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Association of Single Nucleotide Polymorphisms in a Glutamate Receptor Gene (*GRM8*) With Theta Power of Event-Related Oscillations and Alcohol Dependence

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Evidence suggests the P3 amplitude of the event-related potential and its underlying superimposed event-related oscillations (EROs), primarily in the theta (4–5 Hz) and delta (1–3 Hz) frequencies, as endophenotypes for the risk of alcoholism and other disinhibitory disorders. Major neurochemical substrates contributing to theta and delta rhythms and P3 involve strong GABAergic, cholinergic and glutamatergic system interactions. The aim of this study was to test the potential associations between single nucleotide polymorphisms (SNPs) in glutamate receptor genes and ERO quantitative traits. *GRM8* was selected because it maps at chromosome 7q31.3–q32.1 under the peak region where we previously identified significant linkage (peak LOD = 3.5) using a genome-wide linkage scan of the same phenotype (event-related theta band for the target visual stimuli). Neural activities recorded from scalp electrodes during a visual oddball task in which rare target elicited P3s were analyzed in a subset of the Collaborative Study on the Genetics of Alcoholism (COGA) sample comprising 1,049 Caucasian subjects from 209 families (with 472 DSM-IV alcohol dependent individuals). The family-based association test (FBAT) detected significant association ($P < 0.05$) with multiple SNPs in the *GRM8* gene and event-related theta power to target visual stimuli, and also with alcohol dependence, even after correction for multiple comparisons by false discovery rate (FDR). Our results suggest that variation in *GRM8* may be involved in modulating event-related theta oscillations during information processing and also in vulnerability to alcoholism. These findings underscore the utility of electrophysiology and the endophenotype approach in the genetic study of psychiatric disorders.

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INTRODUCTION

Using diagnoses as the sole phenotypes may not be optimal for genetic dissection of complex (non-Mendelian) psychiatric diseases. Psychiatric diagnoses are dichotomous; either an individual is affected or unaffected [Gottesman and Gould, 2003; Begleiter and Porjesz, 2006], with diagnoses based on symptomatic definitions that reflect much heterogeneity in the diseases. In addition, psychiatric diagnoses may adjust over time due to the changes in the diagnostic criteria. In recent decades, research has been directed at identifying the characteristic traits (i.e., endophenotypes or intermediate phenotypes) in subjects with psychiatric diseases as well as their relatives in order to understand the factors underlying the pathogenesis of the disorders. It is believed that these endophenotypes are closer to gene action than diagnostic categories, and that they provide a more powerful strategy in searching for genes involved in producing psychiatric diagnoses [Almasy and Blangero, 1998; Gottesman and Gould, 2003; Porjesz et al., 2005].

Event-related potentials (ERPs) provide a non-invasive tool to explore the characteristics of sensory processes and higher cognitive function in the brain. The P3 (or P300) component is possibly the best-studied ERP. This positive electric potential deflection is elicited approximately 300–500 ms following the occurrence of infrequent stimuli during an oddball experimental paradigm. P3 is highly heritable [O'Connor et al., 1994; Katsanis et al., 1997; Almasy et al., 1999] and it provides quantitative endophenotypes for some complex psychiatric disorders, including disinhibitory disorders such as alcohol or substance-related disorders, conduct disorder, attention-deficit hyperactive disorder (ADHD), antisocial personality disorder (ASP), impulse control disorders [Hesselbrock et al., 2001; Porjesz et al., 2005]. Therefore, identifying specific genetic variants that modulate the P3 and other related electrophysiological measures is a rational strategy to search for genes associated with the relevant psychiatric disorders. By using this strategy, genetic analysis of P300 amplitude data from the Collaborative Study on the Genetics of Alcoholism (COGA) has revealed significant linkage on a number of chromosomes with alcohol dependence and other disinhibitory disorders [e.g., Begleiter et al., 1998; Porjesz et al., 2004].

