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***HTR3B* is associated with alcoholism with antisocial behavior and alpha EEG power—an intermediate phenotype for alcoholism and co-morbid behaviors**

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Abstract

Alcohol use disorders (AUD) with co-morbid antisocial personality disorder (ASPD) have been associated with serotonin (5-HT) dysfunction. 5-HT₃ receptors are potentiated by ethanol and appear to modulate reward. 5-HT₃ receptor antagonists may be useful in the treatment of early-onset alcoholics with co-morbid ASPD. Low-voltage alpha electroencephalogram (EEG) power, a highly heritable trait, has been associated with both AUD and ASPD. A recent whole genome linkage scan in one of our samples, Plains American Indians (PI), has shown a suggestive linkage peak for alpha power at the 5-HT_{3R} locus. We tested whether genetic variation within the *HTR3A* and *HTR3B* genes influences vulnerability to AUD with comorbid ASPD (AUD + ASPD) and moderates alpha power. Our study included three samples: 284 criminal alcoholic Finnish Caucasians and 234 controls; two independent community-ascertained samples with resting EEG recordings: a predominantly Caucasian sample of 191 individuals (Bethesda) and 306 PI. In the Finns, an intronic *HTR3B* SNP rs3782025 was associated with AUD + ASPD ($P = .004$). In the Bethesda sample, the same allele predicted lower alpha power ($P = 7.37 \times 10^{-5}$). Associations between alpha power and two other *HTR3B* SNPs were also observed among PI ($P = .03$). One haplotype in the haplotype block at the 3' region of the gene that included rs3782025 was associated with AUD + ASPD in the Finns ($P = .02$) and with reduced alpha power in the Bethesda population ($P = .00009$). Another haplotype in this block was associated with alpha power among PI ($P = .03$). No associations were found for *HTR3A*. Genetic variation within *HTR3B* may influence vulnerability to develop AUD with comorbid ASPD. 5-HT_{3R} might contribute to the imbalance between excitation and inhibition that characterize the brain of alcoholics.

Keywords

Antisocial Personality Disorder; Serotonin; HTR3; Alcoholism; EEG

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Disclosure/conflict of interest

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Introduction

The inability to defer the immediate pursuit of pleasurable stimuli is a core feature of several psychiatric disorders, including alcoholism and antisocial personality disorder (ASPD). Alcohol use disorders (AUD: alcohol dependence and abuse) and ASPD are common and etiologically complex diseases. According to the National Epidemiological Survey on Alcohol and Related Conditions (NESARC), lifetime prevalences of AUD and ASPD in the United States are 30% and 3.6%, respectively (Compton et al., 2005). These disorders often co-exist within individuals (Compton et al., 2005) and have been shown to share genetic (Kendler et al., 2003) and environmental etiologic factors (Robin et al., 1997). AUD with comorbid antisocial behavior appears to represent a subtype of alcoholism characterized by early onset of substance abuse, family history of alcoholism, increased novelty seeking (Cloninger et al., 1981), increased harm avoidance (Ducci et al., 2007a), polydrug use, and poorer prognosis (Babor et al., 1992). Strong evidence supports a role of serotonergic dysfunction in the etiology of this type of alcoholism. Cerebrospinal fluid levels of the serotonin (5-HT) metabolite 5-hydroxyindoleacetic acid are reduced in alcoholics with comorbid ASPD compared to healthy controls (Fils-Aime et al., 1996; Virkkunen et al., 1996). Reduced 5-HT was also found to be associated with impulsivity-aggression and excessive alcohol intake in nonhuman primates (Higley et al., 1996). Genetic variation within genes belonging to the 5-HT system, such as the 5-HT receptor 1B, 5-HT transporter, and monoamine oxidase A has been associated with alcoholism with comorbid ASPD (Ducci et al., 2007b; Hallikainen et al., 1999; Lappalainen et al., 1998). The 5-HT₃ receptor (5-HT₃R) is of special interest in the context of AUD and ASPD because of its role in reward and because 5-HT₃R is one of several receptors that are responsive to alcohol at concentrations frequently found in drinkers (Lovinger and White, 1991).

5-HT₃R is a ligand-gated ion channel whose activation results in rapid neuronal depolarization. Two subunits of 5-HT₃R (5-HT₃A and 5-HT₃B) have been characterized so far (Davies et al., 1999; Maricq et al., 1991). These subunits are approximately 44% identical in amino acid sequence and are encoded by two genes (*HTR3A* and *HTR3B*) that are co-localized within a 90-Kb region on chromosome 11q23.1. 5-HT₃A and 5-HT₃B are expressed in several brain regions, including the amygdala, caudate, and hippocampus (Davies et al., 1999). Functional 5-HT₃R receptors may be 5HT₃A homopentamers or may be 5-HT₃A/5-HT₃B heteropentamers. When expressed as a homomer, HTR3A is a functional receptor but displays rather weak conductance. However, heteromeric assemblies, including both 5-HT₃A and 5-HT₃B subunits, display a large single-channel conductance and electrophysiological properties that more closely resemble 5-HT₃R found naturally in brain (Dubin et al., 1999). Electrophysiological and microdialysis studies indicate that 5-HT₃R might have a significant role in regulating reward. 5-HT₃R antagonists reduce the number of spontaneously active dopamine (DA) neurons in the ventral tegmental area (VTA) (Minabe et al., 1991; Rasmussen et al., 1991), whereas local application of a 5-HT₃R agonist stimulates somatodendritic release of DA (Campbell et al., 1996). 5-HT₃R antagonists block both alcohol-stimulated (Campbell and McBride, 1995; Campbell et al., 1996) and morphine-stimulated (Imperato and Angelucci, 1989) DA release in the VTA. Furthermore, ethanol potentiates the activation of 5-HT₃R (Lovinger and White, 1991). Recent findings support the efficacy of the 5-HT₃R antagonist ondansetron in the treatment of early-onset alcoholism (Johnson et al., 2000, 2002), suggesting that this receptor might be particularly relevant to the physiopathology of this subtype of alcoholism.

