

Alcohol Dependence and Genes Encoding $\alpha 2$ and $\gamma 1$ GABA_A Receptor Subunits

Insights from Humans and Mice

Cecilia M. Borghese, Ph.D., and R. Adron Harris, Ph.D.

One approach to identifying the causes of alcoholism, particularly without crossing ethical boundaries in human subjects, is to look at the person's genome (and particularly at the variations that naturally arise in the DNA) to identify those variations that seem to be found more commonly in people with the disease. Some of these analyses have focused on the genes that encode subunits of the receptor for the brain chemical (i.e., neurotransmitter) γ -aminobutyric acid (GABA). Different epidemiological genetic studies have provided evidence that variations in certain GABA_A receptor (GABA_A-R) subunits, particularly subunits $\alpha 2$ and $\gamma 1$, are correlated with alcohol dependence. Manipulations of these genes and their expression in mice and rats also are offering clues as to the role of specific GABA_A-Rs in the molecular mechanisms underlying alcoholism and suggest possibilities for new therapeutic approaches. **KEY WORDS:** Alcohol dependence; alcoholism; genetic factors; DNA; genetic theory of alcohol and other drug use (AODU); genetic vulnerability to AODU; genetic variants; γ -aminobutyric acid (GABA); GABA_A receptor (GABA_A-R) subunits; GABRA2; GABRG1; single nucleotide polymorphisms (SNPs); ion channels; neurotransmitters; gene association studies; human studies; animal studies; mice; rats

Even though the consequences of alcohol dependence (AD) clearly are devastating and obvious to observers, the molecular mechanisms involved in the development of the disease are far from clear and understood. The search for these mechanisms is made even more difficult by the vast number of genes, proteins, and pathways in the human body that potentially could be involved, and by the obvious limitations of conducting research with human subjects without crossing ethical boundaries. Yet despite these complexities, various approaches already have allowed researchers to gather much knowledge in recent years, and the essential players in alcohol's mechanisms of action and in the development of AD already may have been identified. Thus, research has found that the primary targets of alcohol seem to be proteins prominently involved in neuronal communication, including:

- Ion channels in the neuronal membrane that are activated by signaling molecules (i.e., neurotransmitters) such as γ -aminobutyric acid (GABA) (i.e., GABA_A receptors), glycine (i.e., glycine receptors), glutamate (i.e., *N*-methyl-D-aspartate receptors [NMDA-Rs]), acetylcholine (i.e., nicotinic receptors), and serotonin (i.e., 5-HT₃ receptors);
- Ion channels regulated by changes in the electric potential across the neuronal membrane (i.e., voltage-gated channels), such as voltage-gated calcium channels; and
- Ion channels regulated by a type of regulatory molecules called G-proteins, such as G-protein-coupled inwardly rectifying potassium channels (GIRKs).

Alcohol's actions on these primary targets trigger the involvement of other systems that ultimately culminate in the development of dependence (Vengeliene et al. 2008).

Many techniques have yielded insight into alcohol's effects on the organism, but perhaps the most challenging field, given the logical ethical constraints, is the study of the neuronal structures and mechanisms that are affected by alcohol and/or which play a role in the development of AD in living humans. One way of circumventing these limitations is by studying how the natural variations (i.e., polymorphisms) between individuals in the genomic DNA relate to AD—that is, whether any specific variants are found more or less commonly than would be expected by chance in people with the disorder. This analysis can provide a glimpse of which genes or gene variants contribute to and shape the development of the disorder.

These natural differences in the genomic DNA between individuals arise from spontaneous mutations of single DNA building blocks (i.e., nucleotides) and are called single nucleotide polymorphisms (SNPs). (For more information on SNPs and their analysis, see the sidebar). In the past 10 years, different genetic association studies in alcohol-dependent subjects have identified several genes linked to this condition. Some examples of proteins that are encoded by genes in which the AD-linked SNPs are located include the following:

- The μ -opioid receptor (encoded by the OPRM1 gene) (Bart et al. 2005; Kim et al. 2004; Nishizawa et al. 2006; Ray and Hutchison 2004; Rommelspacher et al. 2001; Zhang et al. 2006);
- The κ -opioid receptor (OPRK1) (Edenberg et al. 2008; Xuei et al. 2006; Zhang et al. 2008);

Cecilia M. Borghese, Ph.D., is a research associate and R. Adron Harris, Ph.D., is M. June and J. Virgil Waggoner Chair in Molecular Biology and director of the Waggoner Center for Alcohol and Addiction Research, both at the University of Texas at Austin, Austin, Texas.

- Neuropeptide Y (NPY) (Ilveskoski et al. 2001; Lappalainen et al. 2002; Mottagui-Tabar et al. 2005);
- The muscarinic acetylcholine receptor M2 (CHRM2) (Dick et al. 2007; Luo et al. 2005; Wang et al. 2004); and
- The corticotropin-releasing hormone receptor 1 (CRHR1) (Chen et al. 2010).

Another group of genes related to alcohol dependence encode the GABA_A receptors (GABA_A-Rs). This article will

summarize what is known about the role of these receptors in the development of alcohol dependence.

GABA_A Receptors

The GABA_A-Rs are proteins that span the membrane encasing the nerve cells (i.e., neurons) and which are composed of five subunits arranged around a central pore. There are several classes of subunits, including alpha (α), beta (β), gamma (γ), delta (δ), epsilon (ε), pi (π), theta (θ), and rho (ρ) subunits.

Single-Nucleotide Polymorphisms and Their Analysis

What Are Single-Nucleotide Polymorphisms?

Single-nucleotide polymorphisms (SNPs, pronounced “snips”) are spontaneous mutations of single building blocks (i.e., nucleotides) in the genomic DNA. They can occur randomly, in any region of the DNA, including within those regions of the gene that actually encode parts of the resulting protein (i.e., coding sequences), within “silent” regions of a gene that ultimately do not encode parts of the resulting protein (i.e., non-coding regions), or in the regions between genes (i.e., intergenic regions). When a SNP occurs within a coding sequence, it may or may not change the amino acid sequence of the encoded protein. Each amino acid is represented by a three-nucleotide block of DNA (i.e., a codon). Because there are four different nucleotides (represented as A, C, G, and T), 64 possible codons exist; however, these encode only 20 amino acids. As a result, the genetic code is degenerate—that is, several codons may encode the same amino acid (e.g., both ACT and ACC encode threonine). A SNP in which both the original codon and the mutant codon produce the same protein sequence is called a synonymous polymorphism or silent mutation. If a different polypeptide sequence is produced, it is called a replacement polymorphism. This can result either

in the introduction of a different amino acid, which is called a missense mutation, or in a premature stop of the protein, which is called a nonsense mutation (see the figure). Even if the SNP occurs in a noncoding region of the gene, it still may affect regulatory processes that could result, for instance, in altered protein levels.

