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Genetics of GABAergic signaling in nicotine and alcohol dependence

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Abstract

Both nicotine and alcohol addictions are common chronic brain disorders that are of great concern to individuals and society. Although genetics contributes significantly to these disorders, the susceptibility genes and variants underlying them remain largely unknown. Many years of genome-wide linkage and association studies have implicated a number of genes and pathways in the etiology of nicotine and alcohol addictions. In this communication, we focus on current evidence, primarily from human genetic studies, supporting the involvement of genes and variants in the GABAergic signaling system in the etiology of nicotine dependence and alcoholism based on linkage, association, and gene-by-gene interaction studies. Current efforts aim not only to replicate these findings in independent samples, but also to identify which variant contributes to the detected associations and through what molecular mechanisms.

Introduction

Drug addiction is a serious public health concern. According to the World Health Organization (WHO 2002), there are an estimated 2 billion heavy alcohol users, 1.3 billion tobacco users, and 185 million illicit drug users worldwide. There is considerable evidence from family, twin, and adoption studies for the operation of genetic factors in the vulnerability to addiction and that genetics contributes substantially to inter-individual vulnerabilities, with an estimated moderate-to-high heritability for both nicotine dependence (ND) and alcoholism (Goldman et al. 2005; Ho et al. 2010; Li and Burmeister 2009). Estimates of the heritability of alcohol abuse and dependence range from 50 to 70%. Additional studies have revealed a similar high level of heritability across other alcohol-related behaviors, including heavy consumption and “problem” drinking (Gelernter and Kranzler 2009; Goldman et al. 2005; Ho et al. 2010). Similarly, many large twin studies have concluded that genetics contributes significantly to the risk of becoming a regular and dependent smoker. Meta-analysis of a dozen twin studies shows that both genetics and environment play important roles in smoking-related behaviors, with an estimated average heritability for ND of 0.59 in male and 0.46 in female smokers, and an average of 0.56 for the population as a whole (Li et al. 2003a). Nicotine dependence also is influenced by environmental factors, as well as by interaction between genetic and environmental factors (Ho et al. 2010; Lessov-Schlaggar et al. 2008; Li et al. 2003a; Sullivan and Kendler 1999;

Swan et al. 2003). Of the important neurotransmitters in the central nervous system (CNS) implicated in ND and alcoholism, GABA is the main inhibitory one, whose modulatory actions are mediated through two types of receptors: the ionotropic GABA_A receptor and the metabotropic GABA_B receptor (Bettler et al. 2004; Vlachou and Markou 2010). GABA_A receptors form ion channels, whereas GABA_B receptors activate second-messenger systems through G-protein binding and activation. The GABA neurons are part of the mesolimbic dopamine system, critically important in mediating the reinforcing properties of drugs of abuse. Additionally, the GABA system is diffusely expressed in the brain; therefore, areas other than the mesolimbic system may be partly responsible for these effects. Considering the functional importance of GABAergic signaling in CNS, the genes involved in the system have received great attention in human genetic study on addictions, especially to alcohol and nicotine. The primary objective of this communication is to provide an updated review of what we have learned from the genetic epidemiologic studies on the involvement of genes in GABAergic signaling system in drug addictions. However, given the different levels of understanding of the involvement of GABAergic system in the etiology of ND and alcoholism and in the availability of reviews on this signaling system in its relation to ND and alcoholism, we discuss them separately.

Evidence for the involvement of genes in GABAergic signaling in ND

Evidence from genome-wide linkage analysis

During recent years, a significant number of genome-wide linkage studies have been reported for addiction to nicotine, alcohol, and other abused substances (Li and Burmeister 2009), especially for smoking-related behaviors, in which more than 20 such studies have been reported (Han et al. 2010; Li 2008). By examining those reported linkage regions in each study and applying the rigorous criteria proposed by Lander and Kruglyak (1995), 13 regions, located on chromosomes 3–7, 9–11, 17, 20, and 22, were found to be “suggestive” or “significant” in at least two independent samples (Li 2008). Of them, the regions on chromosomes 9, 10, 11, and 17 have received the strongest support, with the regions on chromosomes 9 and 17 being the most interesting, given the primary objective of this report (Bergen et al. 1999; Bierut et al. 2004; Gelernter et al. 2007; Li et al. 2003b, 2006).