Intermediate phenotypes are mediating factors for behaviors that are more heritable and genetically less complex than clinical phenotypes. Their use can enhance the ability to detect genetic effects on complex behaviors (Ducci and Goldman, 2008; Goldman et al., 2005). The resting electroencephalogram (EEG) accesses spontaneous brain electrical activity. Alpha

(8-13 Hz) power is the predominant EEG rhythm in the resting conscious state. Alpha power is a stable trait through healthy adulthood and is highly heritable (0.80) (Van Baal et al., 1996; van Beijsterveldt et al., 1996). Alcoholics appear to have reduced alpha power (Arentsen & Sindrup, 1963; Cogger et al., 1978; Ehlers & Phillips, 2007; Enoch et al., 1999; Jones & Holmes, 1976). In a group of Finnish criminal alcoholics recruited from the same source population as one of the three samples within this study, an overall reduction in alpha power was observed in the waking EEG of participants with ASPD compared with age and gender-matched healthy controls (Lindberg et al., 2005). Similarly, in a mainly Caucasian community-ascertained data set that is included in this report (Bethesda population), alcoholics displayed reduced alpha power (Enoch et al., 1999). Finally, in a recent whole genome linkage scan conducted in a Plains American Indian sample that is also included in this study, a suggestive peak (LOD score = 2.2) for alpha power was found on chromosome 11, in the region where the *HTR3A* and *HTR3B* genes are located, although no peak was observed in the same region for AUD (Enoch et al., submitted for publication). The association between alpha power and AUD was not detected in this population (M.-A. Enoch, personal communication).

The aim of this study was to evaluate whether genetic variation within *HTR3A* and *HTR3B* influence vulnerability to AUD with comorbid ASPD in a large case-control Finnish Caucasian data set that is enriched with early-onset alcoholics with antisocial behavior. To test whether *HTR3A* and *HTR3B* influences an EEG intermediate phenotype for AUD, we evaluated the association of these two genes with resting alpha EEG power, measured in two large and independent datasets including a predominantly Caucasian sample and a sample of Plains American Indians. We hypothesize that genetic variation within *HTR3A/B* genes might specifically moderate the risk of developing AUD with comorbid ASPD and might influence brain electrical activities measured by resting alpha EEG power.

Materials and methods

There were three independent samples in this study, all of whom had lifetime DSM-III-R psychiatric diagnoses: a Caucasian sample from Finland, a predominantly Caucasian sample recruited in Bethesda, Maryland, United States, and a population of Plains American Indians. Resting EEG recordings were available for the Bethesda sample and for the Plains Indians.

Informed consent was obtained according to human research protocols approved by the human research committees of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) for the Plains American Indians and Bethesda samples and the University of Helsinki for the Finnish sample. For the American Indian sample, the Tribal Council also gave approval.

Finns ($N = 518$; males = 100%)—This sample consists of criminal alcoholics and controls collected from the same Caucasian source population in Finland (for a more detailed description, see Lappalainen et al., 1998). The alcoholics were 284 unrelated male criminals who underwent forensic psychiatric examinations at the time of their initial incarceration; 159 were affected by both AUD and ASPD. The controls were 234 unrelated male volunteers who were recruited by advertisements in local newspapers and were compensated for their participation. The mean (standard error [S.E.]) age was 37.53 years (0.56). Mean age of onset (S.E.) of AUD was 19.4 years (0.5) among alcoholics with ASPD and 22.7 (0.5) among alcoholics without ASPD.

Bethesda ($N = 191$; males = 45%)—Volunteers were recruited from the general population of the Washington, DC/Baltimore, Maryland area by newspaper advertisements (Enoch et al., 1999). The only inclusion criterion for probands was that they had to have two living parents and at least two siblings who would, if required, be able to participate in the study. Ethnic

origin was self-identified. There were 175 Caucasians and 16 African-Americans. The mean (S.E.) age was 42.6 years (1.0) (for a more detailed description see Enoch et al., 1999).

Plains American Indians ($N = 306$; males = 42%)—Volunteers were recruited from a Plains American Indian community. Probands were initially ascertained at random from the tribal register, and the families of alcoholic probands were extended. Although most participants derived from one large, multigenerational pedigree, the average sharing of descent was only 0.3%. The average Plains Indian ancestry in our sample was 87% (S.D.: 21%); however, the median and modal values were >99%. The mean (S.E.) age was 42.09 years (0.61) (for a more detailed description see Enoch et al., 2006a).

Exclusion criteria for both the Bethesda and Plains Indian samples were as follows: a history of brain trauma, neurological disease, or recent use (in the past two weeks) of psychotropic drugs that might affect the EEG. Exclusion criteria at the time of testing were a positive breath test for alcohol, signs of alcohol withdrawal, or a positive urine test for psychoactive drugs.

Psychiatric assessment

All analyses used lifetime psychiatric diagnoses that were based on DSM-III-R criteria and assessed using semi-structured psychiatric interviews. The Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-L) (Endicott & Spitzer, 1978) was used in the Bethesda and Plains Indian samples. A clinical social worker (B.A.) experienced in the Plains Indian tribal customs and culture conducted the SADS-L interviews. The Structured Clinical Interview for DSM-III-R (SCID) (Spitzer et al., 1990a, 1990b, 1990c) was used in the Finnish sample.

