Alcoholism: genes and mechanisms

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Alcoholism is a chronic relapsing/remitting disease that is frequently unrecognized and untreated, in part because of the partial efficacy of treatment. Only approximately one-third of patients remain abstinent and one-third have fully relapsed 1 year after withdrawal from alcohol, with treated patients doing substantially better than untreated [1]. The partial effectiveness of strategies for prevention and treatment, and variation in clinical course and side effects, represent a challenge and an opportunity to better understand the neurobiology of addiction. The strong heritability of alcoholism suggests the existence of inherited functional variants of genes that alter the metabolism of alcohol and variants of other genes that alter the neurobiologies of reward, executive cognitive function, anxiety/dysphoria, and neuronal plasticity. Each of these neurobiologies has been identified as a critical domain in the addictions. Functional alleles that alter alcoholism-related intermediate phenotypes include common alcohol dehydrogenase 1B and aldehyde dehydrogenase 2 variants that cause the aversive flushing reaction; catechol-O-methyltransferase (COMT) Val158Met leading to differences in three aspects of neurobiology: executive cognitive function, stress/anxiety response, and opioid function; opioid receptor μ1 (OPRM1) Asn40Asp, which may serve as a gatekeeper molecule in the action of naltrexone, a drug used in alcoholism treatment; and HTTLPR, which alters serotonin transporter function and appears to affect stress response and anxiety/dysphoria, which are factors relevant to initial vulnerability, the process of addiction, and relapse.

Alcoholism is a classic pharmacogenomic disease in the obvious sense that the intake of a drug is essential to the onset and perpetuation of the illness, and because alcoholism, like certain other substance dependencies (nicotine and opioid addiction), is moderately to highly heritable. Large, methodologically sound studies in twins, buttressed by family and adoption studies, have revealed that alcoholism is more than 50% heritable. The inherited vulnerability is both substance-specific and nonspecific [2], as well as both pharmacokinetic (involving differences in drug absorption, distribution, and metabolism) and pharmacodynamic (involving differences in drug response). The challenge for the genetics of alcoholism is to identify functional genetic variants that confer vulnerability or protection. A somewhat overlapping task is to define those polymorphisms that influence the response of an alcoholic to treatment, affecting efficacy or compliance.

Genetic polymorphisms known or predicted to affect the risk for alcoholism or individual response to treatment highlight pharmacokinetic and pharmacodynamic mechanisms but are only examples of the promise of functional genes in alcoholism. The importance of other polymorphisms is not excluded, and because there are many routes to alcoholism vulnerability and many pathways to recovery, it is anticipated that many genetic loci will play a role. The reader is also referred to recent comprehensive reviews that more completely describe, and reference, particular areas; for example, the role of genetic variation in dopamine receptors in humans [3,4] and candidate gene identification in animal models [5]. This paper focuses on common functional variations in humans and mechanisms by which these variations mediate vulnerability.

Genes and mechanisms

Two intermediate phenotypes (mediating traits) or endophenotypes (heritable intermediate phenotypes) that are predictive of the development of alcoholism are the alcohol-induced flushing reaction and acute level of response (LR) to alcohol. Alcohol-induced flushing is an aversive response to alcohol that is common among Orientals, and may include cutaneous flush, increased skin temperature, decreased blood pressure, tachycardia, dizziness, anxiety, nausea, headache, and generalized weakness [6]. The flushing reaction is protective against alcoholism. LR to

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Table 1. Nomenclature of ADH and ALDH genes and functional loci.

Family	Nomenclature new (former)	Polymorphism	Alleles	Peptides
ADH	*ADH1B* (*ADH2*)	Arg47His	Arg47 (*ADH2*1*)	β_1
			His47 (*ADH2*2*)	β_2
		Arg369Cys	Cys369 (*ADH2*3*)	β_3
	ADH1C (*ADH3*)	Arg271Gln	Arg271 (*ADH3*1*)	γ_1
			Gln271 (*ADH3*2*)	γ_2
		Ile349Val		
		Pro351Thr		
ALDH	*ALDH1*: cytosolic			
	ALDH2: mitochondrial	Glu487Lys	Glu487 (*ALDH2*1*)	
			Lys487 (*ALDH2*2*)	

ADH: Alcohol dehydrogenase; ALDH: Aldehyde dehydrogenase.

alcohol is a continuous trait measured as subjective intoxication, motor coordination and steadiness, and endocrine reaction to the drug. LR to alcohol is generally lower in the offspring of alcoholics. Low LR to alcohol in male college students predicted risk of alcoholism irrespective of family history [7]. Heritability of LR to alcohol is 50–60%, as evidenced by studies carried out in both the USA [8,9] and Australia [10]. The origins of variation in these alcoholism-related intermediate phenotypes are partially understood at the gene level. Alcohol-induced flushing is primarily a pharmacokinetic trait and LR to alcohol is primarily a pharmacodynamic trait. Two other intermediate phenotypes, frontal lobe function and anxiety/stress reactivity, will also be discussed.