When aligning DNA sequences from different individuals and comparing them at the same positions (i.e., loci) in the DNA, the occurrence of a SNP results in different “versions” of DNA called alleles. For example, in the figure, the two alleles for the SNP rs279868 are “A” and “G”. Alleles frequently are transmitted from one generation to the next in a larger DNA block, usually from 5,000 to 100,000 nucleotides long. These blocks, which can contain numerous SNPs, are known as haplotypes. Thus, a haplotype specifies markers on one member of a pair of homologous chromosomes (i.e., either the chromosome inherited from the mother or the one inherited from the father).

Haplotypes are not always transmitted from one generation to the next, however, because of a process called recombination that randomly occurs during the formation of germ cells. As a result of recombination, new haplotypes should be formed based on the frequencies of the different alleles involved in the general

population. Sometimes, however, certain combinations of alleles occur more or less frequently in a given population than would be expected from random formation of haplotypes. This nonrandom association of alleles at two or more loci is referred to as linkage disequilibrium. Identification of alleles that are in linkage disequilibrium can be useful for determining genes that are involved in conditions such as alcohol dependence.

How Can SNPs Be Analyzed?

Some genetic studies try to determine whether a certain allele (or haplotype) is present more or less frequently in people who suffer from a medical condition (e.g., alcoholism) than those without the disease; these are called genetic-association studies. One type of genetic-association studies are case-control studies, which include both individuals affected by the condition and disease-free control individuals from the same population. The frequency of alleles then is determined in both case and control subjects. Differences in the frequency of an allele between the two groups suggest that an association exists between the involved gene and the medical condition, with a certain allele conferring an increased or decreased risk for the condition. Identification of the precise nature of this association then requires additional studies.

units. The most commonly found GABA_A-Rs consist of two α , two β , and one γ or δ subunit. For some classes of subunits, several variants exist that are encoded by different genes, including six for the α subunit, three for the β subunit, and three for the γ subunit, allowing for numerous different subunit combinations. When GABA binds to the GABA_A-R, it activates the receptor and the central channel opens, allowing the entrance of negatively charged ions (i.e., anions), specifically Cl⁻, to enter the neuron. This results in an increase in the difference in electrical charge between the inside and outside of the neuron (i.e., hyperpolarization),

which in turn makes it more difficult for the neuron to transmit a nerve impulse, thus ultimately leading to inhibition of neuronal activity. Accordingly, GABA is considered an inhibitory neurotransmitter.

Considerable evidence points to the GABA_A-R as one of the main targets of alcohol (Kumar et al. 2009). The most abundant subunit combination in the brain, $\alpha 1\beta 2\gamma 2$, has been the most studied. Recently, the δ subunit-containing GABA_A-Rs also have been scrutinized in relationship to alcohol (Lobo and Harris 2008).

Single-Nucleotide Polymorphisms and Their Analysis *continued*

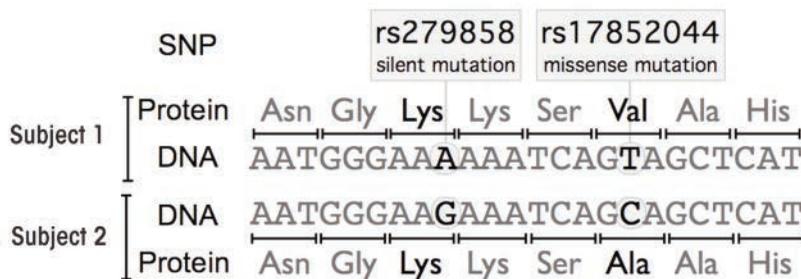
For example, the allele may alter the sequence, the splicing, or the levels of expression of the protein encoded or it may be in linkage disequilibrium with another allele that constitutes the genetic basis for the difference.

A caveat of these studies is that the frequencies of alleles/haplotypes can vary with ethnicity and geography; this is known as population stratification. One way to avoid this problem is to use family-based association designs. In this situation, unaffected family members (e.g., parents or siblings) are used as control subjects for the affected individuals. If an allele increases the risk of having the disease, then that allele would appear more frequently in the affected family members than in the unaffected members.

Other studies, such as twin and adoption studies, focus on the interaction between genes and environment. Twin studies compare the similarity of identical (i.e., monozygotic) and fraternal (i.e., dizygotic) twins. Identical twins generally are more similar than fraternal twins, because they are not only exposed to the same environment but also share a higher genetic similarity. In adoption studies, the adopted individuals are compared with control individuals (i.e., non-adopted individuals either from the adoptive family or the general population or adopted but unrelated children in the adoptive family). By comparing large numbers of twin pairs or adoptees and control subjects (e.g., with respect to the frequency of certain SNPs as well as the disease of

interest), it is possible to better understand the role of genes and environment in the characteristics of a person.

Another approach to using SNPs to identify genes involved in a certain disease is to conduct genome-wide association studies (GWASs). With this strategy, a genetic association with the disease is investigated using many SNPs that cover the entire genome, instead of just a few genes as in the study approaches described above. This allows researchers to identify associations with genes that previously had not been expected to play a role in the disease under investigation. The downside of the GWASs is that they require a much larger number of subjects than do the other studies.



Segment of a single strand of DNA representing a fragment of the coding region from the *GABRA2* gene from two different people. There are two SNPs in this gene region—one in which both variants of the DNA encode the same amino acid (i.e., a silent mutation) and one in which the two variants of the DNA encode different amino acids (i.e., a missense mutation).

