Candidate Genes for Alcohol Dependence: A Review of Genetic Evidence From Human Studies

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FAMILY, TWIN, AND adoption studies have convincingly demonstrated that genes play an important role in the development of alcohol dependence, accounting for approximately 50–60% of the population variance (McCue, 1999). Additionally, patterns of alcohol use seem to be under genetic influence. Twin studies have demonstrated that dimensions of alcohol use, such as quantity of alcohol consumed on a typical drinking occasion, frequency of use, and frequency of intoxication, and alcohol metabolism measures, such as time to peak blood alcohol concentration and rate of elimination, are under substantial genetic influence (Heath, 1995). Furthermore, there is evidence of genetic effects on patterns of alcohol use as early as adolescence, and these effects seem to increase over time (Rose et al., 2001). It is unclear to what extent the genes that influence patterns of alcohol use overlap with those that influence alcohol dependence.

Despite strong evidence for genetic effects contributing to alcoholism susceptibility, detecting the specific genes that increase or decrease the risk for alcoholism has proven difficult. Many factors contribute to the slow progress in isolating the genes involved in drinking behavior. Many genes are thought to contribute to alcoholism susceptibility, and different genes are likely contributing to alcohol dependence in different individuals. Additionally, the environment plays a substantial role in drinking patterns, with nearly half of the variance in drinking patterns and alcohol dependence attributed to environmental factors. Furthermore, these genes and environments probably interact. Data from a Finnish twin study of alcohol use among adolescents demonstrated that the magnitude of genetic influences can vary dramatically between environments, with up to 5-fold differences demonstrated in different environments (Dick et al., 2001). This suggests that some environments may exacerbate the expression of genetic predispositions, whereas others may be protective. Finally, there is substantial phenotypic heterogeneity in the manifestation of alcohol dependence, with alcoholics differing on dimensions such as age of onset of problems, alcohol symptoms, drinking history, and comorbid disorders. Some evidence suggests that genes may be more important in certain subtypes of alcoholics (Cloninger et al., 1981). Other investigators have studied endophenotypes as a means to deal with the substantial heterogeneity involved in alcohol dependence. Endophenotypes are phenotypes that are thought to be intermediaries between a particular disorder and the biological processes that lead to the manifestation of this disorder. For example, brain wave activity, as measured by electroencephalogram (EEG) and event-related potential (ERP), has been studied as an endophenotype for both alcohol dependence and schizophrenia. It is possible that genes act more directly on an endophenotype, as compared with a diagnostic classification, and, therefore, the study of endophenotypes may more efficiently lead to the identification of genes. All of these factors considerably complicate efforts to identify the genes involved in alcohol dependence and to understand the contribution of any specific gene that is identified.

A number of genetic strategies have been used in the study of alcohol dependence. These include both linkage and association studies. Linkage studies involve the ascertainment of families with multiple affected individuals; genotyping of segments of DNA that exhibit variation, called polymorphic markers, is often used to detect chromosomal regions in which affected individuals within a family demonstrate increased sharing of a particular marker allele, suggesting that there may be a gene nearby involved in the disorder. Association studies can use either families or unrelated controls; they test the association between a particular allele at a candidate gene and a specific outcome across families. Association methods typically can detect significant effects over much smaller physical distances as compared with linkage studies. For a more extensive review of the methods used in genetic studies, see Dick and Foroud (2003).

Here we review the evidence for candidate genes that have been implicated in genetic studies of alcohol dependence and related phenotypes, such as quantitative indices of alcohol use, and endophenotypes, such as EEG. This review is not meant to be exhaustive in reporting all candidate genes, but, rather, covers in detail many of the candidate genes currently thought to be most promising.
We focus primarily on evidence from human studies and supplement these findings with evidence from animal studies. More extensive and thorough reviews of findings from the animal literature are available elsewhere (Belknap and Atkins, 2001; Crabbe et al., 1994; McBride and Li, 1998). Additionally, see the accompanying article by Schumann et al. (2003b) for more detailed information on animal studies.