Evidence for association of GABA_B receptor subunit 2 (GABBR2) with ND

Based on the linkage results showing a “suggestive” linkage on chromosome 9 (see Fig. 1) with ND, reported initially by our group in the Framingham Heart Study (FHS) sample (Li et al. 2003b) and verified in independent samples by us (Li et al. 2006) and others (Bergen et al. 1999; Bierut et al. 2004; Gelernter et al. 2004), we conducted positional candidate gene-based association studies on this region for several candidate genes in the Mid-South Tobacco Family (MSTF) sample (Beuten et al. 2005, 2007; Li et al. 2009, 2007). The first gene identified from this linkage region was the subunit 2 gene for GABA_B receptor (GABBR2) (Beuten et al. 2005). Since this earlier report, we have genotyped more SNPs from GABBR2 in the MSTF study with large sample sizes, in which we not only confirmed our earlier finding that GABBR2 was significantly associated with ND but also showed that genetically determined vulnerability to ND was different in subjects of European and African origin (Li et al. 2009).

The GABA_B receptor inhibits neuronal activity through G-protein-coupled second-messenger systems, which regulate the release of neurotransmitters and the activity of ion channels and adenylyl cyclase (Kaupmann et al. 1998; Vlachou and Markou 2010). Although they have not revealed the detailed mechanisms of the involvement of GABA_B receptors in ND or alcoholism, preclinical studies have implicated GABAergic receptors in the rewarding effects of drugs of abuse, including nicotine and alcohol (Corrigall et al. 2000;

Maccioni and Colombo 2009; Vlachou and Markou 2010). Indeed, GABA_B agonists antagonize nicotine-rewarding effects in mice and rats (Fattore et al. 2002) and decrease alcohol consumption and craving in humans and the severity of alcohol-withdrawal symptoms in humans and rats (Colombo et al. 2004; Maccioni and Colombo 2009).

To determine the genetic contribution of GABBR2 variants to the detected linkage signal on chromosome 9, we performed two rounds of linkage analysis, with the first one being considered a regular analysis without correcting for GABBR2 SNPs and the second one a “justified” linkage analysis by including GABBR2 SNPs as covariates (Li 2006). As shown in Fig. 2, we found that the inclusion of GABBR2 SNPs as covariates reduced, but could not completely eliminate, the detected linkage signal on chromosome 9. The inclusion of GABBR2 SNPs decreased the detected linkage signal on chromosome 9 by 36.5, 27.7, and 38.2% for smoking quantity (SQ), the Heaviness of Smoking Index (HSI), and the Fagerstrom test for ND (FTND), respectively. These results indicate that GABBR2 is indeed a candidate gene for ND that contributes to the linkage signal on chromosome 9 for ND detected in our earlier study and there must be other candidate genes within this region that may contribute to our detected linkage signal. This is because GABBR2 SNPs explained only 27.7–38.3% of the detected linkage signal on chromosome 9. Indeed, our further positional candidate gene-based association analyses of this genomic region revealed that neurotropic tyrosine kinase receptor 2 (NTRK2) and Src homology 2 domain-containing transforming protein C3 (SHC3) are significantly associated with ND in the MSTF samples (Beuten et al. 2007; Li et al. 2007).

Interaction of GABBR1 and GABBR2 in affecting ND

Like any other complex trait, addiction is controlled by multiple genetic factors, with each having a relatively modest effect, and by environmental factors, as well as by both gene–gene (epistatic) and gene–environment interactions (Flint and Munafo 2008; Ho et al. 2010; van der Zwaluw and Engels 2009). As documented in the literature (Gelernter and Kranzler 2009; Ho and Tyndale 2007; Lessov-Schlaggar et al. 2008; Li 2008; Li and Burmeister 2009), significant efforts have been made in searching for vulnerability genes to addictions. However, these approaches are effective only for genes with moderate-to-major effects. The ability to identify susceptibility genes for addictions and other psychiatric disorders has been improving, but remains considerably limited by the presence of a diverse array of factors such as epistatic interaction, small-modest genetic effects, small sample size, and heterogeneities. Among these factors, detecting gene–gene and gene–environment interactions appears to be more challenging (Flint and Munafo 2008; Ho et al. 2010; van der Zwaluw and Engels 2009).