Pharmacokinetic domain

A total of ~ 90–98% of the ethanol entering the body is completely oxidized, principally in the liver. Some 80% is metabolized by alcohol dehydrogenases (ADHs), which are zinc-containing oxidoreductases that use NAD as a hydrogen acceptor.

In humans there are five ADH classes (I–V). Three class I ADH enzymes (ADH1A–C), formerly ADH1, ADH2, and ADH3 [11], have a low Michaelis constant (K_m) for ethanol (< 5 mM). ADH1A, -1B and -1C are abundant in the liver [12], constituting ~ 3% of soluble liver protein. Class I ADHs are cytosolic homo/heterodimeric enzymes assembled from sequence-similar α, β and γ subunits encoded by the *ADH1A*, *ADH1B* and *ADH1C* genes (Table 1). Ethanol metabolism in the brain is modest; the class I ADH enzymes are not expressed there to any detectable extent. Substantial first pass elimination of ethanol also occurs in the stomach, where ethanol is metabolized by high V_{max} (velocity of enzyme-catalyzed reaction at infinite concentration of substrate) class IV ADH (formerly ADH7) [13].

Catalase and cytochrome P450 2E1 (CYP2E1) are unlikely to contribute substantially to variation in the overall ethanol elimination rate but seem to play an important role in the brain. Catalase accounts for less than 10% of alcohol catabolism in the liver but for 50–60% in the brain. Peripherally, acetaldehyde is aversive, but in the brain, acetaldehyde may contribute to the psychoactive (rewarding and reinforcing) effects of ethanol [14]. Therefore, higher catalase activity might lead to an increased reward. The *-262C>T* variant (Table 2), a common, functional polymorphism, is found in the promoter region of the catalase gene (11p13) [15]. However, in one study available, to date, this locus was linked to neither response to alcohol nor alcoholism [16].

CYP2E1 is inducible by ethanol up to 10-fold and, therefore, has a more important role in the metabolism of ethanol in alcoholics and heavy drinkers. A polymorphism (an *Rsa*I restriction fragment length polymorphism [RFLP]) in the 5′ flanking region (Table 2) of *CYP2E1* (10q24.3-qter) leads to higher transcriptional activity and increased enzymatic activity, and the higher activity allele (*Rsa*I *c2*) was linked to an increased risk of alcoholism in Mexican–American men [17] and to greater alcohol consumption in middle-aged Japanese men [18]. These findings suggest that a higher rate of acetaldehyde production in the brain might increase the risk for alcoholism.

Acetaldehyde is a chemically reactive and biologically active molecule, which, in peripheral tissues, stimulates the aversive flushing reaction

Table 2. Candidate genes for alcoholism in the pharmacokinetic domain.

Gene	Polymorphism	Functionality	Potential effects	Allele frequencies*
ADH1B	Arg47His	Higher V_{max}	Flushing/protection against alcoholism and fetal alcohol syndrome	0.95/0.05 to 0.99/0.01 0.20/0.80 to 0.40/0.60 (East Asians) 0.75/0.25 (Ashkenazi Jews)
	Arg369Cys	Higher V_{max}	Protection against alcoholism/alcohol-related birth defects	0.75/0.25 (African–Americans) 0.94/0.06 to 0.97/0.03 (Mission Indians)
ADH1C	Arg271Gln	Higher V_{max}	Protection against alcoholism	0.50/0.50 to 0.60/0.40 0.90/0.10 (East Asians) 0.90/0.10 (African–Americans)
	Ile349Val	Unknown		0.60/0.40 (Southwest American Indians)
	Pro351Thr	Unknown		0.74/0.26 (Native Americans)
ALDH2	Glu487Lys	Inactive enzyme	Flushing/protection against alcoholism	0.60/0.40 (East-Asians)
Catalase	-262C/T	Higher enzyme level	Reward	0.72/0.28
CYP2E1	-1053C/T	Higher enzyme activity	Reward	0.95/0.05 0.89/0.11 (Mexican Americans) 0.75/0.25 (East Asians) 0.95/0.05 (African–Americans)
HNMT	Thr105Ile	Reduced enzyme activity	Altered intensity of flushing	0.90/0.10 0.94/0.06 (East Asians)