ALCOHOL DEHYDROGENASE (ADH) GENES

The only genes that have been consistently replicated to contribute to alcoholism susceptibility are polymorphisms in the alcohol-metabolizing enzymes: ADH and aldehyde dehydrogenase (ALDH). ADH oxidizes ethanol to acetaldehyde. There are multiple forms of ADH, but the class I isozymes are thought to play the major role in ethanol metabolism (Edenberg and Bosron, 1997). There are three ADH class I isozymes—ADH1A, ADH1B, and ADH1C (formerly called ADH1, ADH2, and ADH3)—that are closely linked on chromosome 4q22. Genetic polymorphisms have been identified in two of the three ADH class I enzymes: ADH1B has three different alleles, and ADH1C has two different alleles, each of which differs in kinetic properties. The ADH1B*2 and ADH1B*3 alleles and the ADH1C*1 allele code for subunits of proteins which, in vitro, have greater enzymatic activity, suggesting faster conversion of ethanol to acetaldehyde in individuals carrying these alleles. However, the enzymes encoded by the different alleles of the ADH1B gene have a more dramatic effect, altering the kinetic constants more than 30-fold (Bosron et al., 1983).

Allele frequencies for the ADH genes differ substantially among populations. The ADH1B*2 allele is virtually non-existent in blacks and is rare (<5%) in most white populations; however, it predominates in Asian populations (Goedde et al., 1992). The ADH1B*3 allele is largely limited to populations of African descent, occurring with a frequency of 15–20% (Bosron and Li, 1987), and to some American Indian tribes (Wall et al., 1997). The frequency of the ADH1C*1 allele is 55–60% in populations of European decent but is >90% in the Han Chinese (Shen et al., 1997).

A number of studies have reported lower frequencies of both the ADH1B*2 and ADH1C*1 alleles among alcoholics, as compared with nonalcoholics, in a variety of East Asian populations (Chen et al., 1996b; Higuchi, 1994; Maezawa et al., 1995; Muramatsu et al., 1995; Nakamura et al., 1996; Shen et al., 1997; Thomasson et al., 1991, 1994). A meta-analysis concluded that the ADH1B*1 allele is associated with an almost 3-fold increase in risk for alcohol dependence as compared with the ADH1B*2 allele (Whitfield, 1997).

Relatively few studies have found protective effects of the ADH genes among non-Asian populations, in large part due to the rarity of the protective alleles among other populations. However, a study of Jewish men living in Israel found that the ADH1B*2 allele was related to a reduced level of peak weekly alcohol intake (Neumark et al., 1998). Additionally, ADH1B*2 was associated with lower levels of both alcohol dependence and consumption in men, but not in women, in a European population (Whitfield et al., 1998). A genome-wide screen of alcoholism in the Collaborative Study of the Genetics of Alcoholism (COGA), which is largely a Caucasian population, has found evidence of linkage among unaffected individuals to the chromosome 4 region containing the ADH gene cluster (Reich et al., 1998), suggesting that a particular genetic variant may be protective. Additionally, linkage has been reported in this region to the phenotype “maximum number of drinks in a 24-hr period” in the COGA sample (Saccone et al., 2000). Finally, a genome scan comparing the frequency of the alleles at single nucleotide polymorphisms (SNPs) between unrelated control individuals and individuals with histories of illegal substance use and/or dependence found a significant difference for a SNP located near the ADH gene cluster. Together, these findings suggest that the effects of the ADH genotype on alcohol use (and perhaps other drug use) are not unique to Asian individuals. Rather, it seems that the substantial role of the ADH1B locus was detected in the Asian population due to the higher frequency of the protective allele in that group.

There is debate regarding whether the effect of the ADH1C genotype is independent of the ADH1B genotype or whether the observed differences between alcoholics and controls with the ADH1C genotype can be attributed solely to linkage disequilibrium between the ADH1B and ADH1C genes (Whitfield, 1997). A large investigation of 340 alcoholics and 545 controls in a Han Chinese population was undertaken to resolve this question (Chen et al., 1999). That study concluded that polymorphisms at ADH1C exerted no significant effect on the risk for alcohol dependence and attributed previous reports of the effect of ADH1C to linkage disequilibrium with ADH1B. A subsequent analysis of haplotype frequencies in Taiwanese Chinese alcoholic individuals and controls also concluded that the association with alcoholism was due to ADH1B and that associations shown with ADH1C were most likely due to linkage disequilibrium with ADH1B (Osiar et al., 2002).