The genes encoding the GABA_A-R subunits are located in clusters on different chromosomes, including one cluster on chromosome 4 that carries genes called *GABRB1*, *GABRA4*, *GABRA2*, and *GABRG1*, which encode the β1, α4, α2, and γ1 subunits, respectively (see the figure). Previous human genetic studies have linked genetic polymorphisms in two regions of the *GABRA2* gene (i.e., in the middle and at the 3' end of the gene) and in the region between the *GABRA2* and *GABRG1* genes (i.e., the *GABRA2* to *GABRG1* intergenic region) with AD (Agrawal et al. 2006; Covault et al. 2004; Edenberg et al. 2004; Enoch et al. 2006; Fehr et al. 2006; Lappalainen et al. 2005; Soyka et al. 2008). However, the evidence is not unequivocal, because other studies found no association between AD and the SNPs in this area (Covault et al. 2008; Drgon et al. 2006; Matthews et al. 2007). Even among the studies that did find a correlation, some inconsistencies existed. For instance, the first study of the association between *GABRA2* and AD came from the Collaborative Study on the Genetics of Alcoholism (COGA), a vast family-based association study (Edenberg et al. 2004). When Agrawal and colleagues (2006) extended the study of the COGA sample to include illicit drug dependence and comorbid dependence on alcohol and other drugs, the association was found only in subjects with AD and co-occurring drug dependence. On the other hand, Covault and colleagues (2004) found that the association was stronger when alcoholics with comorbid drug dependence were removed from the sample. Despite these inconsistencies, however, most of the clinical and genetic evidence points to *GABRA2* as a major genetic player in AD (see Enoch 2008).

All the SNPs that have been studied in the *GABRA2* and *GABRG1* genes to date are nonfunctional polymorphisms—that is, they do not alter the amino acid sequence of the encoded proteins. An alternative explanation for their role in the development of AD would be that the SNPs may alter the amount of protein that is produced. To address this possibility, researchers have analyzed the levels of an intermediate molecule called messenger RNA (mRNA) that is generated when the information encoded in the DNA is used for the production of a functional protein (i.e., during gene expression). An analysis of α2 mRNA levels in the prefrontal

cortex of AD and control subjects found an association between α2 mRNA levels and the different variants (i.e., alleles) in the SNP rs279858,¹ although mRNA levels did not differ between control subjects and alcoholics (Haughey et al. 2008). Other recent data suggest that the apparent correlation between AD and *GABRA2* may result from a linkage disequilibrium with a not-yet-detected functional variant in the neighboring *GABRG1* gene. These findings on *GABRA2* and *GABRG1* and their association with AD are reviewed in the following sections, focusing on human studies and correlates in genetically modified rodents.

Analyses of the *GABRA2* and *GABRG1* Genes

Genetic-Association Studies

Several gene-association studies have examined the relationship of the *GABRA2* and *GABRG1* genes with AD, with varying results. Two studies—a large twin sample of the Australian population that also investigated the association with smoking and illicit drug use (Lind et al. 2008), and a small case-control study in an Italian sample (Onori et al. 2010)—reported no association between *GABRA2* and AD. Another case-control and family-association study (Sakai et al. 2010) sought to analyze the correlation between *GABRA2* genotype and substance abuse and behavioral problems in adolescents. The investigators only analyzed a single SNP in *GABRA2*, which was found not to be associated with conduct disorder or AD in adolescents.

Enoch and colleagues (2010) conducted a case-control study in African-American men with single and comorbid diagnoses of alcohol, cocaine, and heroin dependence, assessing the *GABRA2* genotype as well as childhood trauma. The exposure to childhood trauma predicted substance dependence.

¹ SNP rs279858 encodes a silent mutation (i.e., a mutation that does not result in an altered amino acid sequence) in the coding region of *GABRA2*.

² A haplotype is a set of closely linked genetic markers (e.g., SNPs) present on one chromosome that tends to be inherited together.



Figure Schematic representation of the cluster of GABA_A receptor genes on chromosome 4. Arrows indicate gene position, size, and direction of transcription. The subunits and the names of the corresponding genes are α2 (*GABRA2*), α4 (*GABRA4*), β1 (*GABRB1*), and γ1 (*GABRG1*).

common in control subjects and seemed to confer resilience to addiction after exposure to severe childhood trauma. These findings suggest that in African-American men, childhood trauma, *GABRA2* SNPs, and their interaction determine (at least in part) the risk of or resilience to substance dependence. However, the data did not show a direct association between *GABRA2* and AD.

In a case–control adoption study of substance abuse (Philibert et al. 2009), the researchers determined the participants' genotypes for SNPs encompassing the *GABRA2* gene and analyzed them with respect to their history of alcohol, nicotine, and/or cannabis dependence. Both *GABRA2* genotype and haplotype were significantly related to vulnerability to all three types of substance dependence, particularly nicotine, and this association was more pronounced in female than in male subjects.

A small study using Japanese subjects (mostly social drinkers) examined the association between genetic variation in *GABRA2* (as assessed via seven SNPs) and subjective responses to alcohol as well as stimulant and sedative effects of alcohol (Roh et al. 2010). Three of these seven SNPs, all of which were located in the middle of the *GABRA2* gene, showed significant associations with subjective effects of alcohol. Specifically, individuals carrying one or two copies of the more common *GABRA2* allele (which is not associated with AD) showed greater subjective responses to alcohol than did individuals carrying two copies of the allele associated with AD. These results are, to some extent, in agreement with previous studies (Haughey et al. 2008; Pierucci-Lagha et al. 2005).

Another study (Kareken et al. 2010) examined the association between *GABRA2* SNPs and the brain's reward system. The participants, which included social drinkers, heavy drinkers, and alcohol-dependent individuals, first were assessed with respect to the brain's responses to alcohol cues (i.e., exposure to the odor of their preferred alcoholic beverage or a control odor) under both alcohol intoxication and control conditions using an imaging technique called functional MRI (fMRI). Then, the subjects were stratified according to their genotype at a SNP in *GABRA2* that previously had been shown to be associated with AD (Edenberg et al. 2004). All participants carried at least one copy of the high-risk allele of the SNP. Under both alcohol intoxication and control conditions, participants with two copies of this allele (i.e., homozygous subjects) exhibited a larger response to alcoholic odors than to control odors in one brain region (i.e., the medial frontal cortical areas), whereas participants with only one copy of this allele (i.e., heterozygous subjects) exhibited a larger response in another brain area (i.e., the ventral tegmental area). Thus, *GABRA2* variants seem to modify the activation of reward-related areas after exposure to alcohol-associated cues. Another study (Villafuerte et al. 2011) used fMRI to analyze the relationship between two *GABRA2* SNPs, the personality trait of impulsivity, and activation of a brain region called the insula cortex during anticipation of reward or loss in a family sample with high numbers of alcohol-dependent individuals. The investigators detected an association of all three variables,

suggesting that *GABRA2* genotype influences insula responses and therefore impulsivity.