Recent research suggests that ERPs are not unitary phenomena but are formed through the superposition of multiple event-related oscillations (EROs) [Makeig et al., 2002; Gruber et al., 2005]. The P3 response is primarily composed of superimposed delta (1–3 Hz) and theta (4–7 Hz) frequency band energy with delta energy more concentrated in the posterior region and theta more fronto-central [Basar-Eroglu et al., 1992; Basar et al., 1999; Schutte et al., 1999; Karakas et al., 2000]. Using ERO data underlying the target visual evoked P3 component, we [Jones et al., 2004] have found significant linkage and linkage disequilibrium on chromosome 7 with theta band (4–7 Hz) EROs. The gene encoding the muscarinic acetylcholine receptor M2 (*CHRM2*) is located under the observed linkage peak. Linkage disequilibrium (LD) analysis revealed significant association between *CHRM2* single nucleotide polymorphisms (SNPs) and both delta (1–3 Hz) and theta (4–7 Hz) band ERO data [Jones et al., 2004]. Recent evidence from COGA indicates that the *CHRM2* gene is not only associated with EROs underlying P3 but also clinical diagnosis, i.e., alcohol dependence and depression [Wang et al., 2004].

The major neurochemical substrates contributing to theta and delta rhythms and P3 involve strong GABAergic, cholinergic and glutamatergic system interactions, and perhaps dopaminergic and noradrenergic influences [e.g. reviewed by Polich and Criado, 2006]. Glutamatergic neurotransmission and its neuroadaptive changes have been proposed as important molecular determinants of craving and relapse [e.g., Cornish and Kalivas, 2000; Tzschentke and Schmidt, 2000]. In particular, it is suggested that a hyperglutamatergic state mediates, at least in part, alcohol relapse behavior [Tsai and Coyle, 1998]. Several studies in animal models and human subjects indicate the possible involvement of NMDA [Holter et al., 2000; Krystal et al., 2003; Bachteler et al., 2005] and metabotropic glutamate receptors during alcohol relapse [Backstrom et al., 2004; Bachteler et al., 2005; Olive et al., 2005]. Acamprosate, a drug used to prevent relapse in alcoholic patients [Mann et al., 2004], is thought to act via suppressing a hyperglutamatergic state in the brain that has been addicted to alcohol [Dahchour and De Witte, 2000; Spanagel and Heilig, 2005].

In the present study, we investigated the potential associations between single nucleotide polymorphisms (SNPs) in glutamate receptor genes and the quantitative trait of event-related theta band energy during processing of target visual signals. *GRM8* was selected because it maps at chromosome 7q31.3–q32.1 within the peak region where we previously identified a significant linkage (peak LOD = 3.5, Fig. 1) using a genome-wide linkage scan of the same phenotype (theta for the target stimuli) [Jones et al., 2004, 2006]. The findings presented here are the first report to link *GRM8* with theta band oscillations underlying the P3 wave using genetic analyses. It is also the first report identifying a positive association between the *GRM8* and alcohol dependence.

METHODS

Subjects

The samples included in this study were recruited and tested as part of the Collaborative Study on the Genetics of Alcoholism (COGA), a large multi-site national study implemented with the purpose of identifying genetic loci linked with the predisposition to develop alcoholism and other related disorders. Data from six COGA sites were included in the analysis: SUNY Downstate Medical Center, Brooklyn, New York; University of Connecticut Health Science Center; Indiana University School of Medicine; University of Iowa School of Medicine; University of California School of Medicine, San Diego; and Washington University School of Medicine, St Louis. Ascertainment and assessment procedures have been outlined previously [Begleiter et al., 1995; Reich et al., 1998; Foroud et al., 2000; Nurnberger et al., 2004].