The ADH1B*3 allele, found almost exclusively in black populations, seems to be protective against alcohol-related birth defects and, indirectly, against alcoholism in this population. Drinking during pregnancy was associated with lower scores on an infant mental development index, but only in the offspring of African American mothers without an ADH1B*3 allele. Children of mothers who drank during pregnancy, but who had an ADH1B*3 allele, scored no differently than children of mothers who did not drink during pregnancy (McCarver et al., 1997). This may illustrate a gene × environment interaction, whereby the deleterious effects of a potentially dangerous in utero environment are enhanced in the presence of a particular genetic
variant. It was proposed that this protective effect was afforded by the more efficient alcohol metabolism provided by the $ADH1B^*$3 allele. In another sample of young adult African Americans, the $ADH1B^*$3 allele was significantly associated with a negative family history of alcoholism (Ehlers et al., 2001). Because family history of alcoholism is a strong predictor of alcohol problems, this association suggests that the $ADH1B^*$3 allele may be protective against the development of alcoholism. Although there was no association between the $ADH1B^*$3 allele and self-reported history of drinking in the sample, more than half of the small, young sample of 97 individuals did not drink regularly, limiting the power to directly test for this association. Finally, in a sample of individuals of mixed ancestry from the Western Cape Province of South Africa, where fetal alcohol syndrome (FAS) is particularly common, the $ADH1B^*$2 allele was significantly increased among individuals in a control group, as compared with individuals with FAS and their mothers (Viljoen et al., 2001). These findings suggest that the $ADH1B^*$2 allele may confer protective effects against FAS in this population. The $ADH1B^*$3 allele was infrequent and not significantly different between groups in this study.

**ALDH Genes**

After the metabolism of ethanol to acetaldehyde by the ADH enzymes, acetaldehyde is converted to acetate by ALDH. There are nine major gene families coding for human ALDH (Agarwal, 2001). Only class I and class II isozymes ($ALDH1$ and $ALDH2$) are thought to be centrally involved in the oxidation of acetaldehyde (Ramchandani et al., 2001). $ALDH2$, the low-$K_m$ form of ALDH found in mitochondria, is thought to be primarily responsible for acetaldehyde oxidation, because it has high catalytic efficiency, which $ALDH1$ does not (Ramchandani et al., 2001). Thus, genetic studies have focused largely on $ALDH2$, which has been localized to chromosome 12 and exhibits notable genetic variation. The enzyme subunit produced by the $ALDH2^*$2 allele renders enzyme tetramers into which it is incorporated comparatively inactive, and, thus, it acts as a dominant negative allele. Heterozygotes have facial flushing and other aversive symptoms when alcohol is consumed. The $ALDH2^*$2 allele is nearly absent in whites and blacks, but it is considerably more common in Asians, with up to 43% of the Japanese population carrying this allele (Goedde et al., 1992). Although the other $ALDH$ gene families have not been studied to the extent that $ALDH2$ has, genetic variation has been found in several additional $ALDH$ genes (Agarwal, 2001), raising the possibility that other ALDH genes may also be involved in alcohol consumption and related disorders.

A potential role for the involvement of $ALDH2$ in alcohol dependence was detected as early as 1982, when Harada et al. (1982) reported that ALDH2 deficiency was substantially lower among Japanese alcoholics, suggesting that the deficient $ALDH2^*$2 allele may play a protective role by reducing the risk of alcohol dependence. Subsequent studies have also reported reduced rates of the $ALDH2^*$2 allele among alcoholics in Asian populations (Chen et al., 1996b; Higuchi, 1994; Lee et al., 2001; Maezawa et al., 1995; Muramatsu et al., 1995; Nakamura et al., 1996; Shen et al., 1997; Thomasson et al., 1991, 1994). $ALDH2^*$2 confers up to a 10-fold reduction in the risk of alcohol dependence (Thomasson et al., 1994), giving it a stronger protective effect than either the $ADH1B$ or $ADH1C$ genes (Chen et al., 1996b; Shen et al., 1997). The effect of the $ADH1B$ genotype seems to be independent from, and additive to, that of the $ALDH2$ locus (Chen et al., 1996b; Nakamura et al., 1996).

**γ-Aminobutyric Acid (GABA) Receptor Genes**

GABA is the major inhibitory neurotransmitter in the human central nervous system. There are two primary types of GABA receptors: GABA$_A$ receptors and GABA$_B$ receptors. The GABA$_A$ receptors act through intrinsic ion channels; the receptor is composed of multiple subunits, designated $\alpha$, $\beta$, $\gamma$, $\delta$, $\rho$, and $\epsilon$, with several identified genes coding for these subunits (Buck, 1996). Most of the GABA$_A$ receptor genes are organized into clusters. Chromosome 4 contains the genes GABRA2, GABRA3, GABRB1, and GABRG1; chromosome 5 contains GABRA1, GABRA6, GABRB2, and GABRG2; and chromosome 15 contains GABRA5, GABRB3, and GABRG3 (National Center for Biotechnology Information, LocusLink). The GABA$_B$ receptors act through $G$ proteins; less is known about their genetic architecture.