Within the Finnish population, 284 participants had a lifetime diagnosis of an AUD and 86% of these had alcohol dependence. One hundred fifty-nine participants had both ASPD and AUD. All subjects with ASPD were also affected by AUD. Controls ($N = 234$) were non-ASPD and nonalcoholic.

In the Bethesda sample, 41 participants had a diagnosis of AUD (68% with alcohol dependence). Only two individuals had AUD comorbid with ASPD. One hundred eighty-two of the 306 Plains Indians had AUD (92% with alcohol dependence); 32 of the alcoholics had comorbid ASPD.

EEG acquisition and analysis

Resting EEG was recorded in the Bethesda and Plains Indians samples. The methods for EEG acquisition and analysis have already been fully described (Enoch et al., 1999, Enoch et al., submitted for publication). In the Bethesda sample, data were recorded from gold-plated electrodes applied to 19 scalp sites identified in the International 10-20 System and at two mastoid sites, all with reference to balanced sternovertrebral electrodes. In the Plains Indians, EEG signals were recorded from a customized fitted electrode cap (Electro-Cap International Inc., Eaton, OH) with pure tin electrodes in a six-channel montage: FZ, P3, PZ, P4, O1, and O2 with reference to balanced sternovertrebral electrodes. Electrode impedance was always below 5 k Ω . Resting EEG was recorded continuously for 3 min with eyes closed. EEG signals were continuously digitized at a sampling rate of 200 Hz and quantitative spectral analysis was performed (Coppola, 1979). Data records were partitioned into consecutive 512-point subunits (2.56 s each). Fast-Fourier transformation of filtered partitions yielded power spectrum estimates in 0.39 Hz steps. Artifact-free data were averaged to yield a composite power spectrum spanning three frequency domains; theta (3-8 Hz), alpha (8-13 Hz), and beta (13-30 Hz). All analyses in this report have been conducted using alpha power recorded posteriorly because alpha power is known to be maximal posteriorly under conditions of relaxed

wakefulness and is also more heritable posteriorly than anteriorly (Zietsch et al., 2007). Moreover, alpha power measures recorded at different scalp regions were highly correlated (pairwise correlations range: 0.89-0.97 in the Bethesda population and 0.60-0.91 among American Indians).

Genotyping

Sixteen SNPs spanning a 90-Kb region within which both *HTR3A* and *HTR3B* genes are located, were selected from the HapMap Project Public Release and genotyped using the Illumina GoldenGate Assay (see web sources listed in the Appendix). The two genes are separated by approximately 30 Kb. We selected SNPs to tag haplotypes in the most diverse HapMap population (African) and to tag all haplotypes with a frequency of at least 0.5% using a double classification tree search algorithm (Zhang et al., 2004). Several polymorphisms found within the coding sequences were forced into the tagging: rs1176744 (*HTR3B* Ser129Tyr); rs17116138 (*HTR3B* Ile183Val); rs1176713 (*HTR3A* Leu459Leu). Within *HTR3A* three markers (rs897692, rs1176724, rs897687) were monomorphic among Caucasians and displayed MAF < 0.005 among Plains American Indians. Therefore, these markers were excluded from all the analyses. Locations of the remaining 13 SNPs are shown in Fig. 1.

Statistical analyses

In the Finnish sample, allele/genotype/haplotype frequencies were compared between individuals with AUD + ASPD versus controls (non-AUD, non-ASPD) using the χ^2 test. The same test was also performed to compare allele/genotype frequencies between alcoholics without ASPD (AUD, no ASPD) and controls across all three datasets.

Genetic association with alpha EEG power was evaluated in the Bethesda and Plains Indian community samples. Absolute resting EEG power was log-transformed to reach normality and compared across genotype/diplotype groups using analysis of variance.

Hardy-Weinberg Equilibrium (HWE) for each locus and linkage disequilibrium (LD) between each pair of markers were computed using Haploview v3.32 (Barrett et al., 2005). Diplotypes were assigned to each individual using PHASE 2.02 (Stephens et al., 2001). Because frequencies of rare haplotypes computed with Phase are imprecise, carriers of haplotypes with frequency lower than 1% were excluded from the analyses.

All statistical analyses were conducted using JMP software v5.1 (SAS Institute, Cary, North Carolina). Criterion for statistical significance was set at 0.05.

Assessment of population stratification using ancestry informative markers (AIMS)

The Finnish and Plains Indian populations are considered to be population isolates, whereas the Bethesda population is more diverse. To characterize each individual for ethnic origin, 186 ancestry informative (AIMS) markers were selected as previously described (Enoch et al., 2006b). In brief, each AIM was a genetically independent HapMap SNP that differed in allele frequency by at least 0.7- and 10-fold between any two continental populations (from among Caucasians, Africans, and Asians). The AIMS were selected to be equally informative for all three continental populations. Genotyping was performed using the same Illumina GoldenGate Assay array described previously. The AIMS were genotyped in the Finnish, Bethesda, and Plains Indians samples but also in the 52 worldwide populations represented in the HGDP-CEPH Human Genome Diversity Cell Line Panel. This includes 1,051 individuals (see web sources listed in the Appendix).