Another type of study called linkage disequilibrium analyzes whether certain alleles located close to each other on the same chromosome are inherited together more or less frequently than would be expected by chance alone.³ Such studies in different populations have focused on *GABRA2* and either *GABRG1* (Ittiwut et al. 2008) or the intergenic region between the two genes (Philibert et al. 2009). The findings of these studies led to the conclusion that associations observed between *GABRA2* and the condition under investigation could be attributable to functional genetic variation at the *GABRG1* locus or that disease-related variants may exist at both loci.

Some new studies have focused on the *GABRG1* gene. Ray and Hutchinson (2009) examined associations between two SNPs of the *GABRG1* gene and alcohol use in hazardous drinkers. The data indicated that variation in one of the SNPs was associated with level of response to alcohol, drinking behavior, and alcohol problems.

Additional evidence of a significant *GABRG1* association with AD was found in a study involving Finnish Caucasian and Plains American Indians that examined both the *GABRA2* and *GABRG1* genes (Enoch et al. 2009). In both populations, there were significant haplotype and SNP associations of *GABRG1*, but not *GABRA2*, with AD. However, in the Finnish study population, the association of three less common haplotypes with AD was determined by *GABRA2*. Taken together, the findings of all of these studies suggest that independent contributions from both *GABRG1* and *GABRA2* likely contribute to the risk of AD.

Genome-Wide Association Studies

In contrast to the approaches used in the studies described above, genome-wide association studies (GWASs) investigate the genetic association with a disease using many SNPs that cover the entire genome instead of just a few genes. GWASs therefore also may discover associations with genes not previously suspected to be involved in the disease. One GWASs (Bierut et al. 2010) identified 15 SNPs that showed a significant association with AD. Moreover, when the investigators performed an independent evaluation for *GABRA2*, they found that five SNPs at that gene showed a modest association with AD.

Another GWASs was carried out in a case–control sample drawn from the families in the COGA, using individuals with AD (56 percent of whom also were dependent on illicit drugs) and individuals who used alcohol but were not dependent on alcohol or illicit drugs (Edenberg et al. 2010). The study identified no single SNP that met genome-wide criteria for significance; however, several clusters of SNPs provided mutual support for an association with the disease. An analysis of SNPs in genes encoding GABA_A-R subunits

³ Even genes located close to each other on a chromosome may not always be inherited together, because of a process called genetic recombination that occurs with a certain probability during the generation of the germ cells.

in this sample found that a SNP in a gene called *GABRR2*, which encodes the GABA_A-R $\alpha 2$ subunit, was highly correlated with AD in this GWAS. This supported previous results, even though the level of significance was not high enough for genome-wide significance (Xuei et al. 2009). Likewise, SNPs in *GABRG1* were associated with AD, consistent with previous studies (Covault et al. 2008; Enoch et al. 2009). However, there was no evidence that the neighboring *GABRA2* gene was associated with AD. Finally, SNPs in other genes encoding GABA_A-R subunits (i.e., *GABRG3*, which encodes the $\gamma 3$ subunit; *GABRA1*, which encodes the $\alpha 1$ subunit; and *GABRG2*, which encodes the $\gamma 2$ subunit) also were associated with AD, again confirming findings of other investigators (Dick et al. 2004, 2006).

It is important to note that there are some inconsistencies in the findings of these genetic studies, which can be attributed to the inherent differences among the study types, the variability (i.e., heterogeneity) of the disease, and the genetic differences among the populations studied. In general, however, evidence continues to accumulate supporting an association between variations in genes encoding GABA_A-R subunits, particularly $\alpha 2$ and $\gamma 1$ subunits, and AD.

Studies in Genetically Engineered Rodents

The studies in humans discussed above have been supplemented with studies of alcohol's effects on behavior in genetically engineered mice carrying mutations in GABA-related genes (Crabbe et al. 2006). For example, Boehm and colleagues (2004) found that mice in which the GABA_A-R $\alpha 2$ subunit had been deleted (i.e., $\alpha 2$ knockout mice) differed from control mice both in behavioral tests conducted without alcohol exposure (e.g., showed decreased spontaneous locomotion when tested for locomotor response to novelty) and in some behavioral responses to alcohol. Thus, the $\alpha 2$ knockout mice showed a shorter duration of alcohol-induced loss of righting reflex (LORR), which is a measure of alcohol's hypnotic effects. However, the sensitivity of the mice to acute ethanol withdrawal seemed unchanged, as did alcohol's anxiety-reducing (i.e., anxiolytic) effects. This lack of an effect of $\alpha 2$ deletion on alcohol's anxiolytic effects was unexpected, because mice genetically modified to possess a benzodiazepine-insensitive $\alpha 2$ subunit called $\alpha 2$ (H101R) (i.e., knockin mice) no longer exhibited anxiolytic behavior when they were treated with the benzodiazepine diazepam (Low et al. 2000). Both alcohol and benzodiazepines are anxiolytic, and both increase GABA_A-R function. If both benzodiazepines and alcohol acted through $\alpha 2$ -containing GABA_A-Rs to produce anxiolytic effects, deletion of the $\alpha 2$ subunit in the knockout mice should reduce alcohol's anxiolytic effects, and that did not happen. Furthermore, accurate assessment of alcohol's anxiolytic effects in the $\alpha 2$ knockout mice was complicated by the altered locomotor responses in these animals. Finally, female $\alpha 2$ knockout mice, but not males, preferred and consumed less alcohol than did the controls. However, interpretation of these find-

ings is complicated by the female mice's greater aversion to bitter-tasting substances.

Another study was conducted in knockin mice carrying $\alpha 1$, $\alpha 2$, $\alpha 3$, or $\alpha 5$ GABA_A-R subunits that are insensitive to benzodiazepines through a mutation in a single amino acid (Tauber et al. 2003). The investigators administered diazepam and alcohol in combination to these mice and then determined the mice's LORR. All animals except for the $\alpha 2$ (H101R) mice showed similar sensitivity (i.e., increased LORR and reduced locomotor activity) to the combined drugs. Furthermore, the $\alpha 2$ (H101R) mice exhibited normal responses to alcohol alone (i.e., normal LORR and locomotor activity) and to a combination of low-dose alcohol and diazepam (i.e., normal locomotor activity). Thus, the benzodiazepine-induced increase in the alcohol-mediated hypnosis depends on the $\alpha 2$ GABA_A-R subunit.

