

## New insights into the genetics of addiction

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**Abstract** | Drug addiction is a common brain disorder that is extremely costly to the individual and to society. Genetics contributes significantly to vulnerability to this disorder, but identification of susceptibility genes has been slow. Recent genome-wide linkage and association studies have implicated several regions and genes in addiction to various substances, including alcohol and, more recently, tobacco. Current efforts aim not only to replicate these findings in independent samples but also to determine the functional mechanisms of these genes and variants.

Addictions are chronic, often relapsing disorders characterized by obsession, compulsion, or physical or psychological dependence. The World Health Organization estimates that there are 2 billion alcohol users, 1.3 billion tobacco users and 185 million illicit drug users worldwide. Twin and family studies provide strong evidence that addictions involve the interplay of genetic and environmental factors<sup>1–5</sup>. Greater knowledge of the genetics underlying addiction therefore is crucial for the development of more effective interventions. For example, after attempting to quit, smokers of European ancestry with the *CYP2B6*\*6 genotype in the cytochrome p450 gene are more likely to relapse than smokers of other genotypes when on placebo, but they can be helped by bupropion treatment<sup>6</sup>.

In recent years, significant progress has been made in identifying susceptibility genes for addictions. Given that most of this progress, which has been focused primarily on addiction to single substances, has been covered in other reviews<sup>1–5,7</sup>, we focus here on the most recent advances made through the use of linkage and association approaches, especially with genome-wide association (GWA) studies; our goal is to provide an updated summary of identified susceptibility loci or genes for multiple substances. Regions on human chromosomes 4, 5, 9–11 and 17 are more likely to harbour susceptibility genes for multiple substances.

Among the susceptibility genes for addiction identified from candidate gene-based or GWA approaches, we limit our focus to genes in the aldehyde dehydrogenase (ADH) gene cluster, and the genes encoding nicotinic acetylcholine receptor (nAChR) subunits, gamma-aminobutyric acid A (GABA<sub>A</sub>) receptor subunit 2 (GABRA2), ankyrin repeat and kinase domain containing 1 (ANKK1) and neurexins. This is because of the influence of these genes on susceptibility to addiction to multiple substances, the strong statistical evidence to support their roles, or the importance of their biological functions in addiction.

We first focus on evidence for the contribution of genetics to addiction, then on the recently identified loci and genes that confer susceptibility to multiple substances, and finally on the knowledge gained from engineered mice.

### Genetic contribution to addiction

Epidemiological studies strongly suggest that genetic factors operate at all steps of addiction, including vulnerability to initiation, continued use and propensity to become dependent (BOX 1). There have been many twin studies investigating the relative contributions of genetic and environmental factors. In addition to estimating genetic liability, twin studies provide information about environmental contributions, including the availability of and exposure to a

substance, and shared and unique environments. The genetic influence on addiction has proved to be substantial, and heritabilities for most addictive disorders are moderate to high.

Numerous large twin studies for alcohol-related behaviours have consistently shown that heritability of alcohol abuse and dependence ranges from 50% to 70%<sup>8</sup>. A similarly high heritability is seen across other alcohol-related behaviours, including heavy consumption and 'problem' drinking<sup>8</sup>. Meta-analysis of the twin studies shows that both genes and environment are important in smoking-related behaviours, with an estimated mean heritability of 0.50 for smoking initiation and 0.59 for nicotine dependence (ND)<sup>4</sup>. In women, genetic factors have a larger role in initiation than in persistence, whereas the opposite is observed in men<sup>4,9</sup>.

Fewer studies have examined genetic influences on illicit drug addiction. Heritability of the use and/or dependence on stimulants, sedatives and heroin in males is 0.33, 0.27 and 0.54, respectively<sup>10</sup>. These values are similar to those seen in females, although such studies are rarer<sup>11</sup>. Moreover, twin studies indicate a large overlap between the genetic predispositions to dependence to most classes of substance. For example, twin studies suggest that nicotine and alcohol dependencies share more than 60% of their genetic vulnerabilities<sup>12</sup>.

Finally, we point out that heritability is specific to the sample under study. Thus, the role of genetic influences may differ across samples, and heritability may be affected by many factors, such as sex, age, education, socioeconomic status and cultural background.