Structure 2.2 (see web sources listed in the Appendix) was used to identify population substructure and compute individual ethnic factor scores. *Structure* was run simultaneously

using the AIMS genotypes from all three populations as well as the 52 populations represented in the HGDP-CEPH Panel. This “anchored” approach was preferred to the unanchored “within population” approach, because it yields a stable and interpretable ethnic factor structure. Number of ethnic clusters (k) was defined by running the data with different K values and computing the probability of $K = n$. The seven-factor solution was optimal. To evaluate the potential impact of ethnic substructure on our association results, ethnic factor scores were compared between cases and controls in the Finnish population using the χ^2 test. In the Finnish population, cases and controls did not differ in ethnic composition ($df = 6$; $\chi^2 = 0.368$; $P = .99$). In the Plains Indian and Bethesda populations, alpha power was correlated with ethnic factor scores modeled as a continuous variable using the Spearman ρ correlation. There were no significant correlations with any of the seven ethnic factors (range of P values in Bethesda = 0.21-0.99; range of P values among Plains Indians = 0.3-0.94). These results indicate that ethnic substructure is unlikely to have an impact on our association analyses and therefore further corrections were not undertaken, nor were the 16 African-Americans in the Bethesda sample excluded from the study.

Results

Behavioral associations of 13 *HTR3A/HTR3B* SNPs were studied in three populations, totaling 1,015 individuals. Allele frequencies differed across populations, especially between Caucasians and Plains Indians as shown in Table 1, where frequencies are given for controls (no ASPD, no AUD). Genotypes at all markers were in Hardy-Weinberg equilibrium.

Single marker association analyses between *HTR3A/B* SNPs and AUD + ASPD (in the Finnish population) and with alpha EEG power (in the Bethesda and Plains Indian populations) are shown in Tables 2 and 3. For easy interpretation, results are also summarized in Fig. 2.

Association of *HTR3A* and *HTR3B* with AUD comorbid with ASPD (single marker analyses)

Genotype and allele frequencies were compared between controls (no AUD, no ASPD) and alcoholics with comorbid ASPD (AUD + ASPD) in the Finnish population (see Table 2 and Fig. 2). The intronic *HTR3B* SNP rs3782025 was significantly associated with AUD + ASPD both at the genotype ($df = 2$; $\chi^2 = 9.59$, $P = .008$) and allele level ($df = 1$; $\chi^2 = 8.28$, $P = .004$). Within *HTR3A*, rs1150226 was significantly associated with AUD + ASPD at the allele level ($df = 1$; $\chi^2 = 4.49$, $P = .03$), although the frequency was low (only five heterozygotes).

Consistent with our a priori hypothesis that 5-HT3 is specifically involved in the pathogenesis of AUD with comorbid ASPD, there were no significant differences in genotype frequencies between controls and alcoholics without ASPD (AUD, no ASPD) across all the datasets: lowest P value: Finns: $P = .13$; Bethesda: $P = .10$, Plains: $P = .18$.

Association between *HTR3A* and *HTR3B* and alpha EEG power (single marker analyses)

Mean $\log_{10}$ alpha power was compared across the three genotype groups for each SNP in the Bethesda and Plains Indian populations (see Table 3 and Fig. 2).

At *HTR3B* SNP rs3782025, the same allele that associated with AUD + ASPD (allele A) in the Finnish population was also associated with reduced alpha power in the Bethesda population ($F = 10.02$; $P = 7.32e^{-5}$) (see Fig. 2).

Among Plains Indians, SNPs rs2276307 ($df = 2$; $F = 3.29$, $P = .03$), adjacent to rs3782025, and rs11606194 ($df = 1$, $F = 4.51$, $P = .03$) were significantly associated with alpha power. SNP rs3782025 was not significantly associated with alpha power among Plains Indians (Table 3).

Haplotype-based analyses

HTR3A and *HTR3B* linkage disequilibrium structure is shown in Fig. 3, where pairwise LD values are given. Across populations, within *HTR3B* we identified two regions of relatively reduced recombination: a 5'-block including five SNPs and a second block located at the 3' of the gene and including four SNPs. In contrast, low D' values were detected between pairs of markers within *HTR3A* and between the two genes.

Haplotype frequencies were computed within *HTR3B*. Haplotype frequencies differed between Caucasians and the Plains Indians (Table 4). In the first haplotype-block, two major haplotypes (TTTGA, CTTGC), accounted for between 91% and 82% of haplotype diversity in the Finnish and Bethesda populations, respectively. Among American Indians, haplotypes TTTGA, CTTGC, and TGTGA accounted for 90% of haplotype diversity. In the second haplotype block, three major haplotypes (GAGT, GAAT, GGAT), accounted for more than 90% of haplotype diversity across all three populations.

Association between *HTR3B* and AUD with comorbid ASPD (haplotype-based analyses)

Haplotype frequencies were compared between alcoholics with comorbid ASPD (AUD + ASPD) and controls (no AUD; no ASPD) in the Finnish population. Within the second *HTR3B* block, haplotype frequencies were overall significantly different between cases and controls ($df = 4$; $\chi^2 = 10.72$; $P = .02$). Haplotypes GAAT ($df = 1$; $\chi^2 = 2.56$; $P = 0.10$) and GGAT ($df = 1$; $\chi^2 = 2.98$; $P = .08$) were more abundant in ASPD + AUD ($df = 1$, $\chi^2 = 2.95$ $P = .08$) and haplotypes AAGA ($df = 1$; $\chi^2 = 1.21$, $P = .27$), and GAGT ($df = 1$; $\chi^2 = 5.19$; $P = .02$) were more abundant among controls. However, the signal of association appeared to be driven by SNP rs3782025 (Table 5).

No differences in haplotype frequencies were detected when controls were compared to non-ASPD alcoholics (Finns: $df = 4$; $\chi^2 = 3.09$; $P = .37$; Bethesda: $df = 4$, $\chi^2 = 5.39$; $P = .24$; Plains: $df = 4$; $\chi^2 = 2.043$; $P = .72$).

No differences in haplotype frequencies were detected between cases and controls within the first *HTR3B* block.