The families used in the analyses are taken from multiplex families recruited from alcoholic probands who were in alcohol or other substance dependence treatment facilities. All probands met DSM-IIIR criteria for alcohol dependence and Feighner definite criteria (COGA criteria). In addition to the probands, the study required two additional first-degree relatives who were alcohol dependent by the same COGA criteria on direct interview

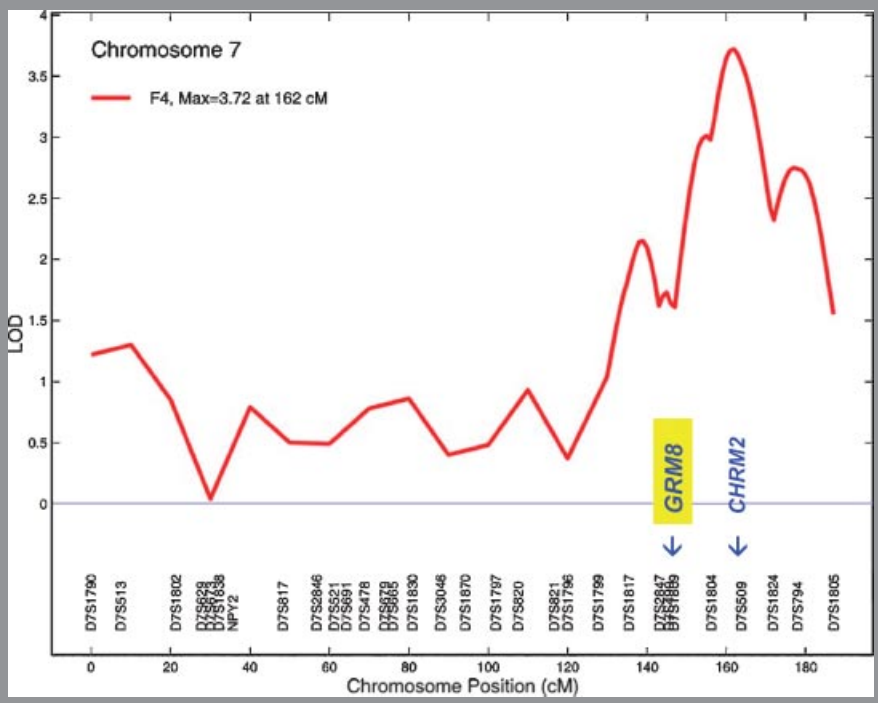

FIG. 1. Linkage curve for the target condition visual evoked theta band oscillation phenotypic data on chromosome *7* at a frontal lead. The approximate locations of the cholinergic muscarinic receptor 2 gene *CHRM2*, and the metabotropic glutamate receptor 8 gene *GRM8* are labeled.

(in person). Semi-Structured Assessment for the Genetics of Alcoholism, SSAGA, a polydiagnostic instrument designed by COGA with well established reliability [Bucholz et al., 1994] and validity [Hesselbrock et al., 1999], was administered in person to determine psychiatric diagnoses in all family members. All subjects completed a neuropsychological battery and family history questionnaire, and EEGs/ERPs were recorded.

A Caucasian-only subset of the sample comprising 209 families with 1049 individuals was used in the genetic association analysis. This subset included 472 individuals diagnosed as alcohol dependent by DSM-IV lifetime criteria, of whom 347 individuals met the diagnostic criteria for alcohol dependence by ICD-10. Blood was obtained for DNA extraction.

Prior to administration of the neurophysiology test battery, alcoholic subjects had been detoxified in a 30-day treatment program and none of the subjects was in the withdrawal phase. All subjects were excluded from the neurophysiology protocol if they manifested any of the following: uncorrected sensory deficits, hepatic encephalopathy/cirrhosis of the liver, history of significant head injury, seizures or neurosurgical procedures, other acute/chronic medical illness, were on medication known to influence brain functioning (e.g., any psychotropic medications), or tested positive for HIV. The subjects were also excluded on the basis of recent (i.e., 5 days) substance and alcohol use, based on self-report as well as breath-analyzer and urine screen. In addition, all subjects were screened for cognitive status, using the Mini Mental State Examination (MMSE, Folstein et al., 1975).