Although null mutant mice that completely lack a certain GABA_A-R subunit provide important contributions to our knowledge of alcohol's targets, the deletion of a receptor in the brain, particularly an important one, is likely to trigger compensatory changes. For example, other receptor subunits could become more abundant and take on the functions of the missing subunit. An alternative approach is to design specific mutations that will render that receptor insensitive to alcohol but normal in every other aspect. One study compared the activities of GABA_A-Rs containing wild-type and mutated $\alpha 2$ subunits expressed in frog egg cells (i.e., *Xenopus* oocytes) (Blednov et al. 2011). With the normal GABA_A-Rs containing the wild-type subunit $\alpha 2$ (SL),⁴ submaximal GABA responses were enhanced (i.e., potentiated) by alcohol. However, this potentiation was absent in the mutant $\alpha 2$ (HA)-containing GABA_A-Rs; there even was a small inhibition of the receptor's activity. In contrast, the mutation did not affect the receptor's sensitivity to GABA or the modulation by zinc, the benzodiazepine flunitrazepam, or the anesthetic etomidate (Werner et al. 2011).

On the basis of these findings, researchers developed and studied two corresponding mouse lines, the $\alpha 2$ SL/SL (i.e., wild-type) mice and the $\alpha 2$ HA/HA (i.e., knockin) mice. The responses to alcohol in these animals then were studied using a variety of tests (Blednov et al. 2011). The analyses found that some typical effects of alcohol (e.g., conditioned taste aversion and motor stimulation) were absent in the knockin mice. Moreover, the knockin animals showed changes in alcohol intake and preference in multiple tests as well as increased alcohol-induced hypnosis. In contrast, the knockin animals exhibited no changes in alcohol's anxiolytic and motor incoordination effects. These altered behavioral responses to alcohol in mutant (i.e., both knockout and knockin) mice may be related to altered subjective effects of alcohol in humans with different $\alpha 2$ -associated SNPs (Kareken et al. 2010; Roh et al. 2010). In summary, the

⁴ The wild-type $\alpha 2$ (SL) subunit carries the amino acid serine at position 270 and the amino acid leucine at position 277. In contrast, the mutant $\alpha 2$ (HA) subunit carried the amino acids histidine at position 270 and alanine at position 277.

study suggests that $\alpha 2$ -containing GABA_A-Rs may be responsible for specific alcohol-induced effects. A subsequent study of the changes in mRNA levels induced by these mutations in the $\alpha 2$ subunit in the outer layer of the brain (i.e., the cerebral cortex) underlines the advantages of using knockin over knockout mice. Of almost 11,000 probes tested, the expression of only three genes was significantly modified in the knockin mice, and the behavioral responses to the sedative agents pentobarbital and flurazepam were unchanged (Harris et al. 2011). This confirms that the introduction of these mutations has minimal impact on the knockin animals compared with controls, minimizing the risk that effects unrelated to the behavior being investigated confound the results.

Another study in mice focused on the role of the GABA_A-R $\alpha 2$ subunit in changes in behavior produced by adaptation to chronic cocaine's effects (i.e., cocaine behavioral plasticity), such as locomotor sensitization, as well as in addiction (Dixon et al. 2010). In GABA_A-R $\alpha 2$ null mutant mice, cocaine did not induce a greater effect after repeated administration (i.e., did not produce behavioral sensitization) as it did in wild-type mice. Conversely, in mice carrying the benzodiazepine-insensitive GABA_A-R $\alpha 2$ (H101R) subunit, an agent called Ro 15–4513 that can increase the receptor responses in this mutant $\alpha 2$ subunit could stimulate locomotor activity if it was delivered into a brain region called the nucleus accumbens and induced behavioral sensitization to this effect after repeated administration. These results suggest that activation of $\alpha 2$ -containing GABA_A-Rs in the nucleus accumbens is sufficient and necessary for behavioral sensitization. Furthermore, the investigators conducted a genetic case–control study in a diverse population (mainly Caucasian) that demonstrated an association of *GABRA2* with cocaine addiction in humans, emphasizing the relevance of $\alpha 2$ -containing GABA_A-Rs in drug dependence.

Finally, researchers used an established animal model of human alcohol abuse, the selectively bred alcohol-preferring (P) rats, to look at the role of GABA_A-R subunits in alcohol's effects (Liu et al. 2011). The levels of GABA_A-R $\alpha 1$ subunits are elevated in a brain region called the ventral pallidum of these rats, and both $\alpha 1$ and $\alpha 2$ levels are increased in another region called the central nucleus of the amygdala (CeA). The study used molecules known as small-interfering RNAs (siRNA), which can interfere with gene expression, to specifically prevent production of $\alpha 1$ and $\alpha 2$ subunits. When siRNA targeted to $\alpha 2$ was infused into the CeA of P rats, both $\alpha 2$ expression and GABA_A-R density were reduced, and this was associated with inhibition of binge drinking. In contrast, siRNA targeted to $\alpha 1$ did not cause any of these changes when introduced in the CeA but did reduce $\alpha 1$ expression and binge drinking when administered into the ventral pallidum. These results highlight that not only the kind of GABA_A-R subunit but also the brain region in which it is located are relevant for alcohol consumption.

Implications of Genetic Findings for Therapeutic Approaches

Extending a previous study (Bauer et al. 2007), Das and colleagues (2010) analyzed the association between a SNP in the *GABRA2* gene and the efficacy of three psychotherapies for alcoholism (i.e., motivational enhancement therapy, cognitive–behavioral therapy, or 12-step facilitation) in preventing extreme drinking in AD patients. The study found that men with a high-risk *GABRA2* allele had a significantly higher probability of extreme drinking than did men without that allele. However, both men and women carrying at least one high-risk allele responded better to the therapy than did those who were homozygous for the low-risk allele. Among the female participants, the most effective therapy was cognitive–behavioral therapy, whereas among male subjects motivational enhancement therapy was most effective.

Tailoring pharmacotherapy to alcohol-dependent patients on the basis of genetic indicators also may be within reach. Two medications, naltrexone and acamprosate, currently are used for the treatment of alcoholism, but often with limited success. In an effort to identify potential associations between genotype and treatment outcome, Ooteman and colleagues (2009) determined SNPs in genes encoding different receptors involved in AD processes (i.e., opioid, dopamine, glutamate, and GABA_A receptors) in alcohol-dependent individuals randomly assigned to acamprosate or naltrexone treatment. The investigators also quantified treatment effectiveness using tests administered the day before treatment initiation and on the last day the medication was administered. The tests included a cue exposure (i.e., participants were exposed to the sight and smell of their favorite alcoholic beverage while listening to a mood-induction script), followed by an assessment of self-reported cue-induced craving and physiological cue reactivity (i.e., heart rate). Significant association effects were found for several SNPs, suggesting that this may be the first step in matching patients to pharmacotherapy based on GABA_A-Rs and other genotypic markers.