Frequency of common allele/frequency of rarer allele in Caucasians if otherwise not stated.
ADH: Alcohol dehydrogenase; ALDH: Aldehyde dehydrogenase; CYP: Cytochrome P450; HNMT: Histamine N-methyltransferase;
V_{max}: Velocity of enzyme-catalyzed reaction at infinite concentration of substrate.

described above. Aldehyde dehydrogenases (ALDHs), principally the low-affinity ALDH1 (K_m = 33 μM), which is a cytoplasmic enzyme, and ALDH2 (K_m = 0.2 μM), which is a high-affinity mitochondrial enzyme [12], play the major role in the catabolism of acetaldehyde. Since the capacity of the ALDH2 to oxidize acetaldehyde exceeds that of the enzymes that produce it, and because acetaldehyde is reactive, acetaldehyde levels are low even after ethanol consumption. Acetaldehyde is undetectable (< 0.5 μM) in the blood of non-flushers and found in concentrations of 50–100 μM in flushers [19,20]. Disulfiram, an irreversible inhibitor of both cytosolic and mitochondrial ALDH isozymes, has been used for the treatment of alcoholism [21]. An important side effect of some drugs, including the antiprotozoal metronidazole, the oral hypoglycemic chlorpropamide and some cephalosporin antibiotics (cefamandole, and cefotetan), is to precipitate a disulfiram-like flushing reaction after the ingestion of ethanol, presumably through the same ALDH inhibitory mechanism.

The flushing reaction is thought to be mediated in part by histamine released from mast cells via the activation of histamine H_1 and H_2 receptors [20,22]. In the periphery, 30–40% of histamine is metabolized by diamine oxidase and 60–70% by histamine N-methyltransferase (HNMT) [20,22]. A common *C314T* (cDNA NM_006895) SNP resulting in a Thr105Ile substitution (Table 2) has been detected in the *HNMT* gene (2q22.1) [23]. The Ile105 allele has 50% lower enzyme activity and might be expected to alter the intensity of the flushing reaction, but no study has yet been conducted to test this hypothesis.

The seven ADH genes are co-localized within a 380-kb region of chromosome 4 (4q21-q25). Linkage to alcoholism was detected to this region by independent genome-wide linkage studies on Southwest American Indians [24] and Caucasians [25].

ADH1B contains two functional polymorphisms, Arg47His and Arg369Cys (Tables 1 and 2), which are a result of transitions *G143A* and *C1108T* (cDNA NM_000668), respectively, in the *ADH1B* gene. The enzyme incorporating His47 (encoded by *ADH2*2*) is superactive (high V_{max}), leading to more rapid accumulation of acetaldehyde. His47 is common in East-Asian populations [26] where it has been linked to alcohol-induced flushing and to a lower risk for alcoholism [6]. However, His47 is also moderately abundant in certain non-Asian populations; for example, in Israelis of Jewish ancestry [27]. Cys369

(designated *ADH2*3*) encodes an enzyme of high V_{max}. It has a high frequency in African–Americans and was also detected in Mission Indians [28], an American population that has experienced some genetic admixture.

ADH1C has three missense variants Arg271Gln, Ile349Val and Pro351Thr (**Tables 1** and **2**), which are caused by SNPs *G815A*, *A1048G* and *C1054A* (cDNA NM_000669), respectively. Arg271, which is predominant among East Asians and African–Americans, encodes an enzyme with higher V_{max}, whereas the Ile349Val variant does not appear to alter enzyme activity [12]. Accordingly, the protective role of the *ADH1C* Ile349 allele found in studies conducted in East Asians seems to be attributable to linkage disequilibrium with the *ADH1B* His47 allele [12,29]. In support of this suggestion, Southwest American Indians, a population in which the *ADH1B* His47 allele is absent, show the opposite effect. In this population the *ADH1C* Ile349 allele was associated with an increased risk for alcoholism [30]. In addition, the Arg271Gln and Ile349Val loci were thought to be in perfect linkage disequilibrium resulting in the polymorphic haplotypes Arg271–Ile349 (designated *ADH3*1*) and Gln271–Val349 (designated *ADH3*2*) an assumption that now appears to be incorrect [31]. The functional significance of Pro351Thr, which is abundant in Native Americans (the Ticuna and Arizona Pima tribes) and almost exclusive to them, is unknown [31].