In the search for determinants of gene–gene and gene–environment interactions, extensive efforts have been expended. Several combinatorial approaches, such as the multifactor dimensionality reduction (MDR) (Ritchie et al. 2001), the combinatorial partitioning method (CPM) (Nelson et al. 2001), and the restricted partition method (RPM) (Culverhouse et al. 2004), are promising tools for detecting gene–gene and gene–environment interactions. Since the original report, MDR has been most widely applied to detect interactions underlying a spectrum of complex disorders. However, these established methods have critical limitations that restrict their practical use. For example, MDR, CPM, and RPM do not allow adjustments for covariates; MDR is applicable only to dichotomous phenotypes; and CPM and RPM cannot handle categorical phenotypes. To overcome the limitations of these existing combinatorial approaches and to meet research needs in determining gene–gene and gene–environment interactions for complex phenotypes, a generalized MDR (GMDR) as well as a pedigree-based GMDR (PGMDR) have been developed for case–control (Lou et al. 2007a) and family-based (Lou et al. 2008) studies, respectively, that

permit adjustments for discrete and quantitative covariates and are applicable to both dichotomous and continuous phenotypes.

Specifically, regarding gene–gene interaction for the GABAergic signaling system for ND, using the PGMDR software, we detected significant interactive effects between the variants in GABBR1 and GABBR2 in ND (Li et al. 2009). This is noteworthy in that a relatively weak association of GABBR1 with ND has been detected (Li et al. 2009) and indicates that a significant interaction exists between variants of GABBR1 and GABBR2 in affecting ND, and the involvement of GABBR1 in modulating ND risk is most likely through its interaction with GABBR2, where GABBR2 polymorphisms directly alter the susceptibility to ND (Li et al. 2009). The reason for failing to detect a significant association of GABBR1 with ND by itself may be attributable to a strong dependence of GABBR1 effects on specific GABBR2 variants or a relatively small marginal effect of GABBR1 variants in the samples studied. More importantly, a significant interaction of GABBR1 with GABBR2 in humans confirms previous findings of pharmacologic studies that showed GABA_B receptor functions as heterodimers of GABA_{B1} and GABA_{B2} subunits (Bettler et al. 2004; Vlachou and Markou 2010).

The involvement of the GABA_B receptor in ND has been reported in many studies using animal models (Bettler et al. 2004), including a recently reported genetic study on zebrafish applying a nicotine behavioral assay in a forward screening of genes mutated through a gene-breaking transposon mutagenesis approach (Petzold et al. 2009). This study used transposons in mutant zebrafish and screened for induced changes in the nicotine-induced locomotive response, which generated two mutant fish lines with significantly attenuated nicotine locomotive response: dbav and hbog with the identified mutations in the chaperonin-containing protein 8 (cct8) and a GABA_B receptor ortholog, gabbr1.2, respectively. This identification of GABA_B receptor involvement in the nicotine response of zebrafish provides further evidence for the role of the GABAergic system in the etiology of ND (Klee et al. 2010). In considering a consistent relation between a reduced reward sensitivity and addiction, these findings point to a potential genetic basis for the involvement of GABA_B receptor signaling in the etiology of ND.

Evidence for association of other genes in GABAergic system with ND

In addition, there is another candidate gene, called GABA_A receptor-associated protein (GABARAP), located in a “suggestive” linkage region on chromosome 17 (see Fig. 1) for ND or other smoking-related behavior (Duggirala et al. 1999; Li 2008; Li et al. 2003b; Wang et al. 2005). GABARAP belongs to a family of microtubule-associated proteins that includes GABARAP, GABA_A receptor-associated protein like 1 (GABARAPL1), GABARAPL2, the yeast protein Apg8p/Aut7, and light chain 3 of microtubule-associated protein 1 (MAP1-LC3) (Kabeya et al. 2000; Lang et al. 1998; Pellerin et al. 1993; Sagiv et al. 2000; Wang et al. 1999). Of the members of the family, GABARAP has been investigated extensively and found to interact with the $\gamma 2$ subunit of the GABA_A receptor. Such interactions among GABA_A receptor, GABARAP, and tubulin promote clustering of the receptor, alter its channel kinetics, and enhance its trafficking to the plasma membrane in neurons (Chen et al. 2000; Leil et al. 2004; Wang et al. 1999). Furthermore, our microarray study indicated that GABARAPL2 was highly regulated by nicotine in multiple rat brain regions in a time- and region-dependent manner (Li et al. 2004).