Summary

Studies of genetically modified mice and rats have demonstrated that manipulation of $\alpha 2$ GABA_A-R subunits produces changes in alcohol-related phenotypes. The findings were more equivocal in human genetic studies, although strong evidence suggests that several GABA_A-R subunits, particularly $\alpha 2$ and $\gamma 1$, have a role in AD in humans. Future studies hopefully will elucidate what exact mechanism creates these variations in the genetic code that affect AD, and how such variations can be used to provide a path to individualized therapy for patients with AD. ■

Acknowledgements

The preparation of this manuscript was supported by National Institutes of Health (NIH) grant AA-06399.

Financial Disclosure

The authors declare that they have no competing financial interests.

References

- AGRAWAL, A.; EDENBERG, H.J.; FOROUD, T., ET AL. Association of GABRA2 with drug dependence in the collaborative study of the genetics of alcoholism sample. *Behavior Genetics* 36(5):640–650, 2006. PMID: 16622805
- BART, G.; KREEK, M.J.; OTT, J.; ET AL. Increased attributable risk related to a functional mu-opioid receptor gene polymorphism in association with alcohol dependence in central Sweden. *Neuropsychopharmacology* 30(2):417–422, 2005. PMID: 15525999
- BAUER, L.O.; COVAULT, J.; HAREL, O.; ET AL. Variation in GABRA2 predicts drinking behavior in project MATCH subjects. *Alcoholism: Clinical and Experimental Research* 31(11):1780–1787, 2007. PMID: 17949392
- BIERUT, L.J.; AGRAWAL, A.; BUCHOLZ, K.K.; ET AL. A genome-wide association study of alcohol dependence. *Proceedings of the National Academy of Sciences of the United States of America* 107(11):5082–5087, 2010. PMID: 20202923
- BLEDNOV, Y.A.; BORGHESE, C.M.; MCCracken, M.L.; ET AL. Loss of ethanol conditioned taste aversion and motor stimulation in knockin mice with ethanol-insensitive alpha2-containing GABA(A) receptors. *Journal of Pharmacology and Experimental Therapeutics* 336(1):145–154, 2011. PMID: 20876231
- BOEHM, S.L.; PONOMAREV, I.; JENNINGS, A.W.; ET AL. gamma-Aminobutyric acid A receptor subunit mutant mice: New perspectives on alcohol actions. *Biochemical Pharmacology* 68(8):1581–1602, 2004. PMID: 15451402
- CHEN, A.C.; MANZ, N.; TANG, Y.; ET AL. Single-nucleotide polymorphisms in corticotropin releasing hormone receptor 1 gene (CRHR1) are associated with quantitative trait of event-related potential and alcohol dependence. *Alcoholism: Clinical and Experimental Research* 34(6):988–996, 2010. PMID: 20374216
- COVAULT, J.; GELERNTER, J.; HESSELBROCK, V.; ET AL. Allelic and haplotypic association of GABRA2 with alcohol dependence. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics* 129B(1):104–109, 2004. PMID: 15274050
- COVAULT, J.; GELERNTER, J.; JENSEN, K.; ET AL. Markers in the 5'-region of GABRG1 associate to alcohol dependence and are in linkage disequilibrium with markers in the adjacent GABRA2 gene. *Neuropsychopharmacology* 33(4): 837–848, 2008. PMID: 17507911
- CRABBE, J.C.; PHILLIPS, T.J.; HARRIS, R.A.; ET AL. Alcohol-related genes: Contributions from studies with genetically engineered mice. *Addiction Biology* 11(3–4): 195–269, 2006. PMID: 16961758
- DAS, S.; HAREL, O.; DEY, D.K.; ET AL. Analysis of extreme drinking in patients with alcohol dependence using Pareto regression. *Statistics in Medicine* 29(11):1250–1258, 2010. PMID: 20225194
- DICK, D.M.; AGRAWAL, A.; WANG, J.C.; ET AL. Alcohol dependence with comorbid drug dependence: Genetic and phenotypic associations suggest a more severe form of the disorder with stronger genetic contribution to risk. *Addiction* 102(7):1131–1139, 2007. PMID: 17567401
- DICK, D.M.; EDENBERG, H.J.; XUEI, X.; ET AL. Association of GABRG3 with alcohol dependence. *Alcoholism: Clinical and Experimental Research* 28(1):4–9, 2004. PMID: 14745296
- DICK, D.M.; PLUNKETT, J.; WETHERILL, L.F.; ET AL. Association between GABRA1 and drinking behaviors in the collaborative study on the genetics of alcoholism sample. *Alcoholism: Clinical and Experimental Research* 30(7):1101–1110, 2006. PMID: 16792556
- DIXON, C.I.; MORRIS, H.V.; BREEN, G.; ET AL. Cocaine effects on mouse incentive-learning and human addiction are linked to alpha2 subunit-containing GABAA receptors. *Proceedings of the National Academy of Sciences of the United States of America* 107(5):2289–2294, 2010. PMID: 20133874
- DIGON, T.; D'ADDARIO, C.; AND UHL, G.R. Linkage disequilibrium, haplotype and association studies of a chromosome 4 GABA receptor gene cluster: Candidate gene variants for addictions. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics* 141B(8):854–860, 2006. PMID: 16894595
- EDENBERG, H.J.; DICK, D.M.; XUEI, X.; ET AL. Variations in GABRA2, encoding the alpha 2 subunit of the GABA(A) receptor, are associated with alcohol dependence and with brain oscillations. *American Journal of Human Genetics* 74(4):705–714, 2004. PMID: 15024690
- EDENBERG, H.J.; KOLLER, D.L.; XUEI, X.; ET AL. Genome-wide association study of alcohol dependence implicates a region on chromosome 11. *Alcoholism: Clinical and Experimental Research* 34(5):840–852, 2010. PMID: 20201924
- EDENBERG, H.J.; WANG, J.; TIAN, H.; ET AL. A regulatory variation in OPRK1, the gene encoding the kappa-opioid receptor, is associated with alcohol dependence. *Human Molecular Genetics* 17(12):1783–1789, 2008. PMID: 18319328
- ENOCH, M.A. The role of GABA(A) receptors in the development of alcoholism. *Pharmacology, Biochemistry, and Behavior* 90(1):95–104, 2008. PMID: 18440057
- ENOCH, M.A.; HODGKINSON, C.A.; YUAN, Q.; ET AL. GABRG1 and GABRA2 as independent predictors for alcoholism in two populations. *Neuropsychopharmacology* 34(5):1245–1254, 2009. PMID: 18818659
- ENOCH, M.A.; HODGKINSON, C.A.; YUAN, Q.; ET AL. The influence of GABRA2, childhood trauma, and their interaction on alcohol, heroin, and cocaine dependence. *Biological Psychiatry* 67(1):20–27, 2010. PMID: 19833324
- ENOCH, M.A.; SCHWARTZ, L.; ALBAUGH, B.; ET AL. Dimensional anxiety mediates linkage of GABRA2 haplotypes with alcoholism. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics* 141B(6):599–607, 2006. PMID: 16874763
- FEHR, C.; SANDER, T.; TADIC, A.; ET AL. Confirmation of association of the GABRA2 gene with alcohol dependence by subtype-specific analysis. *Psychiatric Genetics* 16(1):9–17, 2006. PMID: 16395124
- HARRIS, R.A.; OSTERNDORFF-KAHANEK, E.; PONOMAREV, I.; ET AL. Testing the silence of mutations: Transcriptomic and behavioral studies of GABA(A) receptor alpha1 and alpha2 subunit knock-in mice. *Neuroscience Letters* 488(1): 31–35, 2011. PMID: 21056629
- HAUGHEY, H.M.; RAY, L.A.; FINAN, P.; ET AL. Human gamma-aminobutyric acid A receptor alpha2 gene moderates the acute effects of alcohol and brain mRNA expression. *Genes, Brain, and Behavior* 7(4):447–454, 2008. PMID: 18005236
- ILVESKOSKI, E.; KAJANDER, O.A.; LEHTIMAKI, T.; ET AL. Association of neuropeptide y polymorphism with the occurrence of type 1 and type 2 alcoholism. *Alcoholism: Clinical and Experimental Research* 25(10):1420–1422, 2011. PMID: 11696660
- ITTIWUT, C.; LISTMAN, J.; MUTIRANGURA, A.; ET AL. Interpopulation linkage disequilibrium patterns of GABRA2 and GABRG1 genes at the GABA cluster locus on human chromosome 4. *Genomics* 91(1):61–69, 2008. PMID: 17976953
- KAREKEN, D.A.; LIANG, T.; WETHERILL, L.; ET AL. A polymorphism in GABRA2 is associated with the medial frontal response to alcohol cues in an fMRI study. *Alcoholism: Clinical and Experimental Research* 34(12):2169–2178, 2010. PMID: 20698837
- KIM, S.A.; KIM, J.W.; SONG, J.Y.; ET AL. Association of polymorphisms in nicotinic acetylcholine receptor alpha 4 subunit gene (CHRNA4), mu-opioid receptor gene (OPRM1), and ethanol-metabolizing enzyme genes with alcoholism in Korean patients. *Alcohol* 34(2–3):115–120, 2004. PMID: 15902904
- KUMAR, S.; PORCU, P.; WERNER, D.F.; ET AL. The role of GABA(A) receptors in the acute and chronic effects of ethanol: A decade of progress. *Psychopharmacology (Berlin)* 205(4): 529–564, 2009. PMID: 19455309
- LAPPALAINEN, J.; KRANZLER, H.R.; MALISON, R.; ET AL. A functional neuropeptide Y Leu7Pro polymorphism associated with alcohol dependence in a large population sample from the United States. *Archives of General Psychiatry* 59(9):825–831, 2002. PMID: 12215082