Several lines of evidence suggest that GABA is involved in many of the behavioral effects of alcohol, including motor incoordination, anxiolysis, sedation, withdrawal signs, and ethanol preference (Buck, 1996; Grobin et al., 1998). GABA$_A$ receptor agonists tend to potentiate the behavioral effects of alcohol, whereas GABA$_A$ receptor antagonists attenuate these effects. GABA$_A$ receptors have also been implicated in ethanol tolerance and dependence (Grobin et al., 1998). The precise mechanisms by which GABA reception is involved in these actions of ethanol remain unknown (Grobin et al., 1998). The role of GABA$_B$ receptors in the actions of ethanol has not been studied nearly as extensively as that of GABA$_A$ receptors.

A large genome-wide scan of multiplex families segregating for alcohol dependence has yielded evidence of linkage to a chromosomal region containing genes coding for GABA receptors. Long et al. (1998) found evidence of linkage to chromosome 4p, near the $\beta$ GABA receptor gene ($GABRB1$), among a population of Southwestern American Indians. By use of a case-control design, $GABRB1$ was also significantly associated with alcohol dependence (Parsian and Zhang, 1999); this association remained significant when the analyses were limited to a smaller sample of alcoholics who were characterized by an
earlier age of onset of problems, antisocial behavior, and high novelty seeking (Cloninger, 1987). Association of the GABRB1 gene with alcoholism has also been investigated with a family-based design as part of the COGA study (Song et al., 2003). Family-based association tests avoid the potential problems with population stratification that may exist in population-based, case-control designs. Modest linkage disequilibrium was found between GABRB1 and alcohol dependence, as defined by the COGA criteria of concurrent DSM-III-R and Feighner diagnoses. Additional evidence of association was also observed with the more restrictive ICD-10 criteria for alcohol dependence (Song et al., 2003). A study by Uhl et al. (2001) of SNP differences between drug abusers and controls also found evidence of association with a SNP near the GABAA receptor gene region implicated in the Long et al. (1998) and COGA studies.

The COGA study has also collected electrophysiological data in families of alcoholics. There is evidence suggesting that EEG and ERP differences exist in families of alcoholics (Porjesz et al., 1998). These findings support the use of these brain wave variations as endophenotypes for the study of the genetics of alcoholism susceptibility. Using these quantitative phenotypes in linkage analyses may be more powerful than using a dichotomous disease status (affected/unaffected), for several reasons. First, information from all family members can be used in the genetic analyses, rather than limiting the analyses to only affected individuals. In addition, some endophenotypes, such as the EEG or ERP, have substantially higher heritability as compared with the dichotomous alcoholism phenotype and so may prove to be a more powerful phenotype for genetic studies. In the COGA study, analysis of EEG phenotypes has provided evidence of linkage and association to chromosome 4 at a marker in the GABRB1 gene (Porjesz et al., 2002).

There is also evidence of association between GABAA receptor genes on chromosome 15 and alcohol dependence. In a case-control study of Caucasian alcoholics and controls, an association was reported between GABRB3 and severe alcoholism, as defined by documented alcohol-induced bodily damage, such as cirrhosis (Noble et al., 1998). Furthermore, there was a significant, progressive decrease in the prevalence of the most frequent allele of GABRB3 as one considered nonalcoholics, less-severe alcoholics, and severe alcoholics, respectively. Following evidence from a study demonstrating that GABRB3, GABRA5, and GABRG3 were only expressed from the paternal alleles in hybrid mouse A9 cells containing a single human chromosome 15 (Meguro et al., 1997), Edenberg and colleagues tested for paternal transmission of GABRA5 and GABRB3 in the COGA sample (Song et al., 2003); they found significant evidence of association of both genes with ICD-10–defined alcoholism when only paternal transmission of alleles was studied.