Through a two-stage fine-mapping approach on the basis of linkage analysis findings, we found that two SNPs (i.e., rs222843 and rs17710) in GABARAP are significantly associated with ND in European-American smokers (Lou et al. 2007b). Considering that SNPs rs222843 and rs17710 reside in the promoter and 3′-untranslated region of GABARAP, respectively, we were interested in determining whether they are capable of regulating

GABARAP expression. By using a luciferase reporter assay in human embryonic kidney HEK293 cells, we found that the promoter containing the G allele of rs222843 produced a nearly twofold increase in luciferase activity compared with the one containing the A allele (Fig. 3, right panel). In contrast, we detected no difference in expression of the chimeric reporters containing the A and T alleles of rs17710 (Fig. 3, left panel). This indicates that rs222843, and not rs17710, is functional in causing expression divergence of GABARAP. However, whether this differentially allelic-specific expression can be detected in human smokers remains to be further examined for this functional GABARAP variant.

Evidence for involvement of GABA receptor signaling in ND based on pathway analysis

As mentioned above, both linkage and association analyses have revealed several genes in the GABAergic signaling pathway that are associated with ND or other smoking-related behaviors. However, another study has failed to replicate some of those associations (Agrawal et al. 2008a). Many factors might contribute to difficulty in replicating the findings of linkage and association analysis, which include the presence of substantial heterogeneity, underpowered samples, small genetic effects, inconsistency in defining and assessing the phenotypes of interest, and different study designs and methodologies (Ho et al. 2010; Li 2008; Wang and Li 2010). Generally speaking, a conventional single-gene-based association study reports only the top-ranking SNPs or genes with the smallest statistic and has serious limitations because of functionally critical susceptibility SNPs/genes for a complex trait generally with subtler effects and over-conservative multiple testing correction (Wang et al. 2007). To overcome these limitations, pathway-based association analysis has been proposed (Holmans et al. 2009; Wang et al. 2007), which examines the cumulative impact of a group of genes with modest individual contributions within the same pathway on a phenotype of interest. Compared with single gene-based analysis, pathway-based analysis is thus supposed to reveal more convincing findings, and such findings should be more biologically plausible because a significantly enriched pathway presumably defines a more precise and more specific biological function than a single gene with multiple functions (Holmans et al. 2009). Further, given the fact that Bonferroni correction is considered to be over-conservative for multiple testing and genes with subtler effects could hardly survive such a correction in large-scale association studies, the pathway-based analysis offers an attractive and potentially powerful alternative perspective—a “two-step” testing procedure that first identifies significant clusters of genes, followed by pathway testing within each significant group.

To identify pathways associated with ND and its related behaviors, we recently conducted a comprehensive pathway-based association analysis for three important smoking-related behaviors: smoking initiation, ND, and smoking cessation (Wang and Li 2010). Through searching literature on genetic studies for the behaviors including both candidate gene-based and genome-wide association studies, we identified most, if not all, genes that have been reported to be associated with these phenotypes. We then applied various pathway-based approaches to these associated genes, which revealed 9, 21, and 13 enriched pathways among the genes associated with smoking initiation, ND, and smoking cessation. Of these pathways, we found that GABAergic signaling is significantly associated with ND (Wang and Li 2010). Moreover, we found significant genetic overlap among these three smoking-related phenotypes.

Genetic evidence for the involvement of genes in GABAergic signaling in alcoholism

Alcohol facilitates immediate release of GABA in distinct areas of the human brain (Kelm et al. 2011). The released GABA is then bound to receptors on either side of the synapse,

regulating further release of GABA and hyperpolarization of postsynaptic neurons. Because the involvement of GABAergic signaling in alcoholism has been well covered by many reviews (Edenberg and Foroud 2006; Enoch 2008; Gelernter and Kranzler 2009), in this section, we limit our focus primarily to SNPs and haplotypes in GABA_A receptor subunit genes associated with AD or its related phenotypes identified in multiple studies. Because genetic associations of presynaptic GABA system with AD and related phenotypes are not yet well characterized, their role is only briefly discussed in this communication.