### Identifying susceptibility loci for addiction

Both linkage mapping and association mapping have identified susceptibility loci for addiction-related phenotypes, especially for alcohol dependence (AD) and ND. However, few putative genome linkages have been replicated in independent studies, probably because of genetic heterogeneity.

Despite these limitations, significant progress has been made, especially for AD and ND. Linkage studies have identified a locus on chromosome 4q near the ADH

gene cluster as a major locus for alcoholism in American Indians and Caucasians<sup>13–15</sup>, in addition to the known *ADH2* and aldehyde dehydrogenase 2 (*ALDH2*) variants in Asians and Jewish Americans<sup>16–18</sup>. The ADH locus contains a cluster of seven genes, of which *ADH2* is the most important across populations<sup>19</sup>, although functional variants in *ADH4* and *ADH7* might also be involved<sup>16,20,21</sup>.

One of the most successful examples of the positional candidate gene approach in addiction research has been the identification of *GABRA2* as a susceptibility factor for alcoholism. Early linkage studies in Southwest American Indians<sup>13</sup> implicated chromosome 4p near the *GABRA2*–*GABRB1* cluster; these results were confirmed in some Caucasian studies<sup>22</sup>,

and became particularly convincing when linkage of alcohol-related phenotypes was combined with QTL mapping of electroencephalographic quantitative trait data<sup>23</sup>. Initial association findings for *GABRA2* (REF. 24) in that linkage region were replicated in multiple studies<sup>25–27</sup>, so that *GABRA2* is now considered a confirmed risk locus for AD.

Similarly, variants in *GABBR2* (on chromosome 9q22), which encodes GABA<sub>B</sub> receptor subunit 2, have been associated with ND<sup>28</sup>. Linkage of chromosome 9 with smoking behaviour has been reported in several independent studies<sup>29–32</sup>, and *GABBR2* accounts for 28%–38% of this linkage signal<sup>33</sup>. The involvement of GABA signalling across addictions has been confirmed in animal models (see later section).

Although many linkage scans have been reported for addiction to a specific drug, only a few have investigated the convergence of linkage peaks across drug addictions. Towards this objective, we searched for linkage peaks among multiple abused substances, according to the criteria proposed by Lander and Kruglyak<sup>34</sup>. We found that regions on chromosomes 2–5, 7, 9–11, 13, 14 and 17 have independent evidence of ‘suggestive’ or ‘significant’ linkage, with regions on chromosomes 4, 5, 9, 10, 11 and 17 receiving the strongest support for harbouring susceptibility genes for addictions to multiple drugs (FIG. 1).

Although many candidate genes have been investigated for associations with addiction, only a few findings have been replicated in independent studies for multiple addictions. Instead of giving a detailed review of these candidates individually, our discussion focuses on representative genes that have recently received much attention or are associated with addictions to multiple substances.

## Susceptibility genes for substance addiction

In the past 2 years, the most significant findings in addiction genetics have been the involvement of a few nAChR subunit genes specifically in ND and more generally in substance abuse. As illustrated in BOX 2, nicotine exerts its biological function by binding to nAChRs, which are composed of five subunits. There are 12 nAChR subunit genes: nine alpha ( $\alpha$ 2– $\alpha$ 10) and three beta ( $\beta$ 2– $\beta$ 4). Variants have been associated with dependence on nicotine, alcohol and cocaine, and with lung cancer susceptibility, as explained below.

**nAChR $\alpha$ 4 subunit gene.** A significant association of variants in *CHRNA4*, which encodes the  $\alpha$ 4 subunit, with ND has been detected in three independent studies. The first study revealed significant protective effects of two SNPs against ND in Chinese men<sup>35</sup>. A second study showed that four SNPs, and haplotypes that consist of these SNPs, are significantly associated with ND in European and African Americans, but the effects differed as a function of ethnicity or sex<sup>36</sup>. A third study reported that two SNPs are significantly associated with the subjective response to smoking<sup>37</sup>. Although not all of these associations have been replicated, two SNPs (rs1044396 and rs2236196) are consistent in two of the three studies. Together, these studies provide evidence that variations in *CHRNA4* influence ND.