A number of groups have investigated the role of the GABA_A receptor genes located on chromosome 5, with mixed results. Hsu et al. (1998) found no significant association of GABRG2 with a case-control association design among the Han Chinese in Taiwan. Subsequently, positive findings were reported for GABRG2 by using a case-control design among Japanese individuals meeting criteria for DSM-III-R alcohol dependence, when dependence was comorbid with antisocial personality disorder (Loh and Ball, 2000). GABRG2 has also been linked to alcohol dependence in a Finnish population (Radel et al., 1999). No association was found by Loh and Ball (2000) with the other GABA_A subunit genes located on chromosome 5q: GABRA6, GABRA1, and GABRB2. Sander et al. (1999a) investigated polymorphisms of the chromosome 5 GABA_A receptors in a German population and found an association between GABRA6 and alcohol dependence with comorbid antisocial personality disorder. No associations were reported for GABRB2 and GABRG2. In a small case-control study, Schuckit et al. (1999) found that a genetic polymorphism in GABRA6 was associated with a lower level of response to alcohol and higher rates of alcoholism. In a Scottish population, associations were found between GABRA6 and GABRB2 and alcohol dependence (Loh et al., 1999). The genes encoding GABRA6 and GABRA1 were also investigated by using a family-based design as part of the COGA study (Song et al., 2003); neither of these genes provided evidence of association. Finally, Parsian and Cloninger (1997) compared allele frequencies at the GABRA1 gene on chromosome 5 and GABRA3 on chromosome X between unrelated alcoholics and psychiatrically normal controls. No difference was found for GABRA1; however, a significant difference was found for GABRA3. A small sample of alcoholics and their parents was also genotyped for within-family analyses, and neither gene was significant in the haplotype relative risk analyses, suggesting that the results on chromosome X may be spurious. Notably, neither of the genome-wide screens for alcohol dependence mentioned previously (Long et al., 1998; Reich et al., 1998) found evidence of linkage to the cluster of GABA receptor genes on chromosome 5q.

To our knowledge, only one study has investigated the role of GABA_B receptor genes and alcohol dependence. The GABAB1R1 receptor gene was cloned in 1997 and has been localized to chromosome 6p21.3. The frequencies of three polymorphisms in this gene were investigated in a sample of German ICD-10 alcohol-dependent individuals and controls (Sander et al., 1999c). None of the tested variants differed significantly between the groups, although trends toward association were found with alcoholics who also met criteria for ICD-10 dissocial personality disorder.

Evidence for the involvement of the GABA receptors in alcohol consumption can also be found in animal studies by using strains that differ in alcohol preference and other behaviors related to alcohol consumption (Crabbe et al., 1994). Genes related to alcohol-withdrawal severity have been mapped to murine chromosome 11, which contains...
the Gabra6, Gabra1, and Gabrg2 GABA<sub>A</sub> receptor genes (Buck et al., 1997). A polymorphism has been identified in the Gabrg2 receptor gene that is correlated with alcohol-withdrawal severity, ethanol-conditioned taste aversion, ethanol-induced motor incoordination, and ethanol-induced hypothermia (Buck and Hood, 1998; Hood and Buck, 2000). Linkage has also been reported on mouse chromosome 2, near the Gad1 gene, which encodes the enzyme synthesizing GABA (Buck et al., 1997; Hitzemann et al., 1998; Rodriguez et al., 1995). Additionally, quantitative trait loci involved in alcohol consumption, locomotor activation, and alcohol withdrawal severity have been mapped to murine chromosomes 5, 6, 7, and X in the vicinity of GABA<sub>A</sub> receptor genes [reviewed in Buck (1996)].

Thus, several converging lines of evidence suggest a role of the GABA receptors in alcohol use and dependence. Table 1 summarizes the genetic studies that have investigated the GABA<sub>A</sub> receptor genes in humans; animal studies that found complementary evidence are also included. There is consistent support for the involvement of GABRB1 on human chromosome 4 and the GABA<sub>A</sub> receptor genes on chromosome 15, although only a limited number of studies have investigated these genes. There is less consistent evidence for the involvement of the chromosome 5 GABA<sub>A</sub> receptor genes in humans, although animal work performed on homologous chromosomal regions in mice has provided consistent positive evidence. Further studies are needed to elucidate the exact nature of GABA involvement and the specific receptor genes that are involved.

