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The Role of $GABA_A$ Receptors in the Development of Alcoholism

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Abstract

Alcoholism is a common, heritable, chronic relapsing disorder. $GABA_A$ receptors undergo allosteric modulation by ethanol, anesthetics, benzodiazepines and neurosteroids and have been implicated in the acute as well as the chronic effects of ethanol including tolerance, dependence and withdrawal. Medications targeting $GABA_A$ receptors ameliorate the symptoms of acute withdrawal. Ethanol induces plasticity in $GABA_A$ receptors: tolerance is associated with generally decreased $GABA_A$ receptor activation and differentially altered subunit expression. The dopamine (DA) mesolimbic reward pathway originating in the ventral tegmental area (VTA), and interacting stress circuitry play an important role in the development of addiction. VTA GABAergic interneurons are the primary inhibitory regulators of DA neurons and a subset of VTA $GABA_A$ receptors may be implicated in the switch from heavy drinking to dependence. $GABA_A$ receptors modulate anxiety and response to stress; important elements of sustained drinking and relapse. The $GABA_A$ receptor subunit genes clustered on chromosome 4 are highly expressed in the reward pathway. Several recent studies have provided strong evidence that one of these genes, *GABRA2*, is implicated in alcoholism in humans. The influence of the interaction between ethanol and $GABA_A$ receptors in the reward pathway on the development of alcoholism together with genetic and epigenetic vulnerabilities will be explored in this review.

Keywords

GABA; ethanol; neurosteroids; benzodiazepines; tolerance; withdrawal; reward; VTA; stress; anxiety genes; GABRA2

Introduction

Although alcohol consumption is a social pleasure for many, a significant number of individuals are unable to keep within safe limits and cross over the divide between social drinking and addiction. Alcoholism is common; the 12 month prevalence for alcohol use disorders (dependence plus abuse) is 8.5% (Grant et al., 2004). The essential features of alcoholism are loss of control over consumption, obsessional thoughts about the next drink, and continuation of use despite knowledge of negative health and social consequences (American Psychiatric Association, 1994). A meta-analysis of twin studies has shown that the heritability of all addictive substances ranges from 40 – 70 %; the heritability of alcoholism, derived from nearly 10,000 twin pairs, is 50% (Goldman et al., 2005). Therefore genetic and

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environmental risk factors for alcoholism are almost equally important although they may differ in different populations.

Unlike other addictive drugs that are more specific, alcohol has widespread effects throughout the brain; it acts at a variety of targets within cell membranes and in intracellular signal transduction, inducing effects on neurotransmitter and neurohormone membrane receptors and receptor-gated and voltage-activated ion channels. Alcohol alters the balance between γ-aminobutyric acid (GABA), the primary inhibitory neurotransmitter, and glutamate, the major excitatory neurotransmitter. Genetic vulnerability to alcoholism is therefore likely to be due to numerous genes of small to modest effects in many neurotransmitter systems and signal transduction pathways.

$GABA_A$ receptors undergo allosteric modulation by several structurally unrelated drugs, most with their own binding sites, including ethanol, benzodiazepines (BZs), barbiturates, anesthetics and also endogenous neurosteroids. These drugs have similar anxiolytic, sedative-hypnotic, anticonvulsant, motor-incoordinating, and cognitive impairing effects. $GABA_A$ receptors are implicated in the acute and chronic effects of alcohol including tolerance, dependence and withdrawal, as discussed below. Chronic ethanol consumption results in cross-tolerance to BZs and barbiturates (Kumar et al., 2004). This cross-tolerance, together with the effectiveness of BZs in treating both anxiety and alcohol withdrawal, suggests that $GABA_A$ receptors may play an important role in vulnerability to alcoholism and anxiety, particularly in the mesolimbic dopamine (DA) reward pathway and interacting stress circuitry (Enoch et al., 2003; Enoch, 2007).

$GABA_A$ receptors: structure

$GABA_A$ receptors are composed of five subunits, each of which has several isoforms ($\alpha_{1–6}$, $\beta_{1–3}$, $\gamma_{1–3}$, δ, ε, θ, π, $\rho_{1–3}$) (Barnard et al., 1998). Most receptors consist of two α, two β and one γ subunits (Sigel et al., 2006). There are three main subunit combinations: $\alpha_1\beta_2\gamma_2$ (60%), $\alpha_2\beta_3\gamma_2$ (15%), and $\alpha_3\beta_3\gamma_2$ (10%) (Benke et al., 1994, Michels and Moss, 2007). Expression of the various subunit isoforms varies across brain locations and during development. The receptor subunit composition determines distinct pharmacological and electrophysiological properties (Minier and Sigel, 2004).

$GABA_A$ receptors are ligand-gated, chloride ion channels that confer fast synaptic inhibition. Channel opening is initiated by the extracellular association of agonists with discrete binding pockets leading to conformational changes that result in the opening of a central ion pore (Barnard et al., 1998; Connolly and Wafford, 2004; Wisden and Seeburg, 1992). $GABA_A$ receptors have structural and functional homology with a class of cys-loop ligand-gated ion channel receptors including glycine, 5-HT3 and nicotinic acetylcholine. The topology of this class of receptors consists of a large N-terminus, ligand binding, extracellular domain with a cysteine loop, four transmembrane (TM) domains forming a chloride-ion channel with a large intracellular loop between TM3 and TM4 and a short-extracellular C-terminus (Brejc et al., 2001; Michels and Moss, 2007). The $GABA_A$ receptor ion channel is lined by the TM2 segments from each of the five subunits that form the receptor. There appears be a pocket located between TM2 and TM3 of the $GABA_A$ α subunit that binds both alcohols and anesthetics. It has been shown that within this pocket of the α_1 subunit, serine-270 and alanine-291 are essential not only for the binding of alcohols but also for alcohol-induced conformational changes within the $GABA_A$ receptor (Jung et al., 2005; Jung and Harris, 2006; Mascia et al., 2000; Mihic et al., 1997). However, these studies used very high concentrations of ethanol (200mM) that correspond to anesthetic concentration *in vivo*, and there is so far no evidence that ethanol binds to $GABA_A$ receptors at physiological doses. The exact mechanism by which ethanol enhances GABA responses remains unclear. The BZ

binding site is at the interface of the γ_2 subunit with α subunits excepting α_4 and α_6 (Wafford, 2005; Barnard et al., 1998). Binding sites for GABA (two copies) lies at the interface between α and β (Olsen et al., 2004). Ethanol- and stress-induced neurosteroids potentiate GABA at a binding site located in a cavity formed by α subunit TM domains (Hosie et al., 2006).