ALDH2 (mapped to 12q24.2) contains a *G1510A* (cDNA NM_000690) transition resulting in a Glu487Lys missense polymorphism (**Tables 1** and **2**) that is restricted to Asians. *ALDH2* Lys487 (designated *ALDH2*2*) is a dominantly acting allele that inactivates the ALDH2 tetramer leading to diminished breakdown of acetaldehyde. Glu487Lys heterozygotes have ~ 20% residual enzyme activity substantiating a partial dominance model, which was proposed to explain the changes in enzyme activity [12]. The effects of the *ALDH2* and *ADH1B* protective alleles are 4- to 10-fold, varying between different populations, and the genotype effects on risk are additive [18]. Most highly protective is the *ALDH2* Lys487/Lys487 homozygous genotype, which is nearly completely protective against alcoholism regardless of the presence of *ADH1B* or *ADH1C* polymorphisms.

Pharmacodynamic domain
OPRM1 Asn40Asp
The effects of opioids are mediated via at least three types of opioid receptors: μ, κ, and δ, which are each a G-protein-coupled receptor with a distinct pharmacological profile. Activation of the opioid receptor μ1 (OPRM1) reduces neuronal excitability by inhibiting presynaptic calcium and activating postsynaptic G-protein-coupled, inwardly rectifying potassium (GIRK) channels. OPRM1 is the primary site of action of an endogenous opioid peptide, β-endorphin, and a μ-opioid receptor antagonist, naltrexone. Encoded by the *OPRM1* gene (6q24-q25), the μ-opioid receptor is widely distributed in the brain. It attains its highest levels in the thalamus, where pain/stress responses can be modified at the sensory transmission level, and in components of the limbic system, including the amygdala, nucleus accumbens, and cingulate cortex, where this receptor modulates reward and emotion. The *OPRM1* gene contains an *A118G* (cDNA NM_000914) variant resulting in a functional Asn40Asp (**Table 3**) substitution [32]. The polymorphism is common, with the frequency of the Asp40 allele ranging from 0.1 to 0.15 in individuals of European descent. The frequency of the Asp40 allele shows a high degree of population variation (**Table 3**), which might have important implications for the use of opioid antagonists in different populations. The Asp40 allele has been shown to increase binding affinity for β-endorphin. Additionally, β-endorphin is approximately three times more potent at the Asp40 receptor in activating GIRK channels than at the Asn40 receptor allele [33]. Individuals carrying one or two copies of Asp40 have higher cortisol levels both at baseline and following infusion of the opioid receptor antagonist naloxone [34,35]. Alcoholics with one or two copies of Asp40 and treated with naltrexone had significantly lower rates of relapse and took longer to return to heavy drinking as compared with Asn40 homozygotes [36]. Recently, Asp40 was associated with a more favorable response (higher rate of abstinence, less mood disturbance, and weight gain) to short-term transdermal nicotine replacement [37]. Both ethanol and nicotine increase the level of β-endorphin in a dose-dependent manner. β-Endorphin release mediates part of the rewarding effects of these drugs either directly, by stimulating the μ-opioid receptor, or indirectly, by releasing dopamine [37,38]. Therefore, naltrexone might also be useful for the treatment of nicotine dependence. Indeed, naltrexone augmentation of nicotine replacement therapy has been reported to be beneficial for smoking cessation in a preliminary study [39]. Considering that alcoholism and

Table 3. Candidate genes for vulnerability to alcoholism and treatment response in the pharmacodynamic domain.

Gene	Polymorphism	Functionality	Potential effects	Allele frequencies*
OPRM1	Asn40Asp	Ligand affinity	Naltrexone action, reward, emotion	0.80/0.20 to 0.90/0.10 0.96/0.04 (African–Americans) 0.55/0.45 to 0.80/0.20 (East Asians) 0.84/0.16 (Southwest American Indians) 0.86/0.14 (Hispanics)
HTT	HTTLPR	Transcription	Anxiety, dysphoria, obsessionality	0.50/0.40/0.10
COMT	Val158Met, yin/yang haplotypes	Enzyme activity	Anxiety/dysphoria executive cognition	0.60/0.40
$GABA_{A\alpha6}$	Pro385Ser	Altered binding of benzodiazepines and/or ethanol?	Decreased sensitivity to benzodiazepines and/or ethanol	0.95/0.05
NPY	Leu7Pro	Altered cellular processing and release	Anxiety	0.95/0.05
HNMT	Thr105Ile	Reduced enzyme activity	Anxiety, dysphoria, relapse	0.90/0.10 0.94/0.06 (East Asians)
HTR3B	-100-102AAGins/del	Altered level of expression?	Altered response to ondansetron treatment	0.90/0.10

*Frequency of common allele/frequency of rarer allele in Caucasians if otherwise not stated. HTTLPR is triallelic.