- LAPPALAINEN, J.; KRUPITSKY, E.; REMIZOV, M.; ET AL. Association between alcoholism and gamma-amino butyric acid alpha2 receptor subtype in a Russian population. *Alcoholism: Clinical and Experimental Research* 29(4):493–498, 2005. PMID: 15834213
- LIND, P.A.; MACGREGOR, S.; MONTGOMERY, G.W.; ET AL. Effects of GABRA2 variation on physiological, psychomotor and subjective responses in the Alcohol Challenge Twin Study. *Twin Research and Human Genetics* 11(2):174–182, 2008. PMID: 18361719
- LIU, J.; YANG, A.R.; KELLY, T.; ET AL. Binge alcohol drinking is associated with GABAA alpha2-regulated Toll-like receptor 4 (TLR4) expression in the central amygdala. *Proceedings of the National Academy of Sciences of the United States of America* 108(11):4465–4470, 2011. PMID: 21368176
- LOBO, I.A., AND HARRIS, R.A. GABA(A) receptors and alcohol. *Pharmacology, Biochemistry, and Behavior* 90(1):90–94, 2008. PMID: 18423561
- LOW, K.; CRESTANI, F.; KEIST, R.; ET AL. Molecular and neuronal substrate for the selective attenuation of anxiety. *Science* 290(5489):131–134, 2000. PMID: 11021797
- LUO, X.; KRANZLER, H.R.; ZUO, L.; ET AL. CHRM2 gene predisposes to alcohol dependence, drug dependence and affective disorders: Results from an extended case-control structured association study. *Human Molecular Genetics* 14(16):2421–2434, 2005. PMID: 16000316
- MATTHEWS, A.G.; HOFFMAN, E.K.; ZEZZA, N.; ET AL. The role of the GABRA2 polymorphism in multiplex alcohol dependence families with minimal comorbidity: Within-family association and linkage analyses. *Journal of Studies on Alcohol and Drugs* 68(5):625–633, 2007. PMID: 17690794
- MOTTAGUI-TABAR, S.; PRINCE, J.A.; WAHLESTEDT, C.; ET AL. A novel single nucleotide polymorphism of the neuropeptide Y (NPY) gene associated with alcohol dependence. *Alcoholism: Clinical and Experimental Research* 29(5):702–707, 2005. PMID: 15897713
- NISHIZAWA, D.; HAN, W.; HASEGAWA, J.; ET AL. Association of mu-opioid receptor gene polymorphism A118G with alcohol dependence in a Japanese population. *Neuropsychobiology* 53(3):137–141, 2006. PMID: 16679777
- ONORI, N.; TURCHI, C.; SOLITO, G.; ET AL. GABRA2 and alcohol use disorders: No evidence of an association in an Italian case-control study. *Alcoholism: Clinical and Experimental Research* 34(4):659–668, 2010. PMID: 20102561
- OOTEMAN, W.; NAASSILA, M.; KOETER, M.W.; ET AL. Predicting the effect of naltrexone and acamprosate in alcohol-dependent patients using genetic indicators. *Addiction Biology* 14(3):328–337, 2009. PMID: 19523047
- PHILBERT, R.A.; GUNTER, T.D.; BEACH, S.R.; ET AL. Role of GABRA2 on risk for alcohol, nicotine, and cannabis dependence in the Iowa Adoption Studies. *Psychiatric Genetics* 19(2):91–98, 2009. PMID: 19672139
- PIERUCCI-LAGHA, A.; COVAULT, J.; FEINN, R.; ET AL. GABRA2 alleles moderate the subjective effects of alcohol, which are attenuated by finasteride. *Neuropsychopharmacology* 30(6):1193–1203, 2005. PMID: 15702134
- RAY, L.A., AND HUTCHISON, K.E. A polymorphism of the mu-opioid receptor gene (OPRM1) and sensitivity to the effects of alcohol in humans. *Alcoholism: Clinical and Experimental Research* 28(12):1789–1795, 2004. PMID: 15608594
- RAY, L.A., AND HUTCHISON, K.E. Associations among GABRG1, level of response to alcohol, and drinking behaviors. *Alcoholism: Clinical and Experimental Research* 33(8):1382–1390, 2009. PMID: 19426171
- ROH, S.; MATSUSHITA, S.; HARA, S.; ET AL. Role of GABRA2 in moderating subjective responses to alcohol. *Alcoholism: Clinical and Experimental Research*, 35(3):400–407, 2011. PMID: 21118274
- ROMMELSPACHER, H.; SMOLKA, M.; SCHMIDT, L.G.; ET AL. Genetic analysis of the mu-opioid receptor in alcohol-dependent individuals. *Alcohol* 24(2):129–135, 2001. PMID: 11522434
- SAKAI, J.T.; STALLINGS, M.C.; CROWLEY, T.J.; ET AL. Test of association between GABRA2 (SNP rs279871) and adolescent conduct/alcohol use disorders utilizing a sample of clinic referred youth with serious substance and conduct problems, controls and available first degree relatives. *Drug and Alcohol Dependence* 106(2–3):199–203, 2010. PMID: 19783384
- SOYKA, M.; PREUSS, U.W.; HESSELBROCK, V.; ET AL. GABA-A2 receptor subunit gene (GABRA2) polymorphisms and risk for alcohol dependence. *Journal of Psychiatric Research* 42(3):184–191, 2008. PMID: 17207817
- TAUBER, M.; CALAME-DROZ, E.; PRUT, L.; ET AL. alpha2-gamma-Aminobutyric acid (GABA)A receptors are the molecular substrates mediating precipitation of narcosis but not of sedation by the combined use of diazepam and alcohol in vivo. *European Journal of Neuroscience* 18(9):2599–2604, 2003. PMID: 14622161
- VENGELIENE, V.; BILBAO, A.; MOLANDER, A.; AND SPANAGEL, R. Neuropharmacology of alcohol addiction. *British Journal of Pharmacology* 154(2):299–315, 2008. PMID: 18311194
- VILLAFUERTE, S.; HEITZEG, M.M.; FOLEY, S.; ET AL. Impulsiveness and insula activation during reward anticipation are associated with genetic variants in GABRA2 in a family sample enriched for alcoholism. *Molecular Psychiatry*, Epub ahead of print, 2011. PMID: 21483437
- WANG, J.C.; HINRICHS, A.L.; STOCK, H.; ET AL. Evidence of common and specific genetic effects: Association of the muscarinic acetylcholine receptor M2 (CHRM2) gene with alcohol dependence and major depressive syndrome. *Human Molecular Genetics* 13(17):1903–1911, 2004. PMID: 15229186
- WERNER, D.F.; SWIHART, A.; RAU, V.; ET AL. Inhaled anesthetic responses of recombinant receptors and knockin mice harboring alpha2 (S270H/L277A) GABA(A) receptor subunits that are resistant to isoflurane. *Journal of Pharmacology and Experimental Therapeutics* 336(1):134–144, 2011. PMID: 20807777
- XUEI, X.; DICK, D.; FLURY-WETHERILL, L.; ET AL. Association of the kappa-opioid system with alcohol dependence. *Molecular Psychiatry* 11(11):1016–1024, 2006. PMID: 16924269
- XUEI, X.; FLURY-WETHERILL, L.; DICK, D.; ET AL. GABRR1 and GABRR2, encoding the GABA-A receptor subunits rho1 and rho2, are associated with alcohol dependence. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics* 153B(2):418–427, 2010. PMID: 19536785
- ZHANG, H.; KRANZLER, H.R.; YANG, B.Z.; ET AL. The OPRD1 and OPRK1 loci in alcohol or drug dependence: OPRD1 variation modulates substance dependence risk. *Molecular Psychiatry* 13(5):531–543, 2008. PMID: 17622222
- ZHANG, H.; LUO, X.; KRANZLER, H.R.; ET AL. Association between two mu-opioid receptor gene (OPRM1) haplotype blocks and drug or alcohol dependence. *Human Molecular Genetics* 15(6):807–819, 2006. PMID: 16476706