Presynaptic, postsynaptic and extrasynaptic receptors: phasic and tonic inhibition

$GABA_A$ receptors are ubiquitously distributed at synapses on dendrites, neuronal cell bodies and axons and are also found in extrasynaptic membranes. Most studies of GABA's effects have focused on phasic inhibition; i.e. postsynaptic $GABA_A$ receptors are activated following brief exposure to a high concentration of GABA released from presynaptic vesicles. However GABA that escapes from the synaptic cleft can also activate receptors on pre-synaptic terminals. Accumulating evidence from recent studies has shown that in several brain regions including the cerebellum and the mesolimbic reward pathway, ethanol can enhance GABAergic transmission through effects at both pre- and postsynaptic $GABA_A$ receptors through complex mechanisms (Ming et al., 2006; Roberto et al., 2006; Siggins et al., 2005).

$GABA_A$ receptors can exist as either synaptic or extrasynaptic receptors and this is a dynamic state that may facilitate rapid changes in synaptic inhibition (Thomas et al., 2005). It has recently been shown that low concentrations of extracellular GABA may result in persistent or 'tonic' activation of extrasynaptic $GABA_A$ receptors in hippocampal interneurons, the cerebellum, and the dentate gyrus (Farrant and Nusser, 2005; Kullmann et al., 2005; Semyanov et al., 2003). Other than δ, no $GABA_A$ receptor subunits have so far been found to be exclusively extrasynaptic (Farrant and Nusser, 2005). Neurosteroids potentiate extrasynaptic $GABA_A$ receptors in nanomolar concentrations and decrease neuronal excitability by enhancing tonic conductance mediated by the δ subunit (Fodor et al., 2005; Stell et al., 2003). Some studies have shown that low concentrations of ethanol (1–30mM) potentiate extrasynaptic receptors ($\leq$ 5% of all receptors), predominantly composed of the non-BZ sensitive α_4, α_6 and δ subunits, but have no effect on receptors with γ_2 subunits, i.e. the vast majority of synaptic $GABA_A$ receptors (Hanchar et al., 2006; Krystal et al., 2006; Olsen et al., 2007; Sundstrom-Poromaa et al., 2002). This is relevant because these relatively low concentrations of ethanol are experienced in social drinking; for example, 17mM is the legal upper limit of intoxication in most US States (Lovinger and Homanics, 2007). These findings have not, however, been replicated in other labs (Borghese and Harris, 2007; Korpi et al., 2007). In addition, it is currently a subject of debate as to whether low concentrations of alcohol affect only α_4, α_6 and δ subunits (Lovinger and Homanics, 2007), For example, it has been shown that co-application of low concentrations of ethanol (17mM) influences channel potentiation of at least the $\alpha_1\beta_2\gamma_2$ receptor by neurosteroids (Akk et al., 2007). Moreover, $\alpha_1\delta$ subunit assemblies that are highly sensitive to low concentrations of ethanol and mediate tonic inhibitory currents have recently been detected in hippocampal interneurons (Glykys et al., 2007). Taken together, the importance of tonic inhibition for vulnerability to alcoholism has yet to be clearly elucidated. Moreover, the balance between tonic and phasic inhibition may shift towards the direction of phasic inhibition during chronic ethanol consumption. Rats exposed to the CIE model of binge drinking (described later) showed a net loss of extrasynaptic, and a gain of synaptic, hippocampal $GABA_A$ receptor responsiveness to ethanol concomitant with an increase in central synaptic localization of α_4, but not δ subunits (Liang et al., 2006). The fact that this switch corresponds with the development of tolerance to the sedating / hypnotic effects of alcohol suggests that it may be important in the pathway to addiction (Liang et al., 2006).

$GABA_A$ receptors and the dopamine reward pathway

The mesolimbic dopamine (DA) system is implicated in the development of all addictions and is also stimulated by stress. This “reward” pathway originates in the ventral tegmental area (VTA) of the midbrain and projects to the nucleus accumbens (NAC), the limbic system and the orbitofrontal cortex. The amygdala, hippocampus and medial prefrontal cortex send excitatory projections to the NAC. The feeling of euphoria experienced by humans subsequent to drug ingestion is associated with increased synaptic DA in the reward pathway that is entwined with complex changes in numerous neurotransmitters including GABA, glutamate, serotonin (5-HT), opioid peptides and cannabinoids. $GABA_A$ receptors in the central nucleus of the amygdala appear to be important for oral ethanol self-administration in rodents (McBride, 2002).

GABAergic projections to the VTA come from several regions including the NAC and the ventral pallidum, however the primary inhibitory regulation of DA neurons is by GABAergic interneurons within the VTA (Johnson and North, 1992; Steffensen et al., 1998). It has been shown that VTA GABA neurons are coupled electrically via gap junctions (Stobbs et al., 2004). The predominant $GABA_A$ receptors expressed in the VTA are α_2, α_3, α_4, β_1, β_3, and γ_2 (Okada et al., 2004). In rats, acute ethanol exposure (20–80mM) excites DA neurons directly but also indirectly by reducing the firing rate of VTA GABAergic neurons (Gallegos et al., 1999; Xiao et al., 2007) and by influencing NMDA receptor-mediated excitation (Stobbs et al., 2004). Chronic exposure to ethanol enhances baseline activity of GABA neurons and induces tolerance to ethanol inhibition of the firing rate (Gallegos et al., 1999).

Human and animal studies implicate the opioid system, particularly β-endorphin and its receptor, μ opioid, in sensitivity to the rewarding or reinforcing effects of ethanol. The β-endorphin released by acute ethanol consumption is suppressed in chronic consumption and may be a factor in craving or negative reinforcement (Oswald and Wand, 2004). In the VTA, μ opioid receptors are expressed not on DA neurons but on GABA interneurons. Hyperpolarization of these GABAergic neurons by opiates results in increased firing of DA neurons (Johnson and North, 1992).

In non-drug dependent rodents, opiates can produce their acute rewarding effects through a DA-independent system mediated through brainstem reward circuits (Laviolette et al., 2004) however, in addicted animals the motivational effects of opiates derive from the mesolimbic DA system (Laviolette et al., 2002; Nader et al., 1997). $GABA_A$ receptors in the VTA control this bi-directional reward signaling; chronic opiate exposure and withdrawal induces CREB phosphorylation in a subset of $GABA_A$ receptors that switches their functional conductance properties from an inhibitory to an excitatory signaling mode (Laviolette et al., 2004). This intriguing switching system has been shown to have considerable plasticity in rats and it may have implications for the switch from controllable heavy drug use to addiction in humans.