**DOPAMINE**

**Dopamine Receptors**

Dopamine has long been believed to play an important role in alcoholism due to its involvement in reward behavior (Wise and Rompre, 1989). It is thought that alcohol's rewarding effects are mediated through the mesolimbic dopamine system. There are five dopamine receptors. The dopamine D2 receptor gene (DRD2) is located presynaptically and regulates dopamine release and synthesis. It has been most widely studied in relation to alcohol dependence and has been the focus of substantial controversy. Blum et al. (1990) first reported an association between DRD2 and alcoholism. Studying 70 brain samples from alcoholics and nonalcoholics, the A1 allele of DRD2 correctly classified 77% of the alcoholics, and the absence of the A1 allele correctly classified 72% of the nonalcoholics. This association has been replicated by several groups (Amadeo et al., 1996).

### Table 1. Summary of Evidence From Genetic Studies of GABA<sub>A</sub> Receptor Genes

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<tr>
<th>Chromosome</th>
<th>Gene</th>
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<th>Method</th>
<th>Positive reports</th>
<th>Negative reports</th>
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<td>4</td>
<td>GABRB1</td>
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<td>Linkage analysis</td>
<td>Long et al., 1998</td>
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<td>Alcohol dependence</td>
<td>Case control</td>
<td>Parsian and Zhang, 1999</td>
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<td>Alcohol dependence</td>
<td>Family based</td>
<td>Song et al., 2003</td>
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<td>EEG</td>
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<td>5</td>
<td>GABRG2</td>
<td>Alcohol dependence with ASPD</td>
<td>Case control</td>
<td>Loh and Ball, 2000</td>
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<td>Alcohol dependence with criminality</td>
<td>Linkage analysis</td>
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<td>Alcohol dependence with ASPD</td>
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<td>(11)</td>
<td>Gabrg2</td>
<td>Alcohol withdrawal severity</td>
<td>B6, D2, BXD RI</td>
<td>Buck et al., 1997; Buck and Hood, 1998; Hood and Buck, 2000</td>
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<td>GABRA6</td>
<td>Case control</td>
<td>Sander et al., 1999a</td>
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<td>Alcohol dependence with ASPD</td>
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<td>Alcohol dependence; dependence with Korsakoff's syndrome</td>
<td>Case control</td>
<td>Loh et al., 1999</td>
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<td>15</td>
<td>GABRB3</td>
<td>Severe alcoholism</td>
<td>Case control</td>
<td>Noble et al., 1998</td>
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<td>GABRA5</td>
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The chromosomes for mouse homologs of the corresponding human genes are indicated in parentheses. ASPD, antisocial personality disorder.
that were not subsequently confirmed in the family-based studies, in which they found positive associations with two of the studies also conducted population-based analyses (Neiswanger et al., 1995a; Parsian et al., 1991). Interestingly, negative and found no association between the
two positive studies using both population-based (Dobashi et al., 1997; Parsian et al., 2001) and family-based (Parsian et al., 1997) designs using both population-based (Dobashi et al., 1997; Parsian et al., 1997) and family-based (Parsian et al., 1997) designs for the dopamine D3 and D4 receptor genes. Another group found a positive association between a DRD3 variant and alcohol dependence with delirium, but no associations were found between the D3 or D1 receptor genes and the entire group of alcoholics (Sander et al., 1995). Finally, no association was found between the dopamine D4 receptor gene and alcohol dependence when this was tested in three groups of Taiwanese alcoholics (Chang et al., 1997).

**Dopamine Transporter (DAT)**

Animal studies have found that chronic alcohol consumption alters DAT functioning, suggesting involvement of DAT in the development of alcohol tolerance (Yoshimoto et al., 2000). A small number of groups have investigated the role of DAT in alcohol dependence. Comparing Japanese alcoholics with controls, there was a trend toward an association between DAT and alcohol dependence (Dobashi et al., 1997). Another group tested DAT densities among late-onset type 1 alcoholics versus healthy controls; they found DAT occupancy ratios to be significantly lower among alcoholics (Repo et al., 1999). However, with a family-based approach, no association was detected between DAT and alcoholism, even when alcoholics were stratified on the basis of severity (Franke et al., 1999). Additionally, a study comparing alcohol-dependent individuals who had withdrawal symptoms and healthy controls found no significant difference in a polymorphism in the DAT gene among four aboriginal groups and the Han Chinese in Taiwan (Chen et al., 2001). Additional research is needed to clarify any role of DAT in alcohol dependence.