### Box 1 | Definition of addiction and challenges in addiction genetics research

#### Diagnostic assessment of addiction: nicotine dependence as an example

Drug addiction is characterized by a compulsion to take a substance, with goal-directed behaviour towards excessive substance intake and a loss of control in limiting intake. Clinical addictions generally are diagnosed using the Diagnostic and Statistical Manual IV (DSM-IV)<sup>38</sup> and the International Classification of Disease, Tenth Revision (ICD-10) from the World Health Organization (WHO)<sup>37</sup>. The most recent versions of DSM-IV and ICD-10 recognize two categories of addiction: abuse or harmful use, which is characterized by regular and increasing intake despite adverse consequences; and dependence, which in addition requires symptoms of withdrawal and/or tolerance.

Although there is significant co-morbidity among addiction to different drugs, the measurement of various addictions differs greatly. Here, we use nicotine dependence (ND) to illustrate the complexity of this behaviour. Historically, the assessment of ND has relied largely on the use of the Fagerström Tolerance Questionnaire<sup>38</sup> or a shorter version, the FTND<sup>39</sup>. Since these two scales were introduced, they have been used frequently in both clinical and research settings, partly because of their relationship to treatment outcome. However, the Fagerström scales were developed as unidimensional measures of physical tolerance<sup>38</sup> and thus do not assess some important aspects of ND, such as craving, subjective compulsion to smoke, nicotine withdrawal symptoms, behavioural saliency or behavioural automaticity, which often are regarded as core constructs of ND<sup>40</sup>.

ND also can be assessed by diagnostic criteria based on the DSM-IV<sup>38</sup>. However, these symptoms are ultimately aggregated into a dichotomous classification as ‘dependent’ or ‘not dependent’, which limits its usefulness for research into the causes of addiction. Given that a diagnosis of dependence can be assigned by meeting various combinations of criteria, the diagnosis is conceptually heterogeneous. To expand the breadth of the diagnosis criteria and thereby improve the self-reported assessment of ND, new scales such as the Wisconsin Inventory of Smoking Dependence Motives<sup>41</sup> and the Nicotine Dependence Syndrome Scale<sup>42</sup> were developed. Whether these new scales can capture different aspects underlying ND and reduce the phenotypic heterogeneity among smokers remains to be investigated, especially in non-Caucasian smokers.

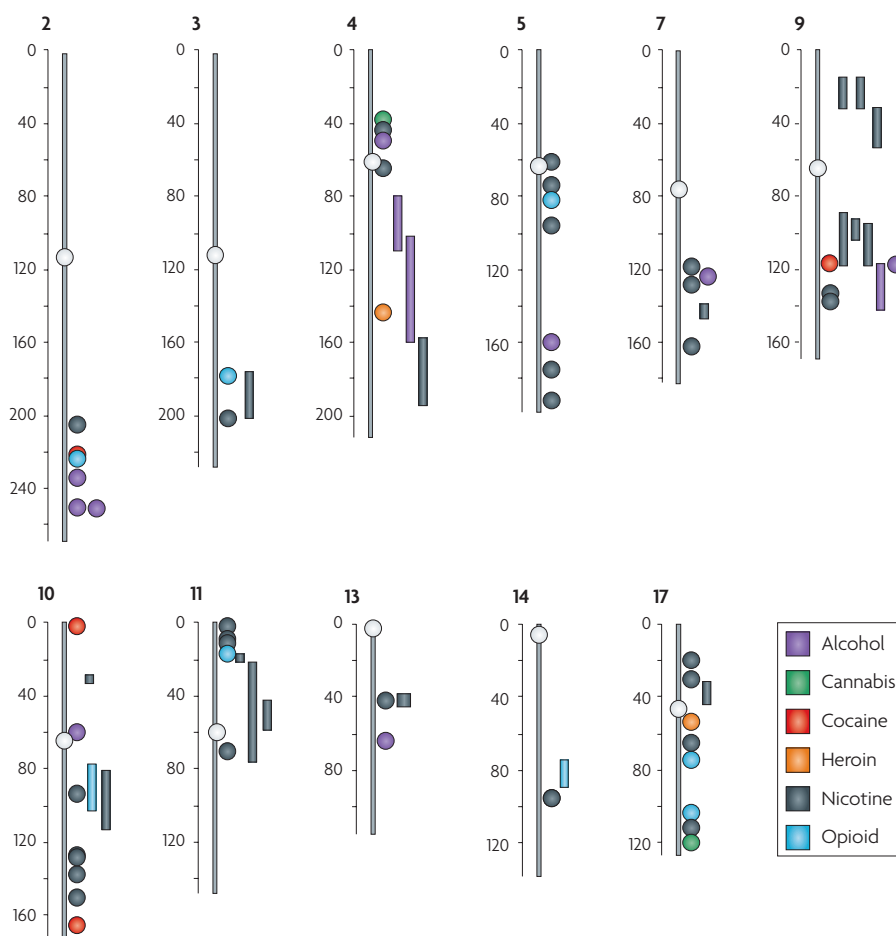
#### Genetic and phenotypic heterogeneity