Plasticity of $GABA_A$ receptor subunits in chronic ethanol consumption and withdrawal

Studies using animal models of addiction or human postmortem brain samples have shown that long-term ethanol use results in differential alteration in $GABA_A$ receptor subunit expression in different brain regions.

The human superior frontal cortex is selectively damaged in chronic alcohol abuse with neuronal loss. Two RT/PCR studies that were controlled for age and post-mortem delay found elevated or trend α_1 mRNA expression in the superior frontal cortex of alcoholics compared with controls but no difference for α_2, α_3 or α_4 expression (Lewohl et al., 1997; Mitsuyama et

al., 1998). Moreover, the total concentration of α subunits was greater in alcoholics compared with controls, in line with findings of increased $GABA_A$ (but not NMDA) binding in alcoholic frontal cortex reported in an earlier study (Dodd et al., 1992). There was one positive study for increased β_3 mRNA expression in alcoholics in frontal cortex (Mitsuyama et al., 1998) but another similar study was negative for both β_2 and β_3 (Buckley and Dodd, 2004).

In contrast, a consistent finding in rodents is that chronic ethanol consumption decreases cortical mRNA and peptide levels of α_1 subunits but increases α_4 levels (Devaud et al., 1996, 1997; Grobin et al., 1998; Matthews et al., 1998). Cortical peptide levels for $\beta_{2/3}$ and γ_1 and mRNA for γ_{2S} increase in ethanol-dependent rats (Devaud et al., 1997).

Cynomolgus macaques who self-administered freely available alcohol at 2g/kg/day on average for 18 months showed significant alterations in current desensitization in the basolateral amygdala that were accompanied by significantly decreased mRNA levels of α_2 and α_3 subunits, a trend decrease of α_1 but no impact on α_4 (Floyd et al., 2004). Moreover mRNA expression of β_1 and γ_2 was reduced in total amygdala samples (Anderson et al., 2007). It has been shown that after 14 days of chronic alcohol consumption there was a decrease of α_1 and α_4 subunit peptide expression in the amygdala, a decrease of α_4 in the NAC but no change in subunit expression in the VTA (Papadeas et al., 2001).

In rats, the chronic intermittent ethanol (CIE) model ($\geq$ 60 doses), with persistent cycles of self-administration and withdrawal, mimics 'binge' drinking in humans (Olsen et al., 2005). CIE rats show $GABA_A$ receptor changes specifically in the hippocampus, including decreased α_1 and δ subunit peptide expression (Cagetti et al., 2003) and elevated mRNA levels of α_4, γ_1 and the short splice variant (γ_{2S}) of γ_2 (Cagetti et al., 2003; Mahmoudi et al., 1997; Petrie et al., 2001). A model of chronic ethanol consumption (40 days) has also been shown to increase α_4 subunit (but no other subunit) peptide expression in the hippocampus (Matthews et al., 1998).

A single-photon emission computed tomographic scan (SPECT) imaging study showed that at one week of abstinence from alcohol, binding of ^{123}I-iomazenil to $GABA_A$ BZ receptors was higher throughout the brain, including the hippocampus and amygdala, but particularly in the frontal cortex, in alcoholic non-smokers compared with controls (Staley et al., 2005). This difference normalized after one month of abstinence. Frontal isotope uptake corresponded with severity of alcohol withdrawal and the number of days since the last drink (Staley et al., 2005). Therefore either the expression of $GABA_A$ receptors increased with chronic drinking in line with the findings in postmortem brain samples (Lewohl et al., 1997) and subsequently declined with abstinence, or $GABA_A$ receptors increased in acute withdrawal before gradually declining. In line with these results, a proton magnetic resonance spectroscopy (^{1}H-MRS) study has shown that non-smoking alcoholics had higher GABA levels at one week of abstinence than controls but after one month, GABA levels were the same (Mason et al, 2006).

Studies have shown that the clinical course of alcoholism differs in smokers and non-smokers. Amongst current alcoholics, 35% are also nicotine dependent (Grant et al., 2004). Either drug may increase the rewarding effects and/or reduce the aversive effects (such as withdrawal) of the other. In contrast to the non-smoking alcoholics described above, $GABA_A$ receptor binding and GABA levels in alcoholic smokers did not differ from controls at one week of abstinence (Mason et al., 2006; Staley et al., 2005). Moreover, a ^{1}H-MRS study in non-alcoholics showed no difference in cortical GABA levels between smokers and non-smokers (Epperson et al., 2005). These results suggest that smoking may ameliorate some of the effects of chronic alcohol consumption, perhaps in part by influencing GABA synthesis and the density of $GABA_A$ receptors that share structural and functional homology with nicotinic acetylcholine receptors. Nicotine is known to stimulate GABA neuronal activity, at least in the hippocampus (Barik

and Wonnacott, 2006). One mechanism for this may be that α_7 nicotinic receptors frequently occur close to synaptic $GABA_A$ receptors and α_7 nicotinic agonists are implicated in the postsynaptic modulation of $GABA_A$ receptors in the hippocampus (Wanaverbecq et al., 2007; Zhang and Berg, 2007). However, other studies have shown that neurobiological recovery in alcoholics (gauged by common brain metabolite concentrations) in the first few weeks of abstinence is adversely affected by chronic smoking (Durazzo et al., 2006). Taken together, these studies suggest that $GABA_A$ receptor subunit plasticity may not underlie the damage observed in the frontal lobes of severe alcoholics. Further studies are needed in this area.

During the peak of behavioral withdrawal (1 day) after chronic ethanol treatment in rats, mRNA expression for β_2, β_3 and $\gamma 1$ significantly increase whereas α_1, α_4 and γ_{2S} expression rebounds back to control levels (Devaud et al., 1996). However, in the CIE model with numerous cycles of drinking and withdrawal that more closely mimic binge drinking in humans, subunit changes (decreased α_1, δ, elevated α_4, γ_1 and γ_{2S}) persist beyond acute withdrawal (2 days) (Cagetti et al., 2003). *GABRG2* has also been associated with alcohol withdrawal severity in mice (Buck and Finn, 2001; Hood et al., 2006).

Taken together, the studies in rodents indicate that chronic ethanol consumption decreases cortical and hippocampal α_1 subunit levels but increases α_4 , γ_1 and γ_{2S} levels however there are regional differences in receptor adaptations, such as in the amygdala and the NAC. There are conflicting results between studies in rodents and human postmortem brain: the latter show increased α_1 mRNA expression in the cerebral cortex. However these postmortem results were from severe alcoholics with considerable phenotypic and comorbidity variability and are not likely to be comparable to controlled animal models.

