investigating genetic variation in addiction with and without comorbidities.

Stress responsivity

The modern concept of stress and its importance for many human diseases was developed by the pioneering neuroendocrinologist Hans Selye, who discovered that various noxious stimuli caused what he called a 'general adaptation syndrome,' mediated in part by the pituitary and adrenal glands.

An important component of the stress-responsive system is the hypothalamic-pituitary-adrenal (HPA) axis. Exposure to stress activates the HPA axis (**Fig. 2**). HPA axis activation or suppression influences addiction[24–27]. The question can therefore be posed: is there a genetic link between HPA axis function and addiction? In addition to the classical feedback regulation of the HPA axis by corticosteroids, clinical studies with opioid antagonists demonstrate that the endogenous opioid system, via both μ and κ opioid receptors, also tonically inhibits the HPA axis. We have proposed that an atypical responsivity to stress and stressors, with a particular focus on the HPA axis, contributes to the continuation of specific addictions, as well as to relapse once the brain has undergone plasticity due to addiction[1,24,25]. We have found that active heroin addicts have a hypo-responsive HPA system and that patients with cocaine dependence, including former heroin addicts in methadone maintenance treatment with ongoing dependence on cocaine, show a hyper-responsive HPA axis[1,26]. In these clinical studies, it is not possible to distinguish whether this atypical stress responsivity preceded (because of underlying physiological and ultimately genetic conditions) or was caused by long-term self-administration of opiates or cocaine.

In animal models of conditioned place preference and drug self-administration, acute and chronic stress affect the HPA axis, as well as other components of stress responsivity in the brain, and may increase the reinforcing effects of drugs of abuse. Stressors can influence the rewarding properties of drugs at each of the stages in laboratory animal self-administration studies, including initiation, maintenance, extinction and reinstatement, which are thought to model human states of initiation and maintenance of addictions, withdrawal and relapse. Thus, in general, stress can enhance acquisition, increase resistance to extinction, and induce reinstatement of self-administration.

Animal studies document physiological and corresponding molecular alterations in components of the HPA axis caused by acute or chronic administration of drugs of abuse. For example, administration of cocaine in a 'binge' protocol for 1 or 2 days to rats causes an increase in plasma corticosterone levels, which is significantly attenuated following chronic 14-day binge cocaine administration. Corticotropin releasing factor mRNA in the hypothalamus is also increased following 1 or 2 days of administration but is significantly reduced after 14 days[28].

In a series of clinical studies, recently abstinent cocaine-dependent subjects were read individually tailored scripts designed to provoke stressful, drug-cue related or neutral, relaxing experiences. Stressful and drug-cue related, but not neutral, scripts evoked increased craving, anxiety and cardiovascular measures, as well as increased plasma levels of ACTH, cortisol, prolactin and norepinephrine, not only indicating involvement of the HPA axis, but also suggesting that the sympatho-adreno-medullary system is involved in cocaine craving during abstinence[29].

The endogenous opioid system, specifically μ and κ opioid receptors, demonstrate inhibitory control over the HPA axis (**Fig. 2**). This is apparently tonic inhibition, rather than feedback and circadian inhibition, as is the case with glucocorticoid regulation of the axis[25]. The μ opioid receptor is the primary target of addictive opioid drugs. Mice lacking the μ opioid receptor gene (*OPRM1*) show dramatically reduced or absent analgesia, reward, physical dependence and respiratory depression in response to opiates, such as morphine (reviewed in ref. 2).

Numerous polymorphisms of the *OPRM1* gene have been identified. The most common coding region polymorphism is the A118G SNP with allelic frequencies that vary widely among populations (allele frequencies from 0.01 to 0.48) and results in an asparagine (Asn) to an aspartic acid (Asp) substitution at amino acid position 40, thereby abolishing a putative glycosylation site in the N terminus[2,11,30]. In *in vitro* studies, we found that the endogenous opioid peptide β-endorphin bound the 118G (Asp40) receptor variant with threefold greater affinity than the prototype 118A (Asn40) receptor[30]. Also, β-endorphin binding to the Asp40 receptors showed threefold greater potency in activation of G protein–coupled inwardly rectifying potassium (GIRK) channels, one of the important intracellular signaling systems of this

receptor[30]. No other agonist tested showed differences in binding to, or GIRK activation of, the variant receptors[30].