COMT: Catechol-O-methyltransferase; GABA: γ-Aminobutyric acid; HNMT: Histamine N-methyltransferase; HTR: Serotonin receptor; HTT: Serotonin transporter; NPY: Neuropeptide Y; OPRM1: Opioid receptor μ1.

smoking are substantially cross-inherited [40], smoking alcoholics carrying Asp40 might be expected to gain the most benefit from naltrexone-augmented nicotine replacement.

HTTLPR

Serotonin (5-hydroxytryptamine [5-HT]) is transported from the synaptic cleft into pre-synaptic serotonergic neurons by an integral membrane protein, the serotonin transporter (5-HTT, SLC6A4, and SERT). 5-HT deficits are classically associated with anxious, dysphoric emotional states and with impulsive and obsessive behaviors. Such states are frequently observed during abstinence and long-term withdrawal, are triggered by life stresses and alcohol cues, and may be antecedent to relapse. The 5-HTT gene (17q11.1-q12) contains a functional polymorphism (HTTLPR) in its promoter [41]. As originally described, the polymorphism had two abundant alleles with frequencies of 0.6 and 0.4 in Caucasians (Table 3) for the L and S alleles, respectively. The S allele contains 14 copies of a 21- to 23-bp imperfect repeat sequence and the L allele contains 16 copies of the repeat (an additional 44 nucleotides). The S allele is lower transcribing, leading to lower 5-HTT expression in vitro in human lymphoblast cell lines [41], in living brain neuroimaged with β-CIT (β-carbomethoxy-3β-[4-iodophenyl] tropane) single photon emission computed tomography (SPECT) [42], and in the post-mortem brain [43]. Recently, this locus has been found to be triallelic: the newly detected L_G allele was a low-expressing variant masquerading as a high-expressing L allele [44].

HTTLPR biallelic genotypes have been repeatedly linked to anxiety and dysphoria. The effect is strongest under conditions of stress and provocation. As evidenced by blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI), individuals who are heterozygous or homozygous for the S allele have heightened amygdala metabolic activation in response to a cognitive fear challenge [45]. The S allele has been associated with depressive symptoms, but only in individuals who had suffered stressful life events [46]. Patients carrying the S allele are more vulnerable to depression, but are less likely to respond to selective serotonin re-uptake inhibitor (SSRI) drugs [47]. Discovery of the triallelic nature of HTTLPR enabled the previously unrecognized highest expressing L_A allele to be linked to obsessive–compulsive disease [44]. Obsessive behaviors are also an aspect of alcohol dependence. The

L allele of *HTTLPR* has been linked to low LR to alcohol in a small Caucasian population [48]. This association was corroborated in a larger population and individuals homozygous for the highest expressing L_A allele had the lowest LR to alcohol [16]. Recently, the *L* allele was linked to more frequent alcohol consumption by another group [49]. These three lines of evidence support the hypothesis that lower synaptic 5-HT leads to higher risk for alcoholism. However, the contribution of *HTTLPR* to vulnerability to alcoholism is controversial. In a number of publications, the *S* allele has been shown to be associated with alcohol dependence [50] and type II alcoholism [51-53], which is characterized by early onset, antisocial behavior and impulsivity, likely to be due to the dysregulation of the serotonergic transmission. Future studies are required to clarify the exact role of the *HTTLPR* in alcoholism or clinical subgroups of alcoholics.

COMT Val158Met and COMT yin/yang haplotypes

The transfer of a methyl group from *S*-adenosyl-*L*-methionine to catecholamines, including the neurotransmitter dopamine, norepinephrine and epinephrine, is catalyzed by catechol-*O*-methyl-transferase (COMT). In addition to its role in the metabolism of endogenous molecules, COMT is also important in the catabolism of catechol drugs. In the prefrontal cortex, where dopamine transporters are expressed at low levels and the uptake of dopamine is slow, COMT activity seems to be crucial to regulating dopamine availability in synapses [54]. Furthermore, in the prefrontal cortex, dopamine plays a critical role in aspects of cognition involved in addictions and recovery, including working memory, and the ability to switch cognitive strategies. Prefrontal cortical dopaminergic tone also underlies differences in behavioral inhibition and the regulation of emotional responses in other parts of the limbic circuit. In the nucleus accumbens, amygdala and other parts of the limbic system, dopamine and norepinephrine play direct roles in anticipatory reward and emotionality. Thus, functional genetic variation at COMT could influence reward, mood and emotionality, and behavioral inhibition processes that ultimately determine the integrated response of an alcoholic to resume drinking or maintain abstinence. Such gene effects could be especially salient in the contexts of therapies taxing the cognitive resources of the patient and following stress.