Association between *HTR3B* and alpha power in the Bethesda and Plains Indian populations (haplotype-based analyses)

Mean Log_{10} alpha power was compared across groups of individuals carrying two, one, or zero copies of each haplotype for each block (see Table 6). Significant differences were detected within the second *HTR3B* block. In the Bethesda population, carriers of two copies of GAAT had significantly reduced alpha power compared with subjects with zero copies and carriers of one copy had intermediate values ($df = 2$, $F = 5.96$; $P = .003$, Table 6). Conversely, carriers of two copies of GAGT had elevated alpha power compared to subjects with zero copies and one copy was associated with intermediate values ($df = 2$; $F = 9.8$; $P = .00009$). Haplotype GGAT was associated with increased alpha power among American Indians ($df = 2$; $F = 3.38$; $P = 0.03$).

No differences in alpha power were detected for haplotypes located in the first *HTR3B* block.

Discussion

Our study has shown that genetic variation within *HTR3B* is associated with AUD comorbid with ASPD in the Finnish population. Consistently with our a priori hypothesis, no significant differences emerged when controls were compared with non-ASPD alcoholics, indicating that 5-HTR3 receptor might be specifically involved in the etiology of early onset alcoholics with

antisocial behavior. *HTR3B* markers were also significantly associated with alpha power in both the Bethesda and Plains Indians populations. Among both of the two mainly Caucasian populations (United States and Finnish), most of the signal appeared to come from an intronic *HTR3B* SNP, namely rs3782025. This SNP significantly predicted AUD with comorbid ASPD in the Finnish population. In the Bethesda population, the same allele was also strongly associated with low alpha power and accounted for 8% of the variance of this resting EEG trait. Significant associations between alpha power and *HTR3B* markers were found among Plains Indians but with different SNPs as compared to Caucasians. No significant findings were reported for *HTR3A*.

Our findings suggest that genetic variation within *HTR3B* may influence vulnerability to develop AUD with comorbid ASPD. Consistent with this idea, the 5-HT_{3R} antagonist ondansetron appears to be effective in the treatment of early onset alcoholics who frequently have ASPD. In a 12-week double-blind, placebo-controlled clinical trial conducted in 321 alcohol-dependent subjects, ondansetron was effective in reducing alcohol consumption and craving in early onset alcoholics (<25 years) but not in late onset (Johnson et al., 2000). Also, ondansetron has been shown to reduce alcohol-heightened aggressive behavior in mice (McKenzie-Quirk et al., 2005). Genetic variation within *HTR3B* has been shown to influence vulnerability to bipolar disorder (Frank et al., 2004) and major depression (Yamada et al., 2006), but to our knowledge this is the first time that an association between AUD with comorbid ASPD and the *HTR3B* gene has been reported. Most of the in vitro electrophysiological studies conducted so far on 5-HT_{3R} have focused on the A subunit. However, more recently, two studies have explored the roles of both subunits and have found that ethanol enhances the activation of HTR3A homomeric receptors, but has no effect on HT3A/B heteromeric receptors (Hayrapetyan et al., 2005; Stevens et al., 2005). Thus, genetic variation within *HTR3B* might influence level of expression of this subunit and in turn the proportion of HTR3A homomeric Vs HTR3A/B heteromeric receptors. Alteration in the subunit composition of 5-HT_{3R} in the brain could in turn affect risk of developing alcoholism moderating ethanol response via this receptor that is known to regulate reward to alcohol and other drugs (Minabe et al., 1991; Rasmussen et al., 1991). Interestingly, a deletion within *HTR3B* has been shown to moderate response to ondansetron in the treatment of nausea (Tremblay et al., 2003). Similarly, one could speculate that genetic variation within *HTR3B* might moderate response to ondansetron in the treatment of alcoholism.

Alterations in brain electrical activity have been described in both alcoholics and ASPD patients (Arentsen and Sindrup, 1963; Coger et al., 1978; Ehlers and Phillips, 2007; Enoch et al., 1999; Jones and Holmes, 1976; Lindberg et al., 2005). In this report, *HTR3B* was found to be associated with low alpha power in the resting EEG, an intermediate phenotype for AUD and ASPD (Lindberg et al., 2005). Our finding indicates that genetic variation within *HTR3B* might be a modulator of neuronal electrical activity and might contribute to the imbalance between excitation and inhibition that characterizes the brain of alcoholics. Consistently, in a whole genome linkage study recently conducted in the Plains Indians sample, an LOD score (2.2) suggestive of linkage to alpha power was found in the same chromosome region where *HTR3A* and *HTR3B* genes are located (Enoch et al., submitted for publication). 5-HT_{3R} is an ion-channel receptor whose activation results in rapid neuronal depolarization. One of the possible mechanisms through which 5-HT_{3R} might influence resting EEG is through the modulation of GABAergic neurons. 5-HT_{3R} is expressed in the subpopulation of GABAergic interneurons that are located within the neocortex, hippocampus, and amygdala (Bloom and Morales, 1998). Activation of GABAergic interneurons via 5-HT_{3R} has been shown to result in a GABA-mediated inhibition of pyramidal cell of the rat hippocampus (Ropert and Guy, 1991) and similar mechanisms are likely to exist in other brain regions.

Associations that we found with both ASPD and alpha power were strongest with marker rs3782025, and stronger than associations with any haplotype. However, a significant association was reported also at the haplotype level. Haplotype GAGT, located in the second *HTR3B* block, was less frequent among alcoholics with ASPD in the Finnish population and was associated with increased alpha power in the Bethesda population. Our results suggest that the *HTR3B* association signal originates from the 3' end of the *HTR3B* gene, which is also upstream of the *HTR3A* gene. Interestingly, SNP rs3782025 is located in a region which appears to be highly conserved across species.