Genetics of post-synaptic GABA_A receptors in alcoholism

Variations in expression of GABA_A receptor subunit isoforms have been implicated in developing alcohol tolerance, alcohol dependence (AD), withdrawal, and self-administration (Kohnke 2008; Krystal et al. 2006). At intoxicating doses, alcohol potentiates synaptic pentameric GABA_A receptors that exist mainly as $\alpha 1\beta 2\gamma 2$ (60%), $\alpha 2\beta 3\gamma 2$ (15%), and $\alpha 3\beta 3\gamma 2$ (10%) subunit combinations in the adult human brain. At lower doses, alcohol potentiates extrasynaptic GABA_A receptors containing $\alpha 6$ and $\alpha 4$ instead of $\alpha 1-3$ and $\alpha 5$ subunits. Molecular studies have indicated increased $\alpha 4$, $\alpha 6$, and $\gamma 2$ and reduced $\alpha 1-3$, $\alpha 5$, and δ mRNA expression in rat and primate brain after chronic treatment with alcohol (Anderson et al. 2007; Cagetti et al. 2003; Grobin et al. 2000).

The 14 of the 16 genes encoding GABA_A receptor subunit isoforms are clustered on four chromosomes: 4p12–q13 ($\alpha 2$, $\alpha 4$, $\beta 1$, $\gamma 1$), 5q31–q35 ($\alpha 1$, $\alpha 6$, $\beta 2$, $\gamma 2$), 15q11–q13 ($\alpha 5$, $\beta 3$, $\gamma 3$), and Xq28 ($\alpha 3$, $\beta 4$, $\epsilon 1$) (Darlison et al. 2005; Enoch 2008). The initial link between GABA_A loci and alcoholism was identified with genome-wide linkage scans in Native Americans and Caucasians, where a locus on chromosome 4p12 was consistently linked with vulnerability to AD (Long et al. 1998; Reich et al. 1998; Zinn-Justin and Abel 1999). Two later studies revealed a much stronger linkage of 4p12 to the alcoholism-related endophenotype beta frequency band of the human electroencephalogram (EEG) (Edenberg et al. 2004; Porjesz et al. 2002).

Association analyses of GABA_A gene cluster on chromosome 4 with alcoholism

The initial fine-mapping of the four genes (GABRA2, GABRA4, GABRB1, and GABRG1) in the chromosome 4p cluster in relation to alcoholism was carried out by Edenberg and colleagues in multiplex families from the Collaborative Study on Genetics of Alcoholism (COGA) sample (Edenberg et al. 2004). Those authors reported significant associations of DSM-IV AD with several SNPs at the individual level and a large, 164-kb, haplotype, all located in GABRA2, within a region starting from intron 3 and expanding over to 58 kb past the 3' end of the gene. Similar findings were reported in subsequent studies conducted in the COGA sample (Agrawal et al. 2006; Dick et al. 2006a), as well as in several independent samples from populations of European (Covault et al. 2004a; Enoch et al. 2006; Fehr et al. 2006; Lind et al. 2008) and Native American (Enoch 2008) ancestry. Table 1 shows a summary of replicated individual SNPs associated with AD and its related phenotypes. Expanding on these findings in GABRA2, Covault and colleagues demonstrated that the large haplotype in the 3' end of GABRA2 is in fact a sub-haplotype of a much larger "risk" region that extends across the intergene region to intron 1 of the adjacent GABRG1 (Covault et al. 2008). Conversely, in a later study, Enoch (2008) reported that the GABRA2 and GABRG1 haplotypes appear to be independent. It is clear that more studies are needed for consensus as to whether the risk alleles of the 3' end of GABRA2 and the 5' end of GABRG1 are transmitted as a block along generations. When examining those identified haplotypes associated with AD, one of the most interesting findings is that a region around axon 5 (from SNP rs279826 to rs279871) appears to be common among all haplotypes (see most intensely colored part of the bar in Fig. 4). This region has been considered to be the region of GABRA2 with the strongest association with AD (Edenberg and Foroud 2006) and

is reported to be associated with AD comorbid with cocaine and marijuana dependence (Agrawal et al. 2006). Moreover, the functional SNP rs279858, which was found to have allele-based mRNA alterations in alcoholic postmortem brain tissue (Haughey et al. 2008), is located on exon 5 in this common haplotype, causing a synonymous change at amino acid residue 132. Perhaps the other function-unknown intronic associations suggest a more prominent role of gene regulation through alternative splicing, mRNA stability, or other post-transcriptional regulatory mechanisms in genetic variability contributing to alcoholism risk, rather than structural changes in GABA_A subunit proteins.