In most tissues, the expression of *COMT* (22q11.21-q11.23) is controlled by two distinct promoters resulting in a short and a long mRNA, which produce the soluble (S-COMT) and the membrane-bound (MB-COMT) isozymes, respectively. By contrast, in the human brain only the long mRNA was detected, but it is thought to code for both the MB-COMT (70%) and the S-COMT (30%) by a leaky scanning mechanism for translation initiation. The K_m value of S-COMT for dopamine is 10–100 times higher (lower affinity) than that of MB-COMT, indicating that MB-COMT accounts for most of the dopamine metabolism at the dopamine concentrations found in the mammalian brain [54,55]. A *G472A* (cDNA NM_000754) transition leads to a Val to Met substitution (**Table 3**) at amino acid 108 (soluble) and 158 (membrane bound). The frequency of Val/Met is 0.58/0.42 in Caucasians. At normal body temperature, Val158 is approximately four-fold more active than Met158, potentially leading to less sustained dopamine signaling, and effecting both cognition and behavior. Normal subjects that are homozygous for Met showed a better performance on prefrontal cognitive tasks and higher metabolic efficiency during tasks; Val/Met heterozygotes were intermediate [56].

In contrast, the *COMT* Met158 allele has been linked to higher levels of anxiety in women [57], and with higher risk of alcoholism and opioid dependency in several large case–control samples [58]. The mechanism of linkage of Met158 to anxiety may be a diminished resiliency to pain/stress. In an imaging/genetics study, Met158 was associated with diminished ability of the brain to respond to a pain/stress challenge by activating endogenous opioid release. Furthermore, Met158 was associated with a lower pain threshold and stronger affective response to pain [59]. Finally, a fascinating aspect of *COMT* genetics is that the Val158 and Met158 alleles reside on haplotypes (patterns of alleles at nearby, linked loci), which are opposite or 'yin/yang' in configuration and that are common in populations worldwide. These aspects of *COMT* population genetics reflect an ancient presence of both Val158 and Met158 alleles and a potential role of selection in their maintenance.

GABA$_{A\alpha6}$ Pro385Ser

The principal inhibitory neurotransmitter in the vertebrate brain is γ-aminobutyric acid (GABA). The heteropentameric GABA$_A$ receptors are

ligand-gated ion channels that mediate fast inhibitory neurotransmission in the CNS by increasing chloride influx, which results in the hyperpolarization of neurons [60]. A range of $GABA_A$ subunits has been divided into six classes based on sequence identity: α (1–6), β (1–4), γ (1–4), δ, ε, and π [60]. The majority of the $GABA_A$ receptors in the mammalian brain are composed of two α, two β and one γ subunit [61]. $GABA_A$ receptors are also the site of action of pharmacologically and clinically important drugs, including sedatives, steroids, convulsants, and anesthetics [62].

Many effects of both acute and chronic exposure to ethanol, including anxiolysis, sedation, motor in-coordination, dependence, tolerance, cross-tolerance to benzodiazepines and barbiturates, and sensitization to inverse agonists, have been shown to be mediated by $GABA_A$ receptors or an alteration in the functional or structural properties of $GABA_A$ receptors [63,64]. Of the approximately 20 $GABA_A$ subunits currently known, the $\alpha 6$ is exclusively expressed in cerebellar granule layers and is the only subunit for which missense variants have been linked to benzodiazepine or alcohol response in animals and humans, to date. In alcohol-non-tolerant (ANT; alcohol-sensitive) rats, a missense variant resulting in an Arg to Gln substitution at amino acid position 100 [65] was found. GABA currents were enhanced by both benzodiazepines and ethanol in mammalian cell lines expressing recombinant receptors incorporating the $\alpha 6$ Gln100 subunit [65]. This suggests that higher ethanol sensitivity of ANT rats might be due to the presence of the $\alpha 6$ Gln100 subunit.

In humans, a *C1210T* (cDNA NM_000811) transition resulting in a Pro385Ser substitution (**Table 3**) was detected in the $GABA_{A\alpha6}$ subunit gene (*GABRA6*), located on 5q34 [66]. Pro385/Ser385 heterozygotes displayed less diazepam-induced impairment of saccadic eye velocity, suggesting that Ser385 may decrease individual sensitivity to benzodiazepines [66]. Furthermore, the Pro385/Ser385 genotype may also predict low LR to alcohol (reflecting decreased sensitivity to ethanol) [16,48]. No association was found between Pro385Ser and alcoholism in a Finnish population [67].

Following up on linkage of alcoholism to the $GABA_A$ gene cluster on chromosome 4 [24], the $GABA_{A\alpha2}$ subunit (*GABRA2*) gene was directly implicated by haplotype-based association analysis [68]. This finding has now been replicated by

at least one other group [69], but the functional locus or loci are unknown.