A significant association between *HTR3B* and alpha power was also found among Plains Indians. However, the signal in this case came from different SNPs, namely rs2276307 that is in LD with rs3782025 and rs11606194 that is in haplotype block 1. Also, among Plains Indians haplotype GGAT was significantly associated with reduced alpha power. This result overall supports the idea that genetic variation within *HTR3B* might be important in moderating alpha power. However, the pattern of association among Plains Indians appears to be different from that observed in the Bethesda and Finnish populations. A possible explanation for these discrepancies might be related to differences in the ascertainment of the Bethesda/Finnish samples as compared to the Plains Indian population. In the latter population, family members of alcoholics' probands were also recruited. As a consequence, many nonalcoholics had a family history of alcoholism, and alcoholism was not associated with alpha power in this population (Enoch et al., submitted for publication). In contrast, association between alpha power and alcoholism were found both in the Finnish (Lindberg et al., 2005) and Bethesda (Enoch et al., 1999) populations. Another possible explanation might be related to genetic heterogeneity; therefore, functional polymorphisms might be different across different populations.

This study has strengths as well as important limitations. Strengths include the use of three relatively large and independent samples, use of clinical phenotypes as well as intermediate phenotypes to increase power to detect gene effects (Goldman and Ducci 2007), and use of ancestry informative markers to rule out ethnic stratification. Limitations include the lack of one large population with measures of both alpha EEG power and a sufficient number of antisocial alcoholics to perform case-control analysis. *P* values presented in the present study were not corrected for multiple testing. Nevertheless, the same SNP and haplotypes were associated with two related phenotypes (ASPD and alpha power) in the same direction in two independent samples of Caucasians. Furthermore, even after applying Bonferroni correction for the number of SNPs tested ($n = 13$), associations between rs3782025 with AUD + ASPD in the Finns and alpha power in the Bethesda population remain significant. Of note, applying Bonferroni correction for the number of SNPs is a very conservative approach because the SNPs tested were not independent (see Fig. 3).

In conclusion, our findings indicate that genetic variation within *HTR3B* may influence vulnerability to alcoholism with comorbid ASPD and may contribute to the low alpha power trait detected among alcoholics as a group. Further research is needed to confirm these findings and, if confirmed, to identify a functional locus or loci in the *HTR3B/HTR3A* region and to explore the molecular biological mechanisms whereby 5-HT_{3R} influences alpha power and vulnerability to disease.

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Appendix

Appendix

Web resources

Illumina GoldenGate Assay: http://www.illumina.com/products/prod_snp_ilmn).

HapMap Project: <http://www.hapmap.org/>

HGDP-CEPH Human Genome Diversity Cell Line Panel:
<http://www.cephb.fr/HGDP-CEPH-Panel>

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*Structure*2.2:<http://pritch.bsd.uchicago.edu/software.html>

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Fig. 1. Distribution of 13 genotyped polymorphisms spanning the 90 kb region where *HTR3A* and *HTR3B* are located.

Fig. 2. Association between *HTR3A/B* SNPs with alcohol use disorder (AUD) co-morbid with antisocial personality disorder (ASPD) in the Finnish population and with an alpha electroencephalogram (EEG) power in the Bethesda and Plains Indian populations.

Fig. 3.

Haplotype-block structure of *HTR3A* and *HTR3B* in the Finnish, Bethesda, and American Indian populations. Color scheme: *black*: $D' = 1$, $\text{LOD} \geq 2$; shades of *grey*: $D' < 1$; $\text{LOD} \geq 2$; *white*: $D' = 1$, $\text{LOD} < 2$; and *light grey*: $D' < 1$; $\text{LOD} < 2$. Block size is proportional to physical distance. SNP rs10502180 is excluded in the Bethesda and Finnish populations because of the low frequency of the rare allele at this SNP among Caucasians.

Table 1MAF of 13 *HTR3A/HTR3B* SNPs among control subjects (without AUD or ASPD), in three populations

		Bethesda (<i>N</i> = 150)	Finns (<i>N</i> = 234)	Plains Indians (<i>N</i> = 124)
<i>HTR3B</i>	SNPs	MAF	MAF	MAF
	rs3758987	0.27	0.30	0.42
	rs10502180	0.01	0.01	0.31
	rs11606194	0.07	0.10	0.07
	rs17116121	0.03	0.10	0.04
	rs1176744	0.27	0.29	0.38
	rs17116138	0.03	0.10	0.04
	rs2276307	0.20	0.17	0.31
	rs3782025	0.48	0.46	0.35
	rs1176761	0.03	0.10	0.05
<i>HTR3A</i>	rs1150226	0.06	0.04	0.04
	rs2276302	0.31	0.37	0.10
	rs3737457	0.01	0.02	0.30
	rs1176713	0.23	0.27	0.35

Abbreviations: MAF = minor allele frequencies; AUD = alcohol use disorders; ASPD = antisocial personality disorder.