**SEROTONIN (5-HYDROXYTRYPTAMINE; 5-HT)**

5-HT is thought to be involved in many aspects of alcohol consumption, abuse, and dependence. Pharmacological agents that increase 5-HT cause a reduction in alcohol self-administration in both rats and humans (Sellers et al., 1992). The gene encoding the 5-HT transporter (HIT) has been mapped to human chromosome 17q11.2 (Gelernter et
It exhibits functional polymorphism, with the shorter allele demonstrating lower transcriptional efficiency. An association between the short allele of HTT and anxiety-related personality traits has been reported (Lesch et al., 1996), supporting the idea that HTT may play a role in alcohol use via its involvement in harm avoidance (Cloninger, 1987).

A number of studies have investigated the role of HTT, with contradictory results (Table 2). In a case-control study of German alcohol-dependent subjects with a history of withdrawal seizure or delirium, the frequency of the short allele was found to be increased among alcoholic subjects (Sander et al., 1997). A subsequent study comparing alcohol-dependent patients and controls also found a higher frequency of the short allele of HTT among patients (Hammoumi et al., 1999). Another study found an increased frequency of the short allele among habitually violent type 2 alcoholics, as compared with type 1 alcoholics and normal controls (Hallikainen et al., 1999). A family-based association study also found support for an association between the short allele of HTT and alcohol dependence (Lichtermann et al., 2000). However, a number of studies have found positive results supporting the role of the long allele of HTT in alcohol use. A small, preliminary study of the level of response to alcohol found that individuals homozygous for the long HTT allele had lower levels of response to alcohol and the sample had a higher proportion of alcoholics (Schuckit et al., 1999). A case-control study of alcoholics also found a higher frequency of the long allele among alcoholics as compared with controls; this association became more significant when limited to type II alcoholics (Parsian and Cloninger, 2001). However, no associations remained significant after correcting for multiple testing (Parsian and Cloninger, 2001). The Schuckit study, previously mentioned in relation to HTT (Schuckit et al., 1999), found no evidence of association with the 5-HT2A gene and alcohol dependence (Cigler et al., 2001; Gorwood et al., 2002), even when limited to alcoholism comorbid with antisociality (Gorwood et al., 2002). However, linkage has been reported to mouse chromosome 9 with a variety of alcohol-related phenotypes, such as alcohol consumption and alcohol-induced hypothermia, in a region containing the 5-HT1B receptor gene (Cigler et al., 1994).

Thus, the role of the 5-HT genes in alcohol use and dependence remains unclear. The role of the HTT gene is controversial, with studies reporting association to alcohol dependence and drinking behavior with each of the two alleles. Furthermore, significant findings with subtypes of alcoholism should be interpreted cautiously due to the possibility of spurious association resulting from multiple comparisons and data mining. There is currently little sup-

<table>
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port for the role of the 5-HT receptor genes and additional genes involved in the serotonergic pathway.

NEUROPEPTIDE Y (NPY)

Relative to genes involved in alcohol metabolism or GABAergic, dopaminergic, and serotonergic function, NPY has only recently been proposed as a candidate gene for alcohol dependence. Studies in selectively bred rats and knock-out mice have provided evidence that genetic variation in NPY contributes to alcohol consumption (Carr et al., 1998; Thiele et al., 1998, 2002). An association of alcohol use and NPY has also been demonstrated in humans: a genetic variant in NPY was associated with higher alcohol consumption in samples ascertained from eastern Finland (Kauhanen et al., 2000). This finding has been replicated in a recent case-control study of European Americans, in which the Pro7 allele of NPY was more than twice as prevalent in two independently collected samples of alcohol-dependent individuals than in controls (Lappalainen et al., 2002). However, another case-control study found that the same genetic variant was significantly lower in alcoholic subjects than in controls (Ilyeskoski et al., 2001). An additional case-control study found no association between genetic variants in NPY in male Japanese alcoholics and controls; however, there was a significant difference between alcoholic patients with and without seizures (Okubo and Harada, 2001). These studies differ methodologically in several ways, including the use of quantitative versus qualitative indices of alcohol use/dependence, the use of nonselected and selected samples, and the use of individuals of different ethnicities. However, the contrasting results underscore the need for further research to elucidate the role of this potentially promising gene in human drinking behavior and alcohol dependence. Recently, it has also been demonstrated that NPY plays a role in the human stress response, providing another interesting pathway by which NPY may be involved in alcohol use (Heilig and Thorsell, 2002; Morgan et al., 2000, 2002).