NPY Leu7Pro

Neuropeptide Y (NPY) is a neurotransmitter involved in alcohol and stress response, and the *NPY* gene (7p15.1) has been linked to alcohol consumption [70]. NPY is a highly conserved inhibitory neuromodulator whose effects are mediated via at least five heterotrimeric G-protein-coupled receptor subtypes: Y_1, Y_2, Y_4, Y_5, and Y_6 [71]. Of the NPY receptors identified, to date, the postsynaptic Y_1 and presynaptic Y_2 autoreceptor (inhibiting NPY release) seem to control voluntary alcohol consumption [72], whereas ethanol-induced sedation appears to be mediated by Y_1 and Y_5 [70,72]. NPY has been reported to be involved in the regulation of a wide variety of physiological and pathological processes, such as stimulation of feeding (orexigenic effect), increased energy storage through lipoprotein lipase activation in white adipose tissue, formation of memory, anxiolysis, and modulation of voluntary alcohol consumption [71]. Pertinent to alcoholism is the anxiolytic property of NPY, which has been consistently reported in all animal models tested, to date [70]. Alcohol preference seems inversely correlated with NPY levels in the brain. NPY-deficient mice display increased alcohol consumption, whereas transgenic mice that overexpress NPY drink less ethanol and are more sensitive to its sedative/hypnotic effects [73]. In humans, a relatively common *T20C* (cDNA NM_000905) transition leads to a Leu7Pro substitution (**Table 3**) in the 28-amino acid signal peptide of prepro-NPY [74]. The Pro7 allele has been associated with approximately 40% higher concentration of exercise-induced NPY in blood [75] which might be due to enhanced intracellular processing of prepro-NPY and/or increased release of the mature peptide [75]. The Pro7 allele has been linked to higher serum cholesterol levels in obese subjects [74], to enhanced carotid atherosclerosis, and to higher blood pressure [76]. NPY Pro7 has also been associated with 34% higher average alcohol consumption in middle-aged Finnish men [77] and with alcoholism in European Americans [78]. Contrary to these findings in alcoholism, the Pro7 variant seemed to be protective against alcoholism in a case-control study that included type 1 and 2 alcoholics [79], and no association was found between the Pro7 allele and alcoholism in two other studies in Finns and Swedes

[80]. Because of the centrality of NPY in stress and anxiety and its role in mediating ethanol intake, additional efforts are underway to identify the functional variants of this gene in humans and other species.

5-HT₃ receptor

The $5\text{-}HT_3$ receptor ($5\text{-}HTR_3$), which mediates rapid excitatory responses, is a ligand-gated ion channel of the cysteine loop-containing superfamily, the structure of which is closely similar to that of nicotinic acetylcholine receptors (nACHR), glycine and $GABA_A$ receptors. Of two $5\text{-}HT_3$ subunits, *5-HTR₄* seems to be indispensable for the expression of the functional receptor, which is involved in mediating some of the rewarding effects of ethanol and other drugs of abuse [81]. The potentiation of $5\text{-}HT_3$ receptor function by ethanol is believed to be the result of an increased potency of 5-HT for receptor activation, as evidenced by a leftward shift of the 5-HT dose–response curve [82]. In addition, ethanol has been shown to prolong the time the $5\text{-}HT_3$ receptor spends in the open state [82]. Numerous polymorphisms have been identified in both the $5\text{-}HTR_{3A}$ [83,84] and $5\text{-}HTR_{3B}$ [85] $5\text{-}HT_3$ subunit genes (11q23.1-q23.2). A $5\text{-}HTR_{3A}$ *C178T* transition (cDNA NM_D49394) has been suggested to be functional based on expression experiments with reporter constructs [86]. Subjects homozygous for the $_{-100\text{-}102}AAG$ deletion (**Table 3**) in the $5\text{-}HTR_{3B}$ promoter who were treated with the $5\text{-}HT_3$ receptor antagonist ondansetron, experienced vomiting more frequently than other patients receiving the same drug regimen [85]. If confirmed, the finding might suggest that individual response to treatment with ondansetron might be influenced and predicted by *HTR3* polymorphisms. It might be of relevance to the treatment of alcoholism for which ondansetron seems to be a promising medication, particularly in early onset alcoholics [87,88].