RETHINKING DRINKING

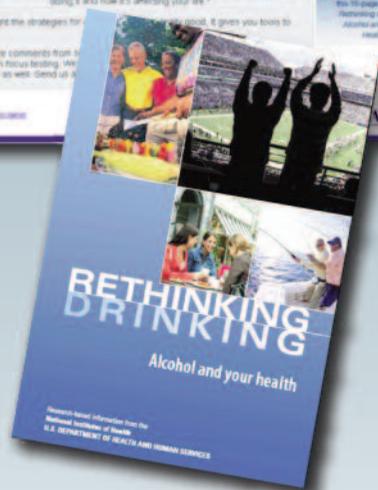
Alcohol and Your Health



Visit NIAAA's Fully Interactive Web site.

Tools to Assess and Change Risky Drinking Habits

- Information about:
 - Risky drinking patterns
 - The signs of an alcohol problem
 - Strategies for cutting back or quitting
- The Rethinking Drinking product set includes:
 - Interactive Web site with quizzes, calculators, and other tools
 - A 16-page booklet



RethinkingDrinking.niaaa.nih.gov

Download a pdf or order online (www.RethinkingDrinking.niaaa.nih.gov) or write to: National Institute on Alcohol Abuse and Alcoholism, Publications Distribution Center, P.O. Box 10686, Rockville, MD 20849-0686 Phone: 1-888-MY-NIAAA Fax: (703) 312-5230.

U.S. Department of Health and Human Services
National Institutes of Health • National Institute on Alcohol Abuse and Alcoholism