One of the most significant challenges for genetic research into addiction concerns heterogeneity at both the phenotypic and genetic levels. Phenotypic heterogeneity is extensive in the manifestation of addiction, with users differing in the magnitude of numerous factors, such as age of onset of problems, drug symptoms, abusing history and co-morbid disorders. One means to reduce phenotypic heterogeneity is to classify more genetically or neurobiologically homogeneous phenotypes or to use endophenotypes as an intermediate phenotype between an addicted phenotype and the biological processes responsible for the manifestation of that disorder. Genetic heterogeneity is another important concern in genetic studies on addiction. Genetic differences exist between different ethnic groups or samples from different geographic locations. One way to minimize the effect on final genetics findings is to increase sample size and use more homogeneous samples.

**Association and interaction analysis of *CHRNA5*.** By contrast, no significant association of *CHRNA5*, which encodes the  $\alpha 5$  subunit, with ND was reported in four independent studies (for example, see REFS 35,36), except for a significant association of rs2072661, found in the 3'UTR of the gene, with ability to quit smoking<sup>38</sup>. However, epistatic analysis using a generalized multifactor dimensionality reduction method<sup>39</sup> revealed that *CHRNA5* has a significant effect on ND when analysed together with *CHRNA4* (REF. 40). The failure to detect significant association of *CHRNA5* with ND by itself may thus be attributable to the small effect of *CHRNA5* or the strong dependency of *CHRNA5* effects on specific *CHRNA4* variants. More importantly, detection of significant interaction of *CHRNA5* with *CHRNA4* in humans is consistent with the formation of functional nAChRs containing these two subunits ( $\alpha 5\beta 2$  nAChRs)<sup>41,42</sup>. A moderate association of *CHRNA5* with early response to alcoholism has also been reported<sup>43</sup>.

The recent development of selective partial agonists of  $\alpha 5\beta 2$  nAChRs as effective smoking cessation agents further supports the role of  $\alpha 5\beta 2$  nAChRs in ND. For example, the partial  $\alpha 5\beta 2$  nAChR agonist varenicline was approved by the US Food and Drug Administration as an aid to smoking cessation. It produces significantly higher quit rates (2–3 times) than other treatments<sup>44</sup>. Varenicline can selectively decrease ethanol consumption and seeking in animals, implying it may be valuable also for treating alcoholism<sup>45</sup>.

**Association of *CHRNA5*, A3 and B4 with ND and lung cancer.** Three recent GWA studies suggested a strong association between lung cancer and variants of *CHRNA5*, *CHRNA3* and *CHRNA4* (encoding subunits  $\alpha 5$ ,  $\alpha 3$  and  $\beta 4$ , respectively) on chromosome 15q24 (REFS 46–48). However, although one study revealed a significant association of a *CHRNA3* SNP with ND<sup>48</sup>, the other two studies suggested association of this and other variants with lung cancer independent of their association with smoking<sup>46,47</sup>. Several other studies provide independent verification of the association between this gene cluster and ND<sup>49–51</sup>, and suggest that power, precision of phenotyping or severity may explain the apparent association with lung cancer rather than a direct, smoking-independent association. We feel that a biological link between variants in the *CHRNA5–A3–B4* cluster and ND is more plausible than a direct effect of these variants on lung cancer.



**Figure 1 | Chromosomal locations of peaks or intervals for addiction to multiple substances.** Summary of chromosomal locations of nominated peaks or intervals for addictions to alcohol, cannabis, cocaine, heroin, nicotine and opioids. Each linkage is shown with either a colour-filled circle or a rectangle, representing a reported linkage peak or region, respectively. 'Significant' or 'suggestive' linkage was determined by independent studies on at least two substances of abuse. The determination of significant or suggestive linkage at each linkage peak or interval was based on the rigorous criteria proposed by Lander and Kruglyak<sup>34</sup>, which define a logarithm of odds (LOD) score greater than 3.6 or a *p* value less than  $2.2 \times 10^{-5}$  as a significant linkage, and an LOD score greater than 2.2 but less than 3.6 or a *p* value less than  $7.4 \times 10^{-4}$  as a suggestive linkage. Each colour represents a type of abused substance. The unit for each chromosome is cM.