Mechanisms for the effects of chronic ethanol on $GABA_A$ subunits include modification of gene transcription or post-translation modification, synaptic or extracellular localization, and ethanol induced neurosteroid changes (Kumar et al., 2004). For example, the ethanol-induced internalization or endocytosis of cortical α_1 leads to decreased cell surface and increased intracellur receptors (Kumar et al., 2003). Also, within rat hippocampus chronic ethanol consumption induces down-regulation of tyrosine kinase phosphorylation of α_1 subunits, up-regulation of β_2 subunits and no change in γ_2 subunits (Marutha Ravindran et al., 2007).

At the moment, there is little evidence from animal models to determine whether receptor plasticity induced by chronic alcohol consumption reverts to pre-drinking levels after protracted withdrawal. On the other hand, the spectroscopy study in humans described above (Mason et al, 2006) showed that after one month of abstinence GABA levels in recovering alcoholics reverted to the same levels as in controls. This would suggest that $GABA_A$ receptor changes induced by chronic ethanol consumption may not be markers of addiction. Further animal and human studies of long-term withdrawal are required before this issue can be addressed.

$GABA_A$ receptors, stress and ethanol

Alcohol may be consumed in excess as a coping mechanism for stress and the altered homeostasis subsequent to addiction can result in stress upon withdrawal (Thomas et al., 2003; Wand, 2005). GABAergic neurotransmission is likely to be important in addiction-associated stress because GABA modulates emotion and response to stress. GABA inhibits, whereas glutamate activates, the hypothalamic-pituitary-adrenal axis (HPA) responses to stress (Herman et al., 2004). Acute stress immediately reduces GABA-stimulated chloride influx in the frontal cortex and amygdala (Martijena et al., 2002). Corticotropin releasing hormone (CRH), the primary mediator of the mammalian neuroendocrine stress response, is localized and co-synthesized within GABAergic neurons in the central amygdala, and in this location

CRH1 receptors have been shown to mediate ethanol enhancement of GABAergic synaptic transmission (Nie et al., 2004). $GABA_A$ receptors in this region play an important role in ethanol self-administration in rodents (McBride, 2002).

Socially isolated rats exhibit anxious behavior accompanied by increased plasma corticosterone together with diminished levels of neurosteroids and brain $GABA_A$ receptor function (Serra et al., 2000). Adult rats that have been subjected to early life stress (maternal separation / handling) have a more active stress response (Hsu et al., 2003). Epigenetic effects on receptors implicated in stress, such as glucocorticoid and $GABA_A$, have been demonstrated in rodents. For example, BZ receptor levels in the adult rat central nucleus of the amygdala are highly correlated with the frequency of maternal licking/grooming over the first week of life (Caldji et al., 1998). Early life stress in rats permanently alters $GABA_A$ receptor subunit expression in the hippocampus such that α_2 subunits predominate in the stressed animals whereas α_1 predominates in emotionally healthy animals (Hsu et al., 2003). Early life maternal neglect also results in increased levels of α_3 and α_4 in the adult rat amygdala whereas enriched maternal care in the first week of life results in increased α_1 and β_3 mRNA levels almost everywhere, including the hippocampus and amygdala, and increased β_2 and γ_2 in the amygdala. These effects can be reversed by cross-fostering (Caldji et al., 2003).

Early life stress affects ethanol consumption and preference in adult male, but not female, rats (Moffett et al., 2007). It is not known whether similar epigenetic effects exist in humans however severe childhood stressors, especially emotional, physical and sexual abuse, have been associated with increased vulnerability to adult psychopathology (Verona and Sachs-Ericsson, 2005; Wilsnack et al., 1997).

Ethanol and neurosteroids

In vivo, $GABA_A$ receptors are exposed to endogenous neurosteroids that include progesterone and its metabolites: 3α-5 α-THP (allopregnanolone or ALLO) and 3α-5 α-THDOC (allotetrahydrodeoxycorticosterone) (Finn et al., 2004a). The effects of ALLO appear to be primarily mediated via its interactions with $GABA_A$ receptors (Herd et al, 2007). Ethanol and stress both stimulate neurosteroid synthesis from cholesterol in the brain. Neurosteroids bind to $GABA_A$ receptors at low (nanomolar) concentrations and potentiate GABA currents (Biggio et al., 2007). Consistent with this action, acute exposure to progesterone or ALLO is anxiolytic (Gulinello and Smith, 2003). In healthy humans, ethanol induces changes in ALLO concentration that correlates with alcohol liking and desire for more alcohol (Pierucci-Lagha et al., 2006). Pre-treatment with finasteride (5α-reductase inhibitor) that reduces the synthesis of ALLO and THDOC, diminishes the subjective response to a moderate dose of alcohol in social drinkers (Pierucci-Lagha et al., 2005). Several studies in rodents have shown that ethanol-induced neuroactive steroids contribute to the acute behavioral effects of ethanol such as sedative-hypnotic (Van Doren et al., 2000; Khisti et al., 2003), anti-depressant (Hirani et al., 2002) and anxiolytic (Hirani et al., 2005). Moreover a relationship exists between the time course of ethanol-induced neurosteroids and specific behavioral and neural effects of ethanol (Morrow et al., 2001). Thus neuroactive steroids contribute to ethanol sensitivity and may influence the risk for addiction (Morrow et al., 2006).

Basal brain and plasma ALLO levels are higher in females than males and are influenced by hormonal fluctuations however stress can elevate male ALLO levels to female levels (Barbaccia et al., 2001). It is therefore not surprising that sexually dimorphic effects have been noted in the interaction of ethanol and neurosteroids. ALLO pretreatment significantly increases voluntary ethanol consumption in male but not female mice (Sinnott et al., 2002) and intra-cranial injection of ALLO modulates the onset and maintenance of ethanol self-administration but does not affect appetitive (ethanol seeking behaviors) (Ford et al., 2007).

In contrast, very high pre-treatment doses of ALLO suppresses ethanol intake (Ford et al., 2005a). Chronic (17 days) self administration of limited access ethanol has been shown to elevate endogenous brain ALLO levels in male but not female mice (Finn et al., 2004b).