Table 2
Associations between *HTR3A* and *HTR3B* SNPs and alcoholism with comorbid ASPD in the Finnish population

Gene	SNPs	G	Controls	AUD + ASPD	χ^2	P value	A	Controls	AUD + ASPD	χ^2	P value
HTR3B	rs3758987	TT	0.47 (109)	0.46 (72)	0.58	.74	T	0.70	0.70	0	.96
		CT	0.45 (103)	0.48 (76)							
		CC	0.08 (18)	0.06 (10)							
	rs10502180	TT	0.98 (227)	0.99 (158)	1.63	.2	T	0.99	1.00	1.44	.42
		GT	0.02 (5)	0.006 (1)							
		GG	0	0							
	rs11606194	TT	0.80 (184)	0.83 (132)	1.61	.44	T	0.9	0.92	0.78	.38
		CT	0.20 (46)	0.17 (27)							
		CC	0.004 (1)	0							
	rs17116121	AA	0.01 (3)	0.06 (1)	1.8	.41	A	0.1	0.07	1.81	.18
		AG	0.17 (39)	0.13 (20)							
		GG	0.82 (191)	0.87 (138)							
	rs1176744	AA	0.50 (115)	0.48 (74)	0.25	.88	A	0.71	0.69	0.21	.64
		AC	0.43 (99)	0.43 (67)							
		CC	0.08 (18)	0.09 (14)							
rs17116138	AA	0.013 (3)	0.063 (1)	1.09	.58	A	0.1	0.08	1.05	.31	
	AG	0.17 (39)	0.14 (22)								
	GG	0.82 (191)	0.86 (136)								
rs2276307	AA	0.69 (158)	0.60 (96)	3.58	.17	A	0.83	0.78	2.95	.08	
	AG	0.29 (67)	0.35 (56)								
	GG	0.02 (5)	0.04 (7)								
rs3782025	AA	0.22 (52)	0.30 (48)	9.59	.008	A	0.46	0.57	8.28	.004	
	AG	0.48 (111)	0.53 (84)								
	GG	0.30 (70)	0.17 (27)								
rs1176761	TT	0.82 (191)	0.86 (136)	1.35	.51	T	0.9	0.93	1.34	.23	
	AT	0.17 (39)	0.13 (21)								
	AA	0.013 (3)	0.006 (1)								
HTR3A	rs1150226	AA	0.043 (1)	0	4.91	.08	A	0.04	0.02	4.49	.03
		AG	0.08 (18)	0.03 (5)							
		GG	0.92 (215)	0.97 (154)							

Gene	SNPs	G	Controls	AUD + ASPD	χ^2	P value	A	Controls	AUD + ASPD	χ^2	P value
	rs2276302	AA	0.39 (90)	0.40 (63)	0.27	.87	A	0.63	0.63	0	1
		AG	0.48 (112)	0.46 (72)							
		GG	0.13 (31)	0.15 (23)							
	rs3737457	AA	0	0	0.35	.56	A	0.02	0.02	0.33	.56
		AG	0.04 (10)	0.03 (5)							
		GG	0.96 (223)	0.97 (154)							
	rs1176713	AA	0.52 (120)	0.54 (85)	3.9	.14	A	0.73	0.71	0.19	.66
		AG	0.42 (97)	0.35 (55)							
		GG	0.06 (15)	0.11 (18)							

Abbreviations: ASPD = antisocial personality disorder; AUD = alcohol use disorders.

Allele (A, $df=1$) and genotype (G, $df=2$) frequencies are compared between cases (AUD + ASPD) and controls (no AUD; no ASPD) using the χ^2 test. SNP alleles are labeled using the “+” DNA strand sequence.

Table 3
Association between markers within *HTR3A* and *HTR3B* genes and alpha power in the Bethesda and American Indian populations

American Indians										
Bethesda										
SNP	G	N	Log alpha power (S.E.)	F	P value	N	Log alpha power (S.E.)	F	P value	
HTR3B	rs3758987	TT	97	4.27 (0.04)	0.01	.99	110	4.28 (0.04)	0.58	.58
		CT	76	4.27 (0.05)			145	4.26 (0.3)		
		CC	14	4.28 (0.11)			51	4.32 (0.05)		
	rs10502180	TT	187	4.27 (0.03)	3.56	.06	140	4.29 (0.03)	0.97	.38
		GT	4	4.67 (0.21)			138	4.24 (0.03)		
		GG	0				28	4.35 (0.07)		
	rs11606194	TT	159	4.25 (0.03)	3.87	.06	270	4.29 (0.02)	4.51	.03
		CT	32	4.41 (0.07)			34	4.14 (0.07)		
		CC	0				0			
	rs17116121	AA	0		1.16	.28	0		1.88	.17
		AG	16	4.38 (0.10)			22	4.16 (0.08)		
		GG	175	4.27 (0.03)			283	4.28 (0.02)		
	rs1176744	AA	97	4.29 (0.04)	0.09	.91	126	4.27 (0.03)	1.16	.31
		AC	72	4.26 (0.05)			138	4.25 (0.03)		
		CC	19	4.26 (0.09)			42	4.36 (0.06)		
	rs17116138	AA	0		1.16	.29	0		1.88	.17
		AG	16	4.38 (0.10)			22	4.16 (0.08)		
		GG	175	4.27 (0.03)			283	4.28 (0.03)		
	rs2276307	AA	125	4.3 (0.06)	0.99	.37	155	4.27 (0.03)	3.29	.03
		AG	55	4.23 (0.06)			119	4.24 (0.03)		
		GG	9	4.14 (0.14)			32	4.44 (0.07)		
	rs3782025	AA	48	4.06 (0.06)	10.02	7.37e⁻⁰⁵	122	4.34 (0.03)	2.87	.06
		AG	96	4.32 (0.04)			146	4.23 (0.03)		
		GG	43	4.42 (0.06)			37	4.25 (0.07)		
	rs1176761	TT	174	4.27 (0.03)	0.41	.52	281	4.28 (0.02)	1.33	.18
		AT	17	4.33 (0.10)			24	4.18 (0.08)		
		AA	0				0			