Association analyses of GABA_A gene clusters on chromosomes 5 and 15 with alcoholism

The chromosome 5q34–q35 GABA_A cluster, encoding the isoforms of the most abundant type of receptor complex (Barnard et al. 1998; Johnson et al. 1992), is linked to alcohol-related phenotypes, but with mixed results in subsequent replication studies. When linkage scans were performed for DSM-IV AD, no significant linkage was found between AD and the variants in the GABA_A cluster in the COGA sample (Reich et al. 1998); however, when more homogeneous intermediate phenotypes such as drinking severity were used, linkage peaks were identified on chromosome 5 (Dick et al. 2006b). Further association studies performed with the COGA sample identified association of AD with GABRG3 (Dick et al. 2004), GABRA5, and GABRB3 (Song et al. 2003). Studies with other samples also found associations of AD with the gene clusters on chromosomes 5 and 15; with GABRB2 and GABRA6 SNPs associated with AD in Finns (Radel et al. 2005), and GABRA6 and GABRB2 SNPs associated with AD in Scottish samples (Loh et al. 1999). In GABRA6, SNP Pro385Ser was found to be associated with AD and a lower response to sedating effects of alcohol, and the 3' UTR T1221C SNP was associated with physiological response to psychosocial stress (Uhart et al. 2004).

Genetics of pre-synaptic GABA receptors and transporters in alcoholism

Synaptic GABA regulators play an important role by modulating the availability of GABA for post-synaptic GABAergic receptors that relay GABA signaling. At higher concentrations, alcohol induces immediate release of GABA in a number of human brain regions, including the central (CeA) and basolateral amygdala, ventral tegmental area (VTA), and hippocampus (Kelm et al. 2011). A number of studies have demonstrated that the alcohol's ability to induce GABA release is facilitated by activation of G_αs or G_αq-coupled receptors such as CRF1 in CeA (Nie et al. 2004, 2009) and 5-HT_{2C} in VTA; in contrast, activation of G_αi-coupled receptors such as GABA_B (Peris et al. 1997; Silberman et al. 2009; Wu et al. 2005), CB1R in the amygdala and hippocampus (Roberto et al. 2010), and OPRD in CeA (Kang-Park et al. 2007) can block GABA release. Thus, it is reasonable to state that the genetic polymorphisms in all of the above-mentioned receptors and their downstream molecules may affect GABA release. However, evidence supporting this hypothesis is limited.

To be consistent with the current theme, we will limit our review to genetic variations in GABA_B receptors that affect GABA release. Compared with studies conducted with GABA_A receptors, only a few studies have performed association analysis of GABA_B receptor subunit polymorphisms with alcoholism, mostly with negative results. An exonic polymorphism T1974C in GABBR1 on chromosome 6p21.3 was shown to be associated with AD, with the T allele being more frequent in alcoholics (Sander et al. 1999).

A number of transmembrane GABA transporters are involved in terminating inhibitory GABAergic signaling through rapid synaptic clearance of GABA by reuptake. The major transmembrane GABA transporter forms present in the human brain are GAT-1, GAT-2 and GAT-3 (Christiansen et al. 2007) encoded by SLC6A1, SLC6A13 and SLC6A11 genes,

respectively. Chronic ethanol administration increased GAT-1 and GAT-3 levels in the hypothalamus and hippocampus of male alcohol-dependent rats (Devaud 2001); the authors of this study concluded that enhanced hypothalamic GABA reuptake resulting in greatly reduced synaptic levels of hypothalamic GABA may have a role in the development of tolerance and withdrawal. As with many other pre-synaptic GABA receptors and regulatory molecules, association analyses of GABA transporter polymorphisms with AD are not reported in literature. However, several recently emerged studies have suggested genetic associations of GABA transporter genes with other psychiatric pathology frequently comorbid with AD; for example, genetic associations of SLC6A1 and SLC6A13 with anxiety (Saus et al. 2010; Thoeringer et al. 2009), SLC6A1 with schizophrenia (Hirunsatit et al. 2009), and SLC6A1 with ADHD (Lasky-Su et al. 2008).