Rats withdrawing from ethanol are sensitized to the anticonvulsant effects of neurosteroids. Potentiation of GABA currents is enhanced up to 50% in withdrawal by ALLO and THDOC (Devaud et al., 1996). THDOC decreases neuronal excitability by enhancing tonic extracellular inhibitory conductance mediated by the δ subunit (Stell et al., 2003). Ethanol withdrawal-prone mice exhibit decreased ALLO levels during withdrawal plus tolerance to ALLO's anticonvulsant effect (Finn et al., 2004a). Pretreatment with finasteride increases acute withdrawal seizures in female mice but decreases withdrawal severity in male mice (Gorin-Mayer et al., 2007). Finasteride initially diminished ethanol consumption in male mice that were established drinkers but heavy drinking eventually re-established indicating compensatory changes in neurosteroid modulation of GABAergic tone (Ford et al., 2005b). Ethanol-induction of neurosteroids is diminished in tolerant and dependent rodents (Morrow et al, 2001). These studies indicate that neurosteroids, synthesized in response to ethanol consumption with effects primarily mediated through $GABA_A$ receptors, appear to ameliorate some of the negative aspects of ethanol, particularly during withdrawal. However, the effects on neurosteroids may be more pronounced in males.

Protein regulation of $GABA_A$ receptors

$GABA_A$ receptors are regulated by several signaling proteins including protein kinase C and A (PKC and PKA) (Chen and Olsen, 2007; Kumar et al., 2004). $GABA_A$ receptor function can be modulated by protein phosphorylation at sites thought to be located in the intracellular domain between TM3 and TM4. The PKC family of serine-threonine kinases influences receptor sensitivity to positive allosteric modulators such as ethanol, neurosteroids and BZs (Hodge et al., 1999; Kumar et al., 2004; Song and Messing, 2005) and has been implicated in animal models of alcohol addiction (Newton and Ron, 2007). PKA regulation of GABA-activated currents occurs through phosphorylation at highly conserved sites in the intracellular domain of β subunits, the effect depending on the particular β subunit isoform (McDonald et al., 1998). PKA has been implicated in anxiety and drinking behavior (Wand, 2005). A range of proteins that associate with individual $GABA_A$ subunits and play important roles in regulation have recently been found and are described elsewhere (reviewed in Chen and Olsen, 2007).

Association between $GABA_A$ receptor genes and alcoholism in humans

Genes for the $GABA_A$ receptor subunit isoforms are clustered in several chromosomal regions, including 4p13-q11 (α_2, α_4, β_1, γ_1), 5q34–q35 (α_1, α_6, β_2, γ_2) and 15q11–q13 (α_5, β_3, γ_3) as well as Xq28 (α_3, β_4, ε_1) (Barnard et al., 1998). Alternative splicing has been demonstrated for most of the $GABA_A$ receptor subunit isoforms indicating complexity in gene expression (Jin et al., 2004; Tian et al., 2005). From Figure 1 it can be seen that there is considerable conservation and linkage disequilibrium extending across long stretches of sequence in the chromosome 4 and 5 clusters but less so in the chromosome 15 cluster. Do these gene clusters have significance? The most abundant $GABA_A$ receptor subunit complex (α_1, β_2, γ_2 (60%)) that has widespread distribution in adult brain originates from the chromosome 5 gene complex. The mRNAs from the chromosome 4 cluster genes predominate in rat embryo but these genes are generally down regulated in the adult rat except in the hippocampus, the majority of DA neurons in the substantia nigra and the VTA where they are highly expressed (Okada et al., 2004; Steiger and Russek, 2004; Wisden et al., 1992). Thus the chromosome 4 cluster of genes are likely to be important in addiction and anxiety and may be vulnerable to epigenetic effects in early development, as indicated above for *GABRA2* (Hsu et al., 2003). Moreover, the

anxiolytic effects of BZs appear to be mediated in part by *GABRA2*; mice with a *GABRA2* knock-in point mutation are insensitive to BZs' anxiolytic effects (Dias et al., 2005; Low et al., 2000).

$GABA_A$ receptor chromosome 4 gene cluster

Earlier genomewide scans in American Indians and Caucasians have provided evidence for linkage of alcohol dependence and relapse-associated β EEG to chromosome 4p at the location of the $GABA_A$ gene cluster (Ghosh et al., 2003; Long et al., 1998; Porjesz et al., 2002; Reich et al., 1998; Zinn-Justin and Abel, 1999). Subsequently, several studies, nearly all in Caucasians, have found haplotype and SNP associations between *GABRA2* and alcoholism. All these studies, together with HapMap (Figure 2), have identified the same two *GABRA2* haplotype blocks, at least within Caucasians, American Indians and Asians. The significant association signals have been within the haplotype block that extends downstream from intron 3. Within this block numerous SNPs are in allelic identity resulting in two major yin-yang haplotypes of similar frequency that account for nearly all of the haplotype diversity in Caucasians and Asians (Figure 3).

The first *GABRA2* association study came from the Collaborative Study on the Genetics of Alcoholism (COGA) that has a dataset compiled from six centers across the USA. COGA performed a family-based association study on 2282 individuals, 41% with lifetime alcoholism and 29% with lifetime illicit drug dependence, from 262 multiplex families with a high density of alcoholism, genotyped across the whole chromosome 4 gene cluster. Probands were treatment-seeking alcoholics. Using the pedigree disequilibrium test, Edenberg et al. (2004) found that the more abundant of the two common haplotypes was a significant risk factor for alcohol dependence and was also associated with β EEG power, an intermediate phenotype for alcoholism. Two subsequent studies using the same COGA dataset showed that the signal for the earlier reported association came only from the alcoholics with comorbid illicit drug dependence (Agrawal et al., 2006) and that *GABRA2* was significantly associated with childhood conduct disorder symptoms, but not alcohol dependence symptoms, in children (Dick et al., 2006a). A recent case-control study in German treatment-seeking alcoholics supports the COGA finding of an association between the more abundant haplotype and alcoholism (Soyka et al., 2008).

In contrast to the above studies, the less frequent of the two yin-yang haplotypes was found to be significantly more abundant in treatment-seeking alcoholics than controls in several case-control studies: U.S. Caucasians (Covault et al., 2004), Russians (Lappalainen et al., 2005), and Germans (Fehr et al., 2006). Covault et al. (2004) found that the association was strongest in the alcoholics who did not have drug dependence or major depressive episode; this was opposite to the COGA finding (Agrawal et al., 2006). Fehr et al. (2006) showed that the frequency of the risk haplotype was positively correlated with alcoholism severity, gauged by withdrawal seizures and early onset of dependence. The G allele of a synonymous exonic *GABRA2* SNP rs279858, a tag allele for this same less frequent alcoholism risk haplotype, was associated with a higher daily probability of drinking and heavy drinking during the 12-week treatment and 12-month post-treatment period in alcoholics from the Project MATCH study (Bauer et al., 2007). This *GABRA2* rs279858 G allele was also associated with decreased pleasurable effects for alcohol in social drinkers (Pierucci-Lagha et al., 2005). Furthermore, the subjective experiences of the social drinkers who had the rs279858 protective AA genotype could be manipulated by pre-treatment with finasteride, a metabolism blocker of the endogenous neurosteroids ALLO and THDOC,that diminished their subjective experiences of alcohol (Pierucci-Lagha et al., 2005). There was no effect of neurosteroid blockade on individuals with the rs279858 G risk allele, possibly because of a floor effect.