		Bethesda		American Indians						
	SNP	G	N	Log alpha power (S.E.)	F	P value	N	Log alpha power (S.E.)	F	P value
HTR3A	rs1150226	AA	4	4.44 (0.21)	1.24	.29	0		0.02	.88
		AG	23	4.16 (0.08)			20	4.29 (0.08)		
		GG	163	4.29 (0.03)			286	4.27 (0.03)		
	rs2276302	AA	82	4.24 (0.05)	1.27	.28	243	4.28 (0.02)	0.04	.95
		AG	83	4.33 (0.05)			57	4.26 (0.05)		
		GG	24	4.22 (0.08)			6	4.30 (0.16)		
	rs3737457	AA	0		2.88	.09	30	4.26 (0.07)	0.41	.67
		AG	4	4.62 (0.21)			130	4.25 (0.03)		
		GG	187	4.27 (0.03)			146	4.29 (0.03)		
	rs1176713	AA	111	4.24 (0.04)	1.38	.25	120	4.30 (0.03)	0.44	.64
		AG	72	4.34 (0.05)			143	4.25 (0.03)		
		GG	7	4.20 (0.16)			43	4.29 (0.05)		

Abbreviation: S.E. = standard error.

SNP alleles are labeled using the “+” DNA strand sequence.

Table 4

Haplotype frequencies within *HTR3B* block one and two in the Finnish, Bethesda, and Plains Indian controls (no AUD, no ASPD). SNP alleles are labeled using the “+” DNA strand sequence

Block	Haplotype	Bethedas (N = 150)	Finns (N = 229)	Plains Indian (N = 124)
1	TTTGA	0.71	0.65	0.26
	CTTGC	0.2	0.17	0.33
	TGTGA	0	0	0.32
	TTTGC	0.02	0.01	0.007
	CTCAC	0.03	0.07	0.04
	CTCGC	0.04	0.03	0
	CTTGA	0	0.03	0.01
	CTCGA	0	0	0.03
2	GAGT	0.45	0.44	0.29
	GAAT	0.33	0.29	0.36
	GGAT	0.19	0.17	0.31
	AAGA	0.03	0.1	0.04

Abbreviations: AUD = alcohol use disorders; ASPD = antisocial personality disorder.

Table 5

Frequencies of haplotypes within HTR3B block 2 are compared between alcoholics with comorbid ASPD (AUD + ASPD) and controls (no AUD, no ASPD) in the Finnish population (χ^2 analyses, $df = 1$)

Haplotype	ASPD (N = 156)	Controls (N = 229)	χ^2	P
AAGA	0.07	0.10	1.21	0.27
GAGT	0.36	0.44	5.19	0.02
GAAT	0.35	0.29	2.65	0.1
GGAT	0.22	0.17	2.9	0.08

Abbreviations: ASPD = antisocial personality disorder; AUD = alcohol use disorders.

SNP alleles are labeled using the “+” DNA strand sequence.

Table 6
Association between *HTR3B* haplotypes and alpha power in the Bethesda and Plains American Indian populations (haplotype-cluster based analyses)

Bethesda		Plains								
		N of copy	N	Log alpha power (SE)	F	P value	N	Log alpha power (SE)	F	P value
B1	TTTGA	0	20	4.29 (0.09)	0.02	.98	158	4.31 (0.03)	2.55	.08
		1	79	4.27 (0.05)			126	4.22 (0.03)		
		2	92	4.27 (0.04)			21	4.38 (0.08)		
	TGTGA	0					140	4.28 (0.03)	1.03	.36
		1					137	4.24 (0.03)		
		2					28	4.35 (0.07)		
	TTTGC	0	180	4.28 (0.03)	1	.32				
		1	11	4.15 (0.13)						
		2								
	CTTGC	0	133	4.30 (0.04)	1.58	.21	147	4.27 (0.03)	1.59	.2
		1	47	4.18 (0.06)			122	4.25 (0.03)		
		2	11	4.31 (0.12)			36	4.38 (0.06)		
CTCAC	0	175	4.27 (0.03)	1.16	.28	282	4.28 (0.02)	1.29	.27	
	1	16	4.38 (0.11)			22	4.16 (0.08)			
	2					1	4.61 (0.39)			
B2	CTCGC	0	177	4.26 (0.03)	1.73	.19				
		1	14	4.42 (0.11)						
		2								
	CTTGA	0					289	4.27 (0.03)	0.34	.56
		1					16	4.33 (0.09)		
		2								
	CTCGA	0					293	4.28 (0.02)	2.53	.11
		1					12	4.09 (0.11)		
		2								
	GAGT	0	53	4.07 (0.06)	9.8	.00009	135	4.32 (0.03)	1.86	.16
		1	103	4.33 (0.04)			146	4.23 (0.03)		
		2	31	4.43 (0.07)			24	4.25 (0.08)		

Bethesda		Plains							
N of copy		N	Log alpha power (SE)	F	P value	N	Log alpha power (SE)	F	P value
GGAT	0	125	4.30 (0.04)	0.84	.42	155	4.27 (0.03)	3.38	.03
	1	53	4.24 (0.06)			118	4.24 (0.04)		
	2	9	4.14 (0.14)			32	4.44 (0.07)		
GAAT	0	88	4.35 (0.04)	5.96	.003	131	4.27 (0.03)	0.4	.67
	1	77	4.27 (0.04)			140	4.27 (0.03)		
	2	22	4.01 (0.09)			34	4.33 (0.07)		
AAGA	0	171	4.27 (0.03)	1.08	.29	282	4.28 (0.02)	1.3	.27
	1	16	4.38 (0.11)			22	4.16 (0.08)		
	2	0				1	4.61 (0.39)		

SNP alleles are labeled using the “+” DNA strand sequence.