Event-Related Potential Data Acquisition and Signal Analysis

Subjects were seated in a comfortable chair located in a dimly-lit sound-attenuated RF-shielded room (IAC, Industrial Acoustics, Bronx, NY) in front of the computer monitor placed 1 m away. EEG activity was recorded on a Neuroscan system (Version 4.1) (Neurosoft, Inc., El Paso, TX) using a multi-channel electrode cap (Electro-cap International, Inc., Eaton, OH), which included 19 electrodes of the 10–20 International System and 42 additional electrode sites (Electrode Position Nomenclature, American Electroencephalographic Association, 1991) as shown previously [Kamarajan et al., 2005]. The electrodes were referenced to the tip of the nose and the ground electrode was at the forehead (frontal midline). A supraorbital vertical lead and a horizontal lead on the external canthus of the left eye recorded eye movements. Electrode impedance was maintained below 5 kΩ. The EEG signals were recorded continuously with a bandpass at 0.02–100 Hz and amplified 10,000 times using a set of amplifiers (Sensorium, Charlotte, VT). The continuous EEG was digitally low-pass filtered at 32 Hz and then segmented into epochs of 100 ms pre-stimulus to 750 ms post-stimulus. The mean EEG activity for 100 ms prior to stimulus onset served as the pre-stimulus baseline. All segments exceeding ±75 μV threshold were automatically excluded from further processing. After correcting eye-movement artifacts, the averaged segments for each individual were screened visually for further artifact rejection. The artifact detection was done on all channels including the electroocculogram (EOG) channels. Artifact-free data obtained

from nine channels located in the central scalp were included for genetic analyses.

Details of the visual oddball paradigm for eliciting event-related potentials employed in the present study have been previously described [Porjesz et al., 1998]. It consists of presentation of three types of visual stimuli ($N = 280$), 60 ms duration, subtending a visual angle of 2.5°, with an inter-stimulus interval of 1.625 sec. The rare target stimulus ($n = 35$) was the letter X, to which the subject was required to press a button as quickly as possible; the responding hand was alternated across subjects to counterbalance any laterality effects due to responding. Speed was emphasized, but not at the cost of accuracy. The frequently occurring non-target stimuli ($n = 210$) were squares and the novel stimuli ($n = 35$) consisted of colored geometric polygons that were different on each trial; the subject was not required to respond to the non-target and novel stimuli.

ERO Energy Band Computation

To obtain estimates of localized power of the generally non-stationary evoked potential time series, we used a recently developed time-frequency representation (TFR) method, the S-transform [Stockwell et al., 1996]. Details of the method for computation of the estimate of localized power were published previously [Jones et al., 2004]. The electrophysiological phenotypes used in the analysis were derived using single trial visual oddball event-related data from the target experimental conditions. The instantaneous amplitudes of the S-transform TFR were averaged across single trials, per individual, to obtain an estimate of event-related total amplitude response (stimulus onset phase locked plus non-phase locked oscillations). The total amplitude response enhances events that occur in a similar time range as related to the stimulus onset, and irrespective of their phase relations. Mean values were calculated from the TFR for use as phenotypes within time-frequency regions of interest (TFROI's) specified by frequency band ranges and time intervals [Lachaux et al., 2003]. This study focused on the evoked oscillation TFROI corresponding to the lower theta (4–5 Hz) frequency band and the 300–500 ms time window range, which is identical to that in our recent study [Jones et al., 2006]. This TFROI was established by examination of target condition grand-mean TFR amplitudes and selecting a region bounding an observed stimulus evoked increase in theta band energy which relates to a subcomponent of the P300 event-related potential. In a similar way to the P300 ERP amplitude the theta band ERO phenotype showed a significant age effect with amplitude decreasing at a rate of 0.05 μV/year (over the 16–75 years age range); therefore, age was included as a covariate in the genetic analyses.