**Association of *CHRNA5*, A3 and B4 with alcohol and cocaine dependence.** Association of the *CHRNA5–A3–B4* gene cluster with alcohol and cocaine addictions has been reported. Wang *et al.*<sup>52</sup> identified a group of SNPs across the *CHRNA5–A3* locus associated with AD. Because these variants are in low linkage disequilibrium (LD) with the SNPs associated with ND and lung cancer, the authors concluded that *CHRNA5–A3* variants are associated with AD, but through different SNPs and mechanisms from those identified in ND and lung cancer. Moreover, Grucza *et al.*<sup>53</sup> found that a non-synonymous polymorphism in *CHRNA5* has the opposite effect on cocaine dependence (CD) than the effect associated

with ND. Such contradictions in allelic associations are also found in other psychiatric disorders<sup>54</sup>. It remains to be seen whether there is true heterogeneity of association (in this case, between ND and CD), or whether some findings will turn out to be false positives.

**Future research on the *CHRNA5–A3–B4* cluster.** Further steps are required to characterize the *CHRNA5–A3–B4* cluster as a susceptibility locus for addictions and lung cancer. First, it is important to determine which variant (or variants) cause such significant associations. Resequencing might identify additional variants that explain the complex patterns, but functional

analysis also will be necessary. The non-synonymous variant rs16969968 causes the substitution of a negatively charged residue (Asp398) with a residue with no charge (Asn). This substitution is in the M3–M4 intracellular loop of *CHRNA5* (BOX 2), a region that is thought to be involved with receptor trafficking. Indeed, the variant form of *CHRNA5* alters receptor function without affecting expression<sup>55</sup>. Second, it is important to determine whether the *CHRNA5*–*A3*–*B4* cluster also has a significant role in non-European populations. Third, more research is needed to determine which smoking-related behaviour drives the association with this cluster. For example, ND was measured by smoking

quantity in two studies<sup>48,50</sup>, whereas another focused on searching for susceptibility genes underlying the transition from regular non-dependent to dependent smokers<sup>49</sup>. Weiss *et al.*<sup>51</sup> found a significant association of the cluster with ND only in early onset smokers. Fourth, other genes in the region, such as the hypothetical locus *LOC123688*, cannot be excluded because of the association with other SNPs in this cluster<sup>47</sup>. However, this issue may be difficult to solve genetically because of the extensive LD structure.

**Associations of other genes with addictions.** Although many candidate genes have been associated with addiction, few findings have

been replicated (see TABLE 1 for verified and [Supplementary information S1](#) (table) for non-verified genes associated with multiple substances, and [Supplementary information S2](#) (table) for genes associated with only one substance). Given the controversial association reports for many genes, we focus on genes that have not only received strong statistical support for association with multiple substances of abuse but that also have greater biological support.

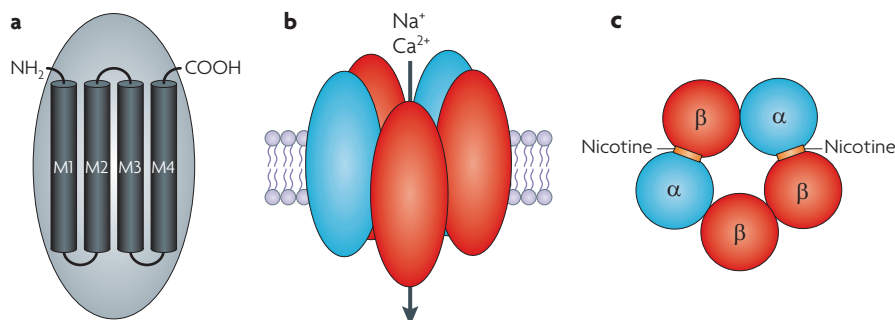
Variants in *GABRA2* are strongly associated with alcoholism<sup>24–27</sup>, but an association has been reported between *GABRA2* variants and addiction to nicotine and polysubstance abuse<sup>56,57</sup>. Furthermore, a linkage study has implicated the region near *GABRA2* in the aetiology of cannabis use<sup>58</sup>. Variants in *ANKK1*, encoding a protein kinase involved in signal transduction<sup>59</sup>, have also been associated with susceptibility to ND<sup>60,61</sup>, AD<sup>62,63</sup>, and co-morbid alcohol and drug dependence<sup>64</sup>. Finally, several GWA studies provide evidence for the involvement of cell adhesion-related genes in vulnerability to addiction and other psychiatric disorders<sup>65</sup>. Representative members of this category include neurexin 1 for ND<sup>66,67</sup> and neurexin 3 for polysubstance<sup>65</sup>, alcohol<sup>68</sup> and opioid abuse<sup>69</sup>. Rare mutations in several members of this gene family, including neurexin 1, also have been associated with autism<sup>34,70</sup> and schizophrenia<sup>71</sup>, implying that the members of this family are important in many psychiatric disorders.