Conclusions and future directions

In sum, significant progress has been made in searching for susceptibility loci and genes for ND and alcoholism. On the basis of the identified linkage peaks on chromosomes 9 and 17 and prior knowledge of the biological functions of the products of each gene, variants in GABRA4, GABRA2, GABRE, GABBR2, and GABARAP are significantly associated with ND. Linkage peaks on chromosomes 4 and 5 harboring GABRA2, GABRG1, and GABRA6 genes were identified to be associated with AD in several independent Caucasian populations. Furthermore, the involvement of the GABAergic signaling pathway, to which these genes belong, in the etiology of ND has been confirmed by pathway-based association analysis.

In spite of this progress in molecular genetic studies of addictions, we still have a long way to go, and there are many challenges that remain to be surmounted (Ho et al. 2010; Li 2010; van der Zwaluw and Engels 2009). These challenges include: (1) further identification and replication of known and unknown genes in GABAergic and other signaling pathways and functional variants (including rare variants) for various addictive disorders through high-throughput approaches such as association study and deep sequencing; (2) study of copy number variations (CNVs) and their impact on gene expression in GABAergic and other addiction-related signaling pathways; (3) better understanding of the mechanisms underlying addictions at the molecular and cellular levels using both in vitro and in vivo approaches; and (4) determining appropriate ways of defining environmental factors such that we can assess how gene–environment interaction affects addictions. An improvement of our understanding of the genetic and environmental factors underlying drug addiction has considerable potential to reduce morbidity and death greatly by providing the most suitable methods for prevention and novel medications for treating different addictive disorders.

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Fig. 1.

Chromosomal locations of nominated regions on chromosomes 9 and 17 for all smoking-related measures with “significant” or “suggestive” linkage score. The linkage results were obtained from the following studies: AA/MSTF African-American sample of the Mid-South Tobacco Family study (Li et al. 2006), EA/MSTF European-American sample of the Mid-South Tobacco Family study (Li et al. 2008), FHS Framingham Heart Study (Li et al. 2003b; Wang et al. 2005), EA/GCOD European-American sample of Genetics of Cocaine or Opioid Dependence study (Gelernter et al. 2007), COGA Collaborative Studies on the Genetics of Alcoholism (Bergen et al. 1999; Bierut et al. 2004; Duggirala et al. 1999), and FSPD Family Study of Panic Disorder (Gelernter et al. 2004)

Fig. 2.
Determination of contribution of GABBR2 SNPs to detected linkage signal on chromosome 9

Fig. 3.

Determination of allelic-specific expression of SNPs rs222843 and rs17710 in GABARAP. The SNPs rs222843 (G/A) and rs17710 (A/T) are located in the promoter and 3' UTR of GABARAP, respectively. Using a luciferase reporter assay, we revealed a significant expression difference between the G and the A alleles of rs222843 ($P < 0.0001$), but not in the A and the T alleles of rs17710. Data shown as mean $\pm$ SD ($N = 4$). ** $P < 0.01$; paired Student's t test

Fig. 4.

Structures of *GABRA2* and *GABRG1* and haplotypes associated with alcoholism. The black bars indicate location of haplotypes in Caucasians. The intensity of the bar corresponds to overlapping haplotypes detected in different association analyses. Therefore, the brightest segments correspond to the most frequently replicated haplotype regions. For both genes, main mRNA isoforms reported on NCBI AceView are shown