Genotyping

Publicly available databases, dbSNP (http://www.ncbi.nlm.nih.gov/snp/) and HapMap (http://www.hapmap.org/) were used to identify SNPs within the *GRM8* gene. Genotyping was performed using Sequenom MassArray technology (http://www.sequenom.com/, San Diego, CA, USA), homogenous MassEXTEND (hME). PCR primers, termination mixes, and multiplexing capabilities were determined with Sequenom MassARRAY Assay Designer software v3.1.2.2. Standard PCR procedures were used to amplify PCR products. All unincorporated nucleotides were deactivated with shrimp alkaline phosphatase. A primer extension reaction was then carried out with the mass extension primer and the appropriate termination mix (hME). The primer extension products were then cleaned with resin and spotted onto a silicon SpectroChip. The chip was scanned with a mass spectrometry workstation (Bruker) and the resulting genotype spectra were analyzed with the Sequenom SpectroTYPER software v3.4. All genotypic data were checked for Mendelian inheritance of marker alleles with the USERM13 [Boehnke, 1991] option of the MENDEL linkage computer programs, which was then used to estimate marker allele frequencies. Chi square tests were used to ensure that all SNPs were in Hardy Weinberg equilibrium.

Genetic Analyses

Family-Based Association Tests (FBAT, Rabinowitz and Laird, 2000] were employed for genetic association analyses. Family-based association designs test for linkage as well as association, and avoid spurious associations caused by admixture of populations. They are appropriate in family samples such as this study. FBAT builds on the original transmission disequilibrium test (TDT) method [Spielman and Ewens, 1996] in which alleles transmitted to affected offspring are compared with the expected distribution of alleles among offspring. It compares the genotype distribution observed in the 'cases' to its expected distribution under the null hypothesis: in this case, given the previous positive finding of linkage (LOD = 3.5) in the same region [Jones et al., 2004, 2006] the null hypothesis tested was ''no association, in the presence of linkage''.

In this study, a quantitative trait, theta band (4–5 Hz) amplitude of EROs, and a dichotomous variable (i.e., alcohol dependent vs. not) were the phenotypes tested. Age and gender were treated as covariates. False discovery rate, FDR [Benjamini and Hochberg, 1995; Storey and Tibshirani, 2003], was calculated to correct for multiple comparisons with the method developed by Storey and Tibshirani [2003].

RESULTS

Table I summarizes the demography of the study subjects. Comorbid psychiatric disorders are distributed similarly between the unaffected and alcohol dependent subjects except cannabis dependence. The alcohol dependent group showed significantly higher co-occurrence with cannabis dependence than the unaffected group in this sample.

Among the 22 SNPs we genotyped, 8 SNPs had very low minor allele frequency (MAF), lower than 0.002. These SNPs were removed for analysis. Their respective locations and MAF are as follows: rs10225567 (Exon10, 0.00028), rs2302165 (Exon 9, 0.00029), rs769198 (Exon7, 0.00167), rs2234947 (Exon6, 0), rs17866780 (Exon2, 0.00084), rs769200 (Exon1, 0.00028), rs769202 (Exon1, 0), rs769194 (Exon1, 0). Pair-wise estimates of linkage disequilibrium [Abecasis and Cookson, 2000] between the remaining 14 *GRM8* SNPs in this study demonstrate that the SNPs showing the strongest association with the theta ERO as well as alcohol dependence phenotypes (Table II) are in strong linkage disequilibrium with one another (detail data not shown). For

TABLE I. Demography of Study Subjects

	Unaffected		Alcohol Dependence (by DSM-IV)		Total	
n	577		472		1,049	
Age	39.30 ± 0.55		39.6 ± 0.61		39.45 ± 0.41	
Gender (M/F)	274/303	0.90 (ratio)	237/235	1.01 (ratio)	511/538	0.95 (ratio)
Psychiatric Comorbidity (by DSM-IIIR)						
ASPD	49	8.49%	53	11.23%	102	
Major depression	92	15.94%	84	17.80%	176	
Bipolar I	3	0.52%	9	1.91%	12	
Bipolar II	6	1.04%	12	2.54%	18	
OCD	7	1.21%	14	2.97%	21	
Social phobia	15	2.60%	23	4.87%	38	
Panic disorder	13	2.25%	22	4.66%	35	
Cannabis dependence*	49	8.49%	191	40.47%	240	*$P = 1.56E-33$
Cocaine dependence	99	17.16%	75	15.89%	174	

ASPD: antisocial personality disorder; OCD: obsessive compulsive disorder.

example, the Lewontin's standardized disequilibrium coefficients |D'| estimated for pairs