The results from the studies described above appear to indicate that both of the two abundant, yin yang haplotypes can be risk factors for alcoholism, at least in Caucasians. Supportive evidence for this apparent paradox comes from a study in non-treatment seeking alcoholics in two population isolates, Finnish Caucasians and Plains American Indians (Enoch et al., 2006). This study showed that although there was no haplotype association with alcoholism per se, alcoholics with high trait anxiety (TPQ harm avoidance) had the highest frequency of the more abundant haplotype, alcoholics with low anxiety had the highest frequency of the less abundant haplotype and non-alcoholics had intermediate frequencies (Enoch et al., 2006). Alcoholics, including those with antisocial personality disorder, have been shown to have higher trait anxiety than non-alcoholics (Ducci et al., 2007; Goodwin and Hamilton, 2003). If trait anxiety does indeed play a role in mediating linkage of *GABRA2* haplotypes with alcoholism then the Enoch et al. (2006) study would predict that COGA alcoholics have higher trait anxiety than the treatment seeking alcoholics of other studies (Covault et al., 2004; Fehr et al., 2006; Lappalainen et al., 2005). Indeed, it has been demonstrated that COGA alcoholics have higher trait anxiety than non-alcoholics (Ducci et al., 2007). Moreover, supportive evidence comes from the fact that in the Covault et al. (2004) study the effect got stronger when alcoholics with major depression (who are likely to have high trait anxiety) were removed. It is noteworthy that the linkage of a disorder with both alleles has been found previously, for example alcoholism has been associated with both the L and S alleles of the serotonin transporter promoter polymorphism *HTTLPR* (Feinn et al., 2005; Hu et al., 2005).

A few studies have found no *GABRA2* association with alcoholism, either in U.S. Caucasians (Covault et al., 2008 (Project MATCH dataset); Drgon et al., 2006; Matthews et al., 2007) or African Americans (Covault et al., 2008). Some of these negative findings may be due to sample selection or lack of power (Drgon et al., 2006; Matthews et al., 2007) although the Project MATCH study included over 700 alcoholics. Another issue may be sexual dimorphism. As described above, Enoch et al. (2006) found significant anxiety-mediated *GABRA2* haplotype associations with alcoholism in Finnish Caucasian and American Indian men but found no association in American Indian women. The negative study in African Americans (Covault et al., 2008) may be attributable to the fact that in this group the two yin yang risk haplotypes are present at much lower frequencies (29% and 27%) compared with other populations (Enoch, unpublished data; Figure 3). Finally, population stratification may be an issue.

Although further studies that include alcoholism subtypes, different ethnic groups and also women are clearly needed, there appears to be good evidence for a fundamental association between *GABRA2* and alcoholism. Many of the SNPs in allelic identity, such as rs279858 (discussed above) and rs279863 (Figure 2), are conserved across species indicating the likelihood of selective pressure for the *GABRA2* region distal to intron 3. One recent study has shown that *GABRA2* mRNA levels in post-mortem prefrontal cortical tissue differed significantly according to genotype (Haughey et al., 2007). However, no functional polymorphism has as yet been found There are numerous alternative splicing isoforms, most of which differ in their 3’ end after exon 3 (Figure 2) and these may be implicated in function. Within the brain there are four major isoforms with alternative 5’ (exon 1A or IB) and 3’ exons (exon 9A or 10) as well as minor isoforms lacking exon 4 or exon 8 (Tian et al, 2005;Jin et al, 2004). The functional significance of these isoforms is at present unknown. SNP rs279827 (Figure 2) is near the acceptor site of intron 4 and thus might affect splicing efficiency.

Studies in the COGA dataset have found no association between alcohol or illicit drug dependence and *GABRA4 (5 SNPs), GABRB1(6 SNPs)* or *GABRG1(6 SNPs)* (Edenberg et al., 2004; Agrawal et al., 2006). However two *GABRG1* SNPs in one COGA study showed trend level association with alcoholism (Edenberg et al., 2004). A recent study has shown significant haplotype and SNP association with alcoholism in a haplotype block that extends from the

intergenic region between *GABRA2* and *GABRG1* (Figure 1) up to *GABRG1* intron 3 in two large groups of U.S. Caucasians but not in African Americans (Covault et al., 2008). As the HapMap block structure indicates, there is extended LD between *GABRA2* and *GABRG1* (Figure 1). Covault et al. (2008) concluded that their earlier *GABRA2* association (Covault et al., 2004) may in part be secondary to LD with risk-related variants in *GABRG1* although there may be separate contributions to alcoholism risk from the two genes. One study found an association between a *GABRB1* intron 8 tetranucleotide repeat and alcoholism (Parsian and Zhang, 1999). A COGA study found consistent, albeit weak, LD between *GABRB1* and alcoholism (Song et al., 2003). A recent study found *GABRB1* haplotype linkage to alcoholism in both Caucasians and American Indians in both proximal and distal haplotype blocks of this large gene (Enoch et al., 2005).

$GABA_A$ receptor chromosome 5 and chromosome 15 gene clusters

The cluster 5 and cluster 15 genes (Figure 1) are not as strong candidates for alcoholism as are the cluster 4 genes for reasons discussed above. Indeed, the results of association studies have been mixed. Although COGA initially found no linkage between chromosome 5 genes and alcoholism (Dick et al., 2005;Song et al., 2003) a secondary analysis showed association of *GABRA1* with measures of drinking severity: history of blackouts, age at first drunkenness, level of response to alcohol (Dick et al., 2006b). A study in two population isolates: Finnish Caucasians and Southwestern American Indians, found sibpair linkage of alcohol dependence to *GABRG2* in the Finns and associations in both populations with *GABRB2* and *GABRA6* SNPs (Radel et al., 2005). Markers in *GABRA6* and *GABRB2* have been associated with alcohol dependence as well as Korsakoff's psychosis in a Scottish study (Loh et al., 1999). The Pro385Ser substitution in *GABRA6* has been associated with a lower level of response to the sedating effects of alcohol (Hu et al., 2005), and to reduced sensitivity to the effects of diazepam in children of alcoholics (Iwata et al., 1999) and a SNP in the 3' UTR (T1521C) has been associated with variation in physiological response to psychological stress (Uhart et al., 2004). There has been a positive association with *GABRG2* and antisocial alcoholism in Japanese (Loh et al., 2000) but negative studies for *GABRB2* and *GABRG2* in Germans (Sander et al., 1999)

Studies using the COGA dataset found evidence of haplotype and SNP association between alcohol dependence and *GABRG3* (Dick et al., 2004) and evidence for imprinting in that paternal, but not maternal, transmission of *GABRA5* and *GABRB3* alleles showed association with alcoholism (Song et al., 2003).