The *in vitro* findings of changes in responses of the 118G variant μ opioid receptor led us to predict that HPA-mediated stress responsivity may be altered in people expressing the variant[30,31]. Although the molecular or cellular mechanisms have yet to be fully elucidated, these predictions have been borne out in clinical studies in which healthy individuals were administered a μ opioid receptor antagonist, naloxone or naltrexone, which causes immediate activation of the HPA axis by blocking the μ opioid receptor; that is, by disinhibition. Subjects heterozygous for the 118G allele showed a greater HPA response to opioid antagonist than did subjects with the only prototype receptor, as measured by serum ACTH and cortisol levels[2,11]. Additionally, people with the 118G variant receptors had a more favorable clinical response to treatment for alcoholism with the opioid antagonist naltrexone[2,11]. This difference in response to treatment may be mediated by differences in HPA axis activation owing to receptor genotype, as modest activation of this axis is desired by at least some alcoholics[27]. This difference in HPA axis responsivity may be a factor in the possible contribution of this variant to the risk for developing opiate addiction and alcoholism reported in some studies[32,33].

A second gene linking the HPA axis, stress response and addiction is *COMT*, which encodes an enzyme that catalyzes the degradative metabolism of the catecholamine neurotransmitters dopamine, norepinephrine and epinephrine, as well as hydroxylated estrogens. A common guanine-to-adenine transition[34] in exon 4 causes the substitution of methionine for valine at residue 158. The methionine form has greater thermolability and a three- to fourfold lower enzymatic activity than the valine form[11]. Genetic linkage and association studies suggest that this polymorphism may be involved in several different psychiatric disorders. The low-activity methionine form is associated with increased risk for alcoholism in several studies.

Genotype at this polymorphism may influence HPA axis function. After administration of naloxone, subjects with the homozygous Met/Met genotype have greater increases in plasma ACTH and cortisol than do people with one or more high-activity valine alleles (Val/Met or Val/Val)[35]. In this study, all subjects were A/A homozygous for the *OPRM1* A118G SNP, as this polymorphism also affects HPA response to opiate antagonist challenge.

Overall, the activity of the HPA axis seems to undergo extensive plasticity as a result of exposure to drugs of abuse. Furthermore, HPA responsivity is affected by genetic variants. Along with the finding that stress is a precipitating factor in relapse, these results point to the importance of more extensive studies of genetic variants in the HPA axis and drug addiction.

Environmental factors

The expression of a genetic predisposition may be, in part, conditional on exposure to environmental determinants. In twin studies, environmental factors, including family environment, influence the development of alcohol dependence in individuals with a relatively high genetic risk. The influence of family and non-family environmental factors also contribute to abuse of or dependence on other drugs of abuse[4,5]. Among maltreated children, those with the *MAOA* variant that directs high expression levels were not as likely to develop antisocial problems in adulthood as children with the low-expression variants[36]. MAOA metabolizes a variety of neurotransmitters, including serotonin, norepinephrine and dopamine; defects in the *MAOA* gene have been linked to aggression. Although the environment contributes to the development of antisocial traits, in these children, the resultant antisocial behavior was moderated by genetic factors.

Another association study investigated why stressful events may lead to depression in some individuals but not in others[37]. *SERT* has a repeat polymorphism in the promoter region, with the long form of the repeat polymorphism expressing higher levels of *SERT* mRNA. At 26 years of age, people with the long or short form of the *SERT* promoter polymorphism had similar depressive symptoms and episodes, as well as suicidal ideations, if they lacked 'life events' such as employment, relationship or health stressors from age 21 to 25. However, in people who had experienced stressful life events and had two copies of the short *SERT* alleles, depression and suicidal ideation increased at a much higher rate, whereas an intermediate increase was observed in heterozygous subjects. These results suggest that common genetic variants maintained at a high frequency in the population promote resistance to environmental stressors. Furthermore, the lack of replication in many genetic studies may be due to the specific gene-environment interaction that must occur for an effect to be observed. On the other hand, these studies may have to be revisited in light of the recent report of an A → G variant in the long repeat of the *SERT* promoter polymorphism that affects expression[38]. These two specific variants (the *MAOA* and *SERT* promoter polymorphisms) are each associated with alcoholism (**Table 1**). Childhood abuse also increases the risk of developing alcoholism or other drug addiction. These studies point to the critical interaction between specific genetic variants and the environment as leading to associations with addiction.