Expert opinion

Pharmacogenetics is ultimately the study of the role of inherited genetic variants that underlie individual differences in the response to a drug or treatment regimen. A unique feature of the pharmacogenetics of addictive diseases, such as alcoholism, is the crucial role of multiple neurobiological mechanisms that influence anxiety, dysphoria, sedation, craving, executive cognitive function, and reward. Most effects of ethanol are determined by the interaction of several gene products, creating the possibility for action of multiple genetic variants. For instance, the binding of β-endorphin to the μ-opioid receptor might be altered by the OPRM1 Asn40Asp variant. Endorphin released by ethanol is directly rewarding or indirectly rewarding via dopamine release. In the frontal cortex, the sustainability of dopamine signaling appears to be moderately influenced by the COMT Val158Met polymorphism, which is simultaneously a determinant of the activation of the μ-opioid system in various brain regions. Therefore, a single functional polymorphism might have multiple direct and indirect effects. Several functional gene variants (e.g., variants of *COMT, HTTLPR,* and *OPRM1*) have already been identified as having important consequences in behaviors relevant to alcoholism treatment response, and OPRM1 Asn40Asp has actually been linked to response to naltrexone. We strive to rapidly advance knowledge of the pharmacogenetics of alcoholism aiming at identifying genetic variants that influence the vulnerability to this common neuropsychiatric disease and the response to its treatment.

Outlook

Alcoholism is a lifelong relapsing/remitting disease for which treatment is only moderately effective. The detection of moderate, but clinically significant, treatment effects in clinical trials on alcoholism and other psychiatric diseases will be enhanced by the incorporation of a pharmacogenetic component in these studies. For example, a pharmacogenetic component is included in COMBINE, an ongoing multi-center treatment study evaluating combined cognitive/behavioral intervention, naltrexone, and acamprosate (homotaurine) [89]. Acamprosate, the mechanism of whose clinical effects is unknown, was recently approved in the USA for alcoholism treatment. Acamprosate interacts with a wide variety of neurotransmitters and neuromodulators, including GABA, taurine, dopamine, and opioids [90]. The efficacy of acamprosate is thought to be due primarily to its ability to reduce craving. Craving is determined by the anticipation of euphoric effects and relief from withdrawal symptoms; therefore, the response to acamprosate treatment is likely to be influenced by functional polymorphisms in the pharmacodynamic domain. Cognitive/behavior therapy and naltrexone, the other treatment modalities in this study, would seem to access somewhat different neurobiologies and, therefore, may be sensitive to the effects of other functional polymorphisms, or sets of polymorphisms.

Highlights

- Alcoholism, a common neuropsychiatric disease, and level of response to alcohol (an intermediate phenotype) both have heritabilities of ~ 50%.
- Currently, long-term treatment of alcoholism is unsatisfactory because available drugs, including disulfiram, SSRIs, naltrexone, and ondansetron, have only partial efficacy; relapse rate is high [1]; and most alcoholics do not seek treatment.
- Knowledge of genetic polymorphisms might help identify individuals who are more or less vulnerable to alcoholism.
- Genetic variants are also likely to influence response to the treatment either by affecting function of medication-specific molecular targets, including enzymes, receptors or transporters, or indirectly and through factors that are not medication specific: anxiety, dysphoria, sedation, and cognition.
- In the pharmacokinetic domain, the protective role of ADH1B His47 and ALDH2 Lys487 are best known in East Asians, but ADH1B His47 seems to be moderately frequent and protective in Ashkenazi Jews and Europeans. Other genes, such as *HNMT*, might also alter the intensity of alcohol-induced flushing or modulate other alcoholism-associated behaviors, such as anxiety.
- Neurobiologies of reward, executive cognitive function, anxiety/dysphoria and neuronal plasticity have been identified as critical domains in treatment response and appear to be affected by functional variants of several genes (e.g., *COMT* and *HTT*).
- In the pharmacodynamic domain, level of response to alcohol has been associated with variants of several genes (e.g., *HTT* and *GABRA6*), but the findings require confirmation.
- HTTLPR, NPY Leu7Pro polymorphisms and the *GABRA2* gene have been associated with alcoholism but their definitive roles remain to be clarified.
- The Asp40 allele of OPRM1 has been associated with more favorable response to naltrexone in alcoholics and to transdermal nicotine replacement therapy in smokers.

In clinical practice, genotype may become a component of a diagnostic algorithm useful for assigning patients to optimal treatment. For example, if the lower relapse rate in naltrexone-treated alcoholics carrying the *OPRM1* Asp40 allele is confirmed in larger populations, this information can be used to identify individuals who are more likely to respond to naltrexone therapy. Likewise, if any of the polymorphisms in the pharmacodynamic domain is proven to be predictive of failure to respond, rapid clinical genotyping prior to initiating the therapy will be a valuable tool to select those patients who will likely benefit most from an alternative therapy. Finally, the inconsistencies between the results of clinical trials addressing the efficacy of various drugs, such as ondansetron, SSRIs and acamprosate, might be partly explained by the different genotypes of the participants.

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