## Murine behavioural studies

**Knockout (KO) models of nAChR subunit genes.** Genes encoding nAChR subunits mediate variability in many of the behavioural effects of nicotine. KO mice for nAChR subunits  $\alpha 3$ – $\alpha 9$  and  $\beta 2$ – $\beta 4$  have shown that the deletion of these genes alters many effects of nicotine. Unlike wild-type animals, mice lacking the  $\alpha 4$  and  $\beta 2$  subunits do not self-administer nicotine and do not show nicotine-induced increases of dopamine levels in the ventral stratum<sup>72,73</sup>. In addition,  $\beta 2$  KO mice self-administer cocaine but fail to maintain self-administration when cocaine is switched to nicotine<sup>72</sup>, and  $\alpha 4$  KO mice exhibit a prolonged motor response to cocaine<sup>73</sup>. Together, the findings from  $\alpha 4$  and  $\beta 2$  KO mice provide evidence that  $\alpha 4\beta 2^*$  (\* indicates another undetermined subunit) nAChRs are required for proper regulation of dopamine release and for maintenance of self-administration and reinforcement.

KO mice of  $\alpha 5$  (REF. 74) and  $\beta 4$  (REF. 75) subunits, as well as  $\alpha 3$  KO heterozygous

### Box 2 | Composition and function of nAChRs in the brain



Neuronal nicotinic acetylcholine receptors (nAChRs) belong to a superfamily of ligand-gated ion channels that also includes receptors for gamma-aminobutyric acid (GABA), glutamate, glycine and serotonin. nAChRs are pentameric complexes assembled from an extensive family of subunits. In vertebrates, 12 nAChR subunit genes with a common ancestor have been cloned and classified into two subfamilies of nine alpha ( $\alpha 2$ – $\alpha 10$ ) and three beta ( $\beta 2$ – $\beta 4$ ) subunits. There is considerable subunit diversity among nAChR subtypes and, consequently, receptor subtypes are commonly referred to by their subunit composition.

nAChRs are widely distributed in the brain, and their subunit composition is cell and region specific. Most nAChRs in the nervous system contain one type of  $\alpha$  and one type of  $\beta$  subunit, with  $\alpha 4\beta 2$  receptors accounting for 90% of the high-affinity nAChRs in the brain;  $\alpha 3\beta 4$  receptors are the main subtype in the autonomic ganglia and adrenal medulla and in subsets of central nervous system neurons. The  $\alpha 4\beta 2$  and  $\alpha 3\beta 4$  subtypes also can contain the  $\alpha 5$  subunit, which is believed to increase the rate of channel desensitization and calcium permeability.

Because nAChRs are the primary site of action for nicotine in the brain, they are important targets for the development of therapeutic agents that facilitate smoking cessation efforts. nAChRs are composed of five subunits that combine to form a functional receptor (see the figure, part a). Each subunit comprises four membrane-spanning domains, which are arranged in concentric layers around a central aqueous pore with the M2 domains lining the pore. Following nAChR activation by an endogenous agonist (acetylcholine, for example) or exogenous agonist (such as nicotine), the domains of each of the five nAChR subunits rearrange such that the central pore opens and permits cationic trafficking (see the figure, part b). Nicotine binds at the interface between the  $\alpha$  and  $\beta$  subunits in heteromeric nAChRs to activate the receptor (see the figure, part c). Activation of nAChRs can potentiate neurotransmitter release (when expressed at presynaptic terminals) and neuronal excitability (when expressed at postsynaptic terminals) throughout the brain. As a result, nAChRs contribute to a wide range of brain activities, including cognitive functions, and neuronal development and degeneration. Nicotine dependence is initiated through the activation of nAChRs. Chronic nicotine exposure produces the long-lasting physiological and behavioural changes associated with addiction, including nAChR upregulation and gene expression alteration, and long-term potentiation and depression induction at glutamatergic synapses.