$GABA_A$ receptors as therapeutic targets for alcoholism

Benzodiazepines are commonly used to ameliorate the symptoms of acute withdrawal from alcohol, however there is an increasing trend to instead use anti-convulsants such as carbamezepine that are more effective in preventing rebound withdrawal symptoms and reducing post-treatment drinking than BZs (Malcolm et al., 2002; Mueller et al., 1997). The sedative-anticonvulsant chlomethiazole that may act at the anesthetic binding site on $GABA_A$ receptors (Usala et al., 2003) is widely used in Europe in acute withdrawal. Other, newer anti-convulsants that are considered to act primarily via an effect on GABA may prove useful in the treatment of alcoholism (Czuczwar and Patsalos, 2001). For example, topiramate, which has a complex effect on $GABA_A$ receptors (Gordey et al., 2000) has been shown to reduce the percentage of heavy drinking days and other drinking outcomes in recovering alcoholics (Johnson et al., 2007). Finally, as this review has indicated, neuroactive steroids are likely to be promising new drug targets for the treatment of alcoholism.

Conclusion

The modulation of $GABA_A$ receptors by ethanol is complex and involves numerous interactions including with neurosteroids, protein regulators, stress hormones and neurotransmitters such as opioids. Although substantial progress has been made in recent years we are still far from understanding the $GABA_A$ receptor changes associated with the switch from heavy drinking to alcohol addiction. Recent studies are beginning to shed light on associations between $GABA_A$ receptor genes and alcoholism although functional polymorphisms have yet to be determined. The relatively new field of epigenetics is emerging and studies of gene × environment interactions are likely to yield further insights into the role that $GABA_A$ receptors play in the development of alcoholism.

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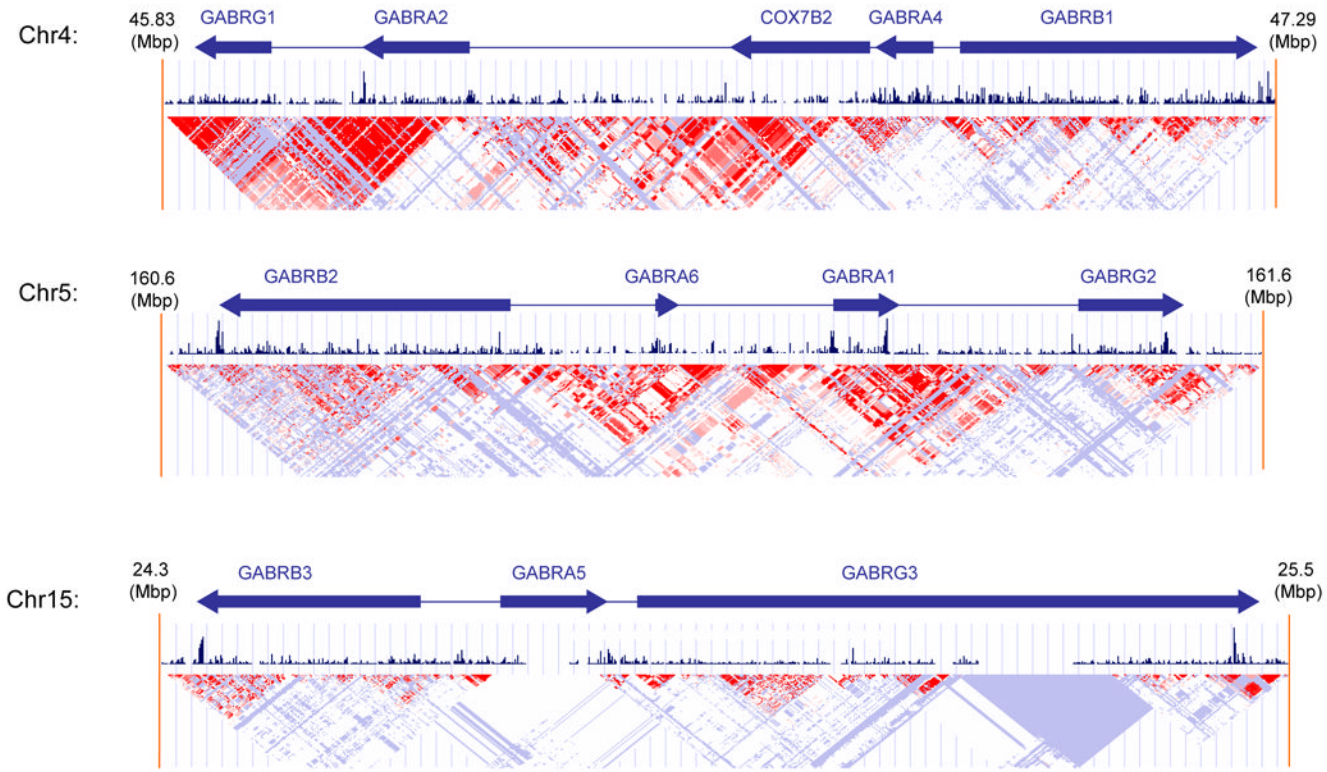

FIGURE 1. Chromosomal clusters for $GABA_A$ receptor genes in the HapMap Caucasian population

Arrows indicate gene position, size and direction of transcription. Red triangles show areas of linkage disequilibrium with D' ≥ 0.8. Conservation (vertical lines) across 17 vertebrate species, from zebrafish to humans is show across the chromosomal regions.