Genetic factors directly associated with addiction

As noted previously, genetic factors account for 30–60% of the overall variance in the risk for the development of drug addictions, but there may be different influences of environmental or genetic factors at different stages[4–6]. The potential influences of the personality traits of impulsivity and risk-taking, of stress responsivity, and comorbid psychiatric conditions, along with potential gene variants involved in each of these factors, have been discussed above. We will now highlight direct genetic studies of addiction to alcohol, opiates and cocaine and other stimulants. That is, these studies focus on genetic variants and addictive diseases without analyzing the personality traits mentioned above. Many of the genes for which there is evidence of association or linkage are those already discussed as potentially contributing to impulsivity, risk-taking, anxiety, depression and stress-responsivity (**Table 1**).

Linkage studies have been conducted to identify genetic determinants of addictive diseases[2,11,39–41]. The Collaborative Study on the Genetics of Alcoholism (COGA), a multi-center effort to identify genes involved in alcoholism, was an early project[7]. New techniques allow association studies to be done on thousands of genes using microarray technology. A multiple pooling technique with a 1,494-SNP microarray identified 42 chromosomal regions that may be involved in vulnerability to drug abuse in African-Americans and European-Americans. All the affected subjects had polysubstance abuse, including nicotine and alcohol abuse or addiction, so the regions identified may contain genes that are involved in addictions to multiple substances[41]. This study of polysubstance abuse showed that at least 15 large chromosomal regions were shared with regions identified in one or more other linkage studies of alcoholism and nicotine addiction, suggesting that there may be general genetic factors for addiction[40].

Genetic variants may also contribute to opiate addiction. One promising candidate is the μ opioid receptor gene (*OPRM1*). Several individual variants and haplotypes at the *OPRM1* locus are associated with opiate dependence[2,11,31]. The 118G allele of the common functional A118G SNP was associated with heroin addiction in two relatively non-admixed populations, one of Han Chinese and the other in central Sweden[2]. In the latter study, the population-attributable risk for the 118G allele was 21% for Swedish individuals with

two Swedish parents[32]. Some studies of the A118G SNP, as well as of other polymorphisms in this gene, have not identified association or linkage to an addiction with this locus, possibly owing to differences in the genetic makeup of the populations under study, differences in population substructure or the use of different assessment criteria. Another association of the *OPRM1* 118G allele with alcohol dependence has been reported in Swedish individuals from central Sweden, further indicating the importance of ethnic/cultural background[33].

We found an association between a single SNP and also a specific haplotype of variants of the κ opioid receptor gene (*OPRK1*) and opiate addiction[42]. Prodynorphin is the precursor to dynorphin peptides, the endogenous ligands of the κ opioid receptor that can attenuate cocaine-induced increases in perisynaptic dopamine levels in reward-related areas of the brain[1]. We found that a 68-base repeat polymorphism in the promoter of the dynorphin gene was associated with cocaine abuse or dependence and also with cocaine-alcohol dependence[2]. The specific associations of addictive status with μ and κ opioid receptor systems can be viewed in the context of the importance of these two systems in the neurobiology of reinforcement and reward by different drugs of abuse (including opiates and psychostimulants[1]).

Alleles of the *DRD2* gene are associated with alcoholism, cocaine dependence, psychostimulant abuse or polysubstance abuse[2]. Some studies report the *DRD4* gene to be associated with opiate addiction or alcoholism, although these findings have not always been replicated[12].

The high-activity Val158 allele of the *COMT* gene V158M polymorphism is associated with polysubstance abuse[43], with alcoholism[11] and, in a family-based haplotype relative-risk study, with heroin addiction[44]. Functional magnetic resonance imaging shows that individuals with the high activity valine/valine genotype of the *COMT* gene have enhanced prefrontal cortex function when given amphetamine during a working memory task, whereas amphetamine caused deterioration of cortical efficiency in individuals with the methionine/methionine genotype (see ref. 11 for review). Alleles of the *DRD4* and *COMT* genes also interact with methamphetamine abuse[45].

Cocaine-induced psychosis is associated with a potentially functional variable nucleotide tandem repeat in the 3′ untranslated region of *DAT* (reviewed in ref. 2). Variants of this gene have also been associated with amphetamine-induced psychosis[2] and with alcoholism[11]. A functional polymorphism in the promoter region of *DBH* that causes lower plasma dopamine β-hydroxylase activity is associated with cocaine-induced paranoia[46].