Table 1 | **Candidate genes associated with at least one drug addiction that are verified by meta-analysis**

Gene symbol	Gene name	Biological function	Chromosomal location	Drug (phenotype)	Evidence from knockout animal model	References for meta-analysis
5HTT (also known as SERT)	5-hydroxytryptamine transporter	Neurotransmitter transport	17q11.1–q12	Alcohol (i, d, c), cocaine (d, c), heroin (d), methamphetamine (d), nicotine (d)	Increased sensitivity to alcohol-induced sedation and hypnosis; motor-coordination deficits in response to alcohol; reduced gross alcohol intake; altered behavioural responses to cocaine and alcohol	93,94
CYP2A6	Cytochrome P450, family 2, subfamily A, polypeptide 6	Oxidation reduction	19q13.2	Alcohol (d), nicotine (i, d, c)	None	94
DAT1	Dopamine transporter	Neurotransmitter transport	5p15.3	Alcohol (d, c), cocaine (d), heroin (d), methamphetamine (d), nicotine (i, d, c)	Reduced alcohol preference in female mice; cocaine-induced stereotypy (repetitive behaviour)	94,95
DRD2	Dopamine receptor 2	Synaptic transmission, dopaminergic	11q23.1–q23.2	Alcohol (d, c), cocaine (d), heroin (d), nicotine (i, d, c)	Alcohol preference and alcohol-induced ataxia; reduced rate of high-dose self-administration of cocaine	96–99
IL10	Interleukin-10	Cytokine activity	1q31–q32	Alcohol (d)	None	100
BDNF	Brain-derived neurotrophic factor	Regulation of synaptic plasticity	11p13	Alcohol (i, d, c), nicotine (d), cocaine (d), methamphetamine (d)	Increased alcohol intake; increased preference for cocaine	101

The genes reported here have one or more variants that have been associated with more than one addiction. This table may only represent a partial list of genes that have been investigated for association with addictions. c, cessation or withdrawal; d, abuse or dependence; i, initiation.

mice<sup>76</sup>, show that each of the three genes in a cluster on chromosome 15 mediates similar effects of nicotine; that is, the animals show resistance to nicotine-induced seizures, and the  $\alpha 5$  and  $\alpha 3$  subunits mediate both the locomotion-suppressant effects of nicotine and the somatic signs of nicotine withdrawal.

**Knockin (KI) model of nAChR subunit  $\alpha 4$  in nicotine addiction.** To better understand the roles of  $\alpha 4$ -containing nAChRs, a KI approach was adopted with the goal of

making a hypersensitive nAChR that might generate more noticeable phenotypes. One of the most successful examples of this approach was the generation of two lines of  $\alpha 4$  KI mice by introducing a point mutation into the M2 transmembrane region of the  $\alpha 4$  subunit<sup>77,78</sup>. In Leu9/Ser KI mice, this mutation produces  $\alpha 4$ -containing nAChRs with an approximately 30-fold increase in sensitivity to acetylcholine and nicotine. Heterozygous KI mice exhibit greater anxiety, impaired motor learning, excessive ambulation that is eliminated by

small amounts of nicotine, and reduction of dopaminergic function on ageing<sup>78</sup>. By contrast, both homozygous and heterozygous Leu9/Ala KI mice are exceptionally sensitive to nicotine and ND-related behaviours, including reward, tolerance and sensitization<sup>77</sup>. These findings suggest that genetic variability in the  $\alpha 4$  subunit can produce dramatic changes in nicotine sensitivity, implying that a variant (or variants) in the human *CHRNA4* gene alters sensitivity to nicotine and vulnerability to ND.

## Glossary

### Behavioural automaticity

Control of behaviour by external stimuli and events in the immediate environment, often without knowledge or awareness of such control.