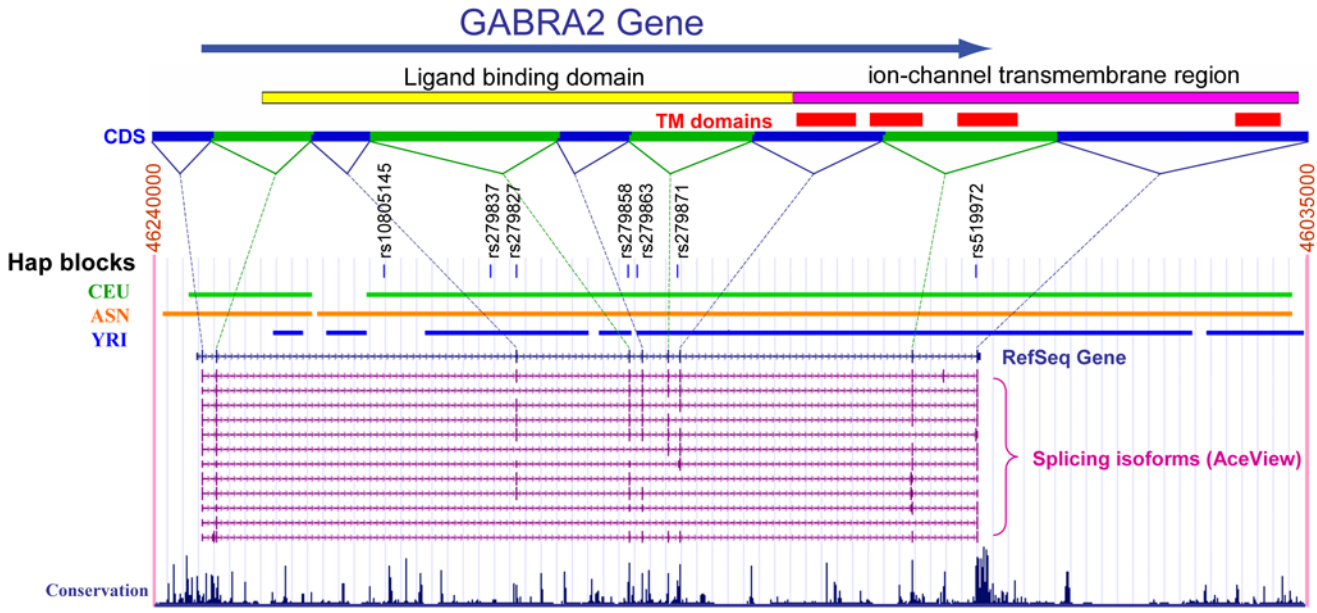

FIGURE 2. ***GABRA2*** **gene structure**

The large arrow indicates the direction of transcription. CDS = coding sequence are shown, linked by dotted lines to the relevant exons. TM domains = transmembrane domains, numbering 1 through 4 from left to right. The locations of the TM domains within the coding sequences are shown. HapMap population blocks are indicated: CEU = Caucasian; ANS = Asian; YRI = African. The structure of 12 isoforms is shown. Conservation across 17 vertebrate species, from zebrafish to humans is indicated. Information derived from HapMap and the UCSC genome browser: www.genome.ucsc.edu

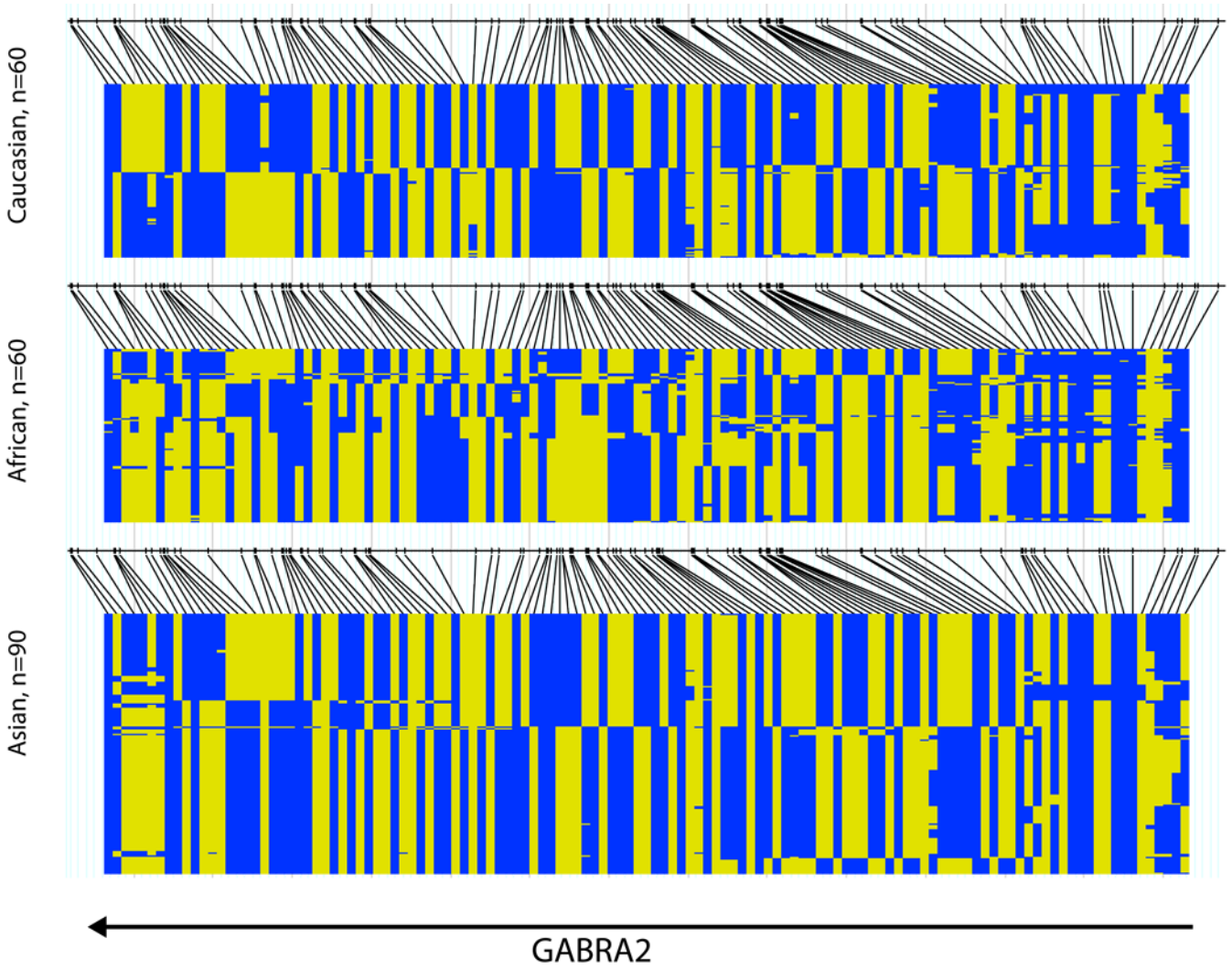

FIGURE 3. ***GABRA2*** **common haplotypes for HapMap Asian, African and Caucasian samples**
Black lines indicate the genotyped SNPs; the two colors indicate the two alleles for each SNP. All 3 populations show 2 haplotype blocks with the block boundary in intron 3. The Asian and Caucasian populations both have 2 common haplotypes in the distal haplotype block. The Africans show more haplotype diversity.