CONCLUSIONS

Although a substantial genetic component for drinking behavior and alcohol dependence has been established, the complexity of alcohol-related traits has made the path to specific gene identification arduous. Despite this, several promising candidate genes are emerging. \textit{ADH1B} and \textit{ALDH2} remain the only genes with definitively established contributions to alcohol dependence. The effects of these genes seem to be additive, with \textit{ALDH2} having a stronger effect. There is also a preliminary suggestion that the \textit{ADH1B}^{*3} allele, found primarily in black populations, and, perhaps, the \textit{ADH1B}^{*2} allele may be protective against adverse alcohol-related outcomes, such as impaired mental ability and FAS.

Evidence from both human and animal studies is accumulating to implicate the GABA\textsubscript{A} receptor genes as likely candidates in alcohol use and dependence. There is consistent evidence for the involvement of the GABA\textsubscript{A} receptor genes on chromosomes 4 and 15, and there is more mixed evidence for the GABA receptors on chromosome 5, with both positive and negative reports. Only a limited number of studies have investigated the chromosome 4 and 15 GABA\textsubscript{A} receptor genes; clearly, continued research is needed on these potentially promising genes.

There has been considerable debate regarding the role of the dopamine D2 receptor in alcohol dependence. The bulk of the current evidence, especially accumulating negative reports from family-based studies, does not seem to support a strong role for \textit{DRD2}; rather, it seems that if \textit{DRD2} is involved in alcohol dependence it is through a broader, as yet undefined, addictive/compulsive phenotype. There is little evidence to support the role of other dopamine receptor genes in alcohol dependence. Modest evidence of association with \textit{DAT} in a small number of studies warrants further research to understand whether \textit{DAT} plays a role in alcohol dependence.

The role of the \textit{HTT} gene in alcohol dependence remains controversial, with several positive reports of association to each of the \textit{HTT} alleles and with other investigators finding no association at all. If \textit{HTT} plays a role in alcohol dependence, it is clearly a complex one, of which we have limited understanding. There is currently no evidence of association with the 5-HT receptor genes that have been studied.

Promising new candidate genes, such as \textit{NPY} (Carr et al., 1998; Thiele et al., 1998, 2002), cyclic adenosine monophosphate/protein kinase A (Heberlein, 2000), and protein kinase C (Bowers et al., 1999), are also emerging from the animal literature, and their involvement in drinking behavior and alcohol dependence in humans warrants further study. Although this review has concentrated on the candidate genes that have received the most study thus far, other candidate genes certainly exist and are only beginning to be characterized. For example, a variant in the enzyme fatty acid amide hydrolase has been recently associated with problematic drug and alcohol use in humans (Sipe et al., 2002). Additionally, a cannabinoid CB1 receptor antagonist recently has been demonstrated to abolish the increase in alcohol intake that occurs in rats after a period of imposed abstinence, suggesting that this receptor may play a role in alcohol relapse (Serra et al., 2002). An analysis of genetic variations of protein tyrosine kinase fyn showed an association of a fyn genotype with alcohol dependence in a group of 430 patients and 365 controls (Schumann et al., 2003). Protein tyrosine kinase fyn has been shown to modulate the activity of the alcohol-sensitive NR2A and NR2B subunits of the NMDA receptor (Cheung and Gurd, 2001), suggesting that it may be important for mediating the glutamatergic effects of ethanol. There is also some suggestion that genetic variation in the glutamate transporter \textit{EAAT2} gene might contribute to vulnerability to risk-taking behav-
ior in alcoholics (Sander et al., 2000). Thus, many other promising genes likely still have yet to be defined.

Until recently, researchers have been limited in their genetic studies by the availability of potential candidate genes in their linked regions. In addition, very little was known of the genetic variation within potential candidate genes. The sequencing of the human genome will make the cataloging of human genes and genetic variation available to all researchers. It is anticipated that this will then rapidly advance the association of candidate genes with alcoholism (see discussion of methodology in the accompanying Schumann article). Once replicable associations are established, it will still remain a challenge to identify the causative genetic variant responsible for the role of that gene in alcohol dependence. In vitro studies will be needed to conclusively demonstrate that a genetic variant is causally related to variation in a gene’s product, function, or level of expression. However, identifying these genes and understanding their pathways may lead to early intervention for individuals at risk for alcohol dependence and may lead to the development of more effective treatment of alcoholic patients, making this an important research pursuit.

REFERENCES