### Behavioural saliency

In the context of this article, the likelihood and the degree to which a stimulus elicits a reaction or response.

### Depression induction

A procedure that induces a state of depression.

### Endophenotype

In psychiatry, a biomarker for a behavioural symptom that has a clear genetic connection.

### Genetic heterogeneity

Causation of a disorder or trait by different genetic variants in different samples. This can arise when

participants of different ethnic origins are included, genetic effects or samples are small, and marker density is low.

### Haplotype

A combination of the alleles at different loci on the same chromosome.

### Heritability

The proportion of phenotypic variance that can be attributed to variance of the genotype.

### Linkage mapping

Linkage refers to the tendency of two close loci on the same chromosome to co-segregate within a pedigree. Genome-wide linkage scanning can identify loci that are involved in conditions for which there is no *a priori* reason to suspect any contribution.

### Long-term potentiation

The long-lasting improvement in communication between two neurons that results from stimulating them simultaneously.

### Multifactor dimensionality reduction method

A data reduction approach for detecting combinations of attributes or independent variables that interact to influence a binary outcome.

### Positional candidate gene approach

A candidate gene-based association study that focuses on a genome region identified from linkage analysis.

### QTL mapping

The statistical study of the genetic loci that contribute to variations in a quantitative trait or phenotype.

**Mouse models of GABA genes in alcohol and other addictions.** Rodent models of addiction have been studied for decades<sup>79</sup>. QTL mapping of alcohol withdrawal identified several loci reproducibly: for example, one replicated locus involves non-synonymous variants in *GABRA2* (REF. 80). Deletion of GABA<sub>A</sub> receptor  $\alpha 1$  or  $\beta 2$  subunits leads to reduced loss of the righting reflex (which tends to bring the body into a normal position) in response to alcohol, as well as reduced consequences in response to other drugs<sup>81</sup>. Conversely, sensitivity to alcohol can be manipulated by overexpression or reduction of the activity of the GABA transporter<sup>82</sup>.

Overall, many other mutants, including KI, KO and transgenic mice of various GABA subunit genes, ABA in mediating the behavioural consequences of alcohol<sup>79</sup> and other drugs<sup>83</sup>. The specific genes and alleles may differ from those associated in humans but, overall, animal models have confirmed and extended findings of the involvement of GABA in AD and other addictions<sup>79,83</sup>. The molecular mechanisms of the effects of GABA are being established by microarray analysis<sup>79,84</sup>.

## Conclusions

Significant progress has been made in identifying susceptibility loci and genes for addictions. Comparison of linkage peaks for addictions to various substances confirms what we have learned from genetic epidemiological studies, namely that genetic vulnerability to different substances in part overlaps. Variants in several genes, including aldehyde dehydrogenases, *GABRA2*, *ANKK1*, and *neurexins 1 and 3*, have been associated with addictions to multiple drugs. Most recently, variants of the *CHRNA5-A3-B4* cluster on chromosome 15 were found by GWA and targeted studies to be associated not only with addictions to tobacco, alcohol and cocaine, but also with lung cancer. These genes have effects on nicotine responses in animal models and can thus be considered established targets for these studies. Nevertheless, the exact nature of the variants and their functions are still unknown. Functional studies as well as studies in other ethnic populations with different LD structures may be revealing. Confirmation of candidate gene findings by GWA studies is reassuring; this is similar to the robust findings in type II diabetes<sup>85</sup> but lies in sharp contrast to GWA results in other psychiatric disorders, which so far have failed to replicate any candidate findings<sup>54</sup>. Such candidate genes with prior biochemical

evidence and animal models allow a quicker move to functional studies. Well-designed GWA studies with large samples should help to identify additional genes, including genes that implicate novel biological pathways involved in addiction.

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## DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>  
 ADH2 | ADH4 | ADH7 | ALDH2 | ANKK1 | CHRNA3 | CHRNA4 | CHRNA5 | CHRNA2 | CHRNA4 | GABBR2 | GABRA2

## FURTHER INFORMATION

Margit Burmeister's homepage: <http://www.mbn.med.umich.edu/mbni/faculty/burmeister/burmeister.html>  
 Ming D. Li's homepage: <http://www.healthsystem.virginia.edu/internet/addiction-genomics/People/ml2km.cfm>

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