Two studies reported an association of heroin dependence with polymorphisms in *SERT*, but this finding was not replicated in other studies (reviewed in ref. 2). Variants in *SERT*, *TPH2* and *MAOA* and genes encoding serotonin receptors 5-HT_{1B} and 5-HT_{2A} have all been associated with alcoholism[11]. Alcohol dependence is associated with variants of the *GABRA2* gene, which codes for the α2 subunit of $GABA_A$; this gene is located in a region of chromosome 4p, which is linked and associated with alcoholism[8].

The endogenous cannabinoid system is also implicated in genetic studies of addictions. A trinucleotide repeat polymorphism in the 3′ flanking region of the cannabinoid receptor 1 (*CNR1*) gene is associated with intravenous drug abuse (cocaine, amphetamine or heroin)[47]. A synonymous coding region SNP in the 3′ untranslated region is associated with symptoms of delirium in alcohol withdrawal[48]. A study of 22 polymorphisms in *CNR1* identified a haplotype in an intronic 5′ region of the gene that is associated with substance (cocaine, opiate, alcohol or other drug) abuse[49]. Fatty amide acid hydrolase, encoded by the *FAAH* gene, is an enzyme that metabolizes endogenous ligands of the cannabinoid receptors. A functional SNP that alters the sensitivity of the enzyme to protease *in vitro* is associated with drug and alcohol abuse[50].

A pharmacokinetic gene product, the cytochrome CYP450 gene *CYP2D6*, has been studied in codeine dependence. This enzyme biotransforms codeine and several of its congeners into metabolites with greater opioid potency. The *CYP2D6* gene is highly polymorphic, resulting in large differences in enzyme activity[11].

As detailed above, variants of genes involved in specific neurotransmitter systems are implicated in vulnerability to alcoholism; genes involved in biotransformation or degradation of alcohol are also implicated[11]. The alcohol-metabolizing enzymes alcohol dehydrogenase (*ADH1B* and *ADH1C*), and aldehyde dehydrogenase (*ALDH*) genes have variants that are protective against alcoholism[11]. The reports of associations of these alcohol-metabolizing gene variants with protection from alcoholism are diverse, robust and exhaustively reviewed elsewhere[11].

Some studies in **Table 1** were primarily designed to test for associations between genetic variation and addictive diseases. Other studies focus on the association of genetic variants with personality traits such as impulsivity and risk taking. Many of these variants are associated in independent studies with both addictive diseases and personality traits (for example, *SERT*, *TPH1*, *TPH2*, *COMT*). One major focus for the future could be integrated studies on the role of personality trait variants in addictive diseases.

Summary and conclusions

Addiction is a complex disorder with interacting factors, including environmental factors, drug-induced neurobiological changes, comorbidity, personality traits and stress responsivity. Clearly, multiple genetic variants that affect these factors may work in concert to affect vulnerability and severity of addiction. As a concrete example, a functional SNP in the *OPRM1* gene (A118G) influences the μ opioid receptor, as defined by molecular and cellular studies and in human studies, and results in clinically observable changes in stress responsivity, vulnerability to opiate addiction and alcoholism in defined populations, as well as in response to a specific addiction pharmacotherapy.

The advent of more modern technologies, such as SNP microarrays, enhances our capacity to study genetic influence on the addictive diseases. Several important challenges remain for the near future; in particular, the refinement of phenotyping in the addictive diseases, which may focus on clinically relevant aspects of this disorder, such as age of initiation, speed of progression to regular drug use, severity of dependence or withdrawal, vulnerability to relapse, and response to specific pharmacotherapeutic treatments. Molecular resequencing of new and previously studied genes is of critical value in the discovery of genetic variants of potential interest. A relative standardization across laboratories in phenotyping and statistical approaches (and the sharing of these data) is desirable to assess more directly replicability and generalization across different populations.

Without such relative standardization, meta-analyses of studies using highly disparate methodologies are difficult. Meta-analyses focus on particular questions (such as an association between a genetic variant and a personality trait or an addiction) and combine results from multiple studies into a coherent summary. Analyses are based on individual or aggregate patient data, with the former being the preferred type, although the use of the latter is more common. Phenotypic assessments, ethnic/cultural group studies and statistical methods used must be similar to decrease heterogeneity in the combined data. Hence, the results of meta-analyses of apparently similar studies may not be directly compared, and meta-analyses of disparate studies may be misleading.

Additional information, including references for additional reading, is available in the **Supplementary Note** online.

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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