Table 1

Replicated GABRA2 SNP associations for AD and related QTL

Phenotype	dbSNP ID	Chromosome position	*P _{Meta} value	Design (references)	Associated risk allele/genotype	Race/ethnicity
AD (diagnosed using DSM-IV criteria for AD)	rs490434	46,193,279	**2.45 × 10 ⁻⁴	Family (Agrawal et al. 2006; Edenberg et al. 2004) [†]	NR	EA (83%), AA (13%), other (4%)
	rs144013	46,201,920	7.78 × 10 ⁻⁴	CC (Bierut et al. 2010; Covault et al. 2008)	A	EA
	rs567926	46,241,769	9.12 × 10 ⁻⁶	CC (Covault et al. 2008)	C	EA
				CC (Fehr et al. 2006)	C	European-German
	rs572227	46,251,393	2.00 × 10 ⁻²	CC (Bierut et al. 2010)	A	EA
				Family (Edenberg et al. 2004)	NR	EA (83%), AA (13%), other (4%)
	rs534459	46,256,805	2.21 × 10 ⁻⁵	CC (Bierut et al. 2010; Covault et al. 2004a; Covault et al. 2008)	C	EA
	rs279871	46,305,733	2.50 × 10 ⁻⁵	CC (Lydall et al. 2011)	A	European-UK
				CC (Fehr et al. 2006)	A	European-German
				Family (Agrawal et al. 2006; Dick et al. 2006a; Edenberg et al. 2004)	A	EA (83%), AA (13%), other (4%)
	rs279863	46,313,022	**3.19 × 10 ⁻³	Family (Edenberg et al. 2004)	NR	EA (83%), AA (13%), other (4%)
				CC (Enoch et al., 2009)	A	European-Finnish
	rs279858	46,314,593	2.20 × 10 ⁻²	CC (Bierut et al. 2010; Covault et al. 2004a; Covault et al. 2008)	G	EA
				CC (Enoch et al. 2009)	AA and GG	European-Finnish
				CC (Fehr et al. 2006)	G	European-German
				CC (Lydall et al. 2011)	G	European-UK
				CC (Lappalainen et al. 2005)	G	European-Russian
				Family (Agrawal et al. 2006; Edenberg et al. 2004) [†]	NR	EA (83%), AA (13%), other (4%)
				Family (Villafuerte et al. 2011)	G	EA (98.2%), Other (1.8%)

Phenotype	dbSNP ID	Chromosome position	*P _{Meta} value	Design (references)	Associated risk allele/genotype	Race/ethnicity
				Family (Lind et al. 2008)	A	European-Australian (>90%)
	rs279844	46,329,655	7.71×10^{-3}	CC (Covault et al. 2004a; Covault et al. 2008)	T	EA
	rs279826	46,334,209	$**3.35 \times 10^{-5}$	Family (Agrawal et al. 2006; Edenberg et al. 2004) [‡]	NR	EA (83%), AA (13%), other (4%)
	rs279837	46,339,323	8.00×10^{-3}	Family (Edenberg et al. 2004)	NR	EA (83%), AA (13%), other (4%)
				CC (Covault et al. 2004a)	C	EA
	rs279841	46,340,763	2.70×10^{-2}	CC (Bierut et al. 2010)	A	EA
				Family (Edenberg et al. 2004)	NR	EA (83%), AA (13%), other (4%)
Excessive beta EEG fast activity	rs548583	46,263,344	$**4.33 \times 10^{-3}$	CC (Lydall et al. 2011)	C	European-UK
				Family (Edenberg et al. 2004)	NR	EA (83%), AA (13%), other (4%)
	rs279871	46,305,733	$**1.39 \times 10^{-2}$	CC (Lydall et al. 2011)	A	European-UK
				Family (Edenberg et al. 2004)	NR	EA (83%), AA (13%), other (4%)
	rs279863	46,313,022	$**2.71 \times 10^{-2}$	CC (Lydall et al. 2011)	C	European-UK
				Family (Edenberg et al. 2004)	NR	EA (83%), AA (13%), other (4%)
	rs279841	46,340,763	$**2.93 \times 10^{-3}$	CC (Lydall et al. 2011)	G	European-UK
				Family (Edenberg et al. 2004)	NR	EA (83%), AA (13%), other (4%)
Sensitivity to acute effects of alcohol	rs279858	46,314,593	3.33×10^{-3}	Human alcohol challenge study (Haughey et al. 2008)	AA	EA (>90%)
				Human alcohol challenge study (Roh et al. 2010)	A	Japanese
				Pharmacogenetics (Pierucci-Lagha et al. 2005)	AA	EA (82%), Hispanic (18%)

All chromosome positions are determined based on the NC_000004.11

EA European-American, AA African-American, NR not reported, CC case-control study design, Family Family-based study design

[‡]AD comorbid with other drug dependencies

* Meta-analysis was performed with the metal (Wilier et al. 2010) or Fisher's combined probability test (indicated with '**'), where allelic association data were not available; both positive and negative associations were considered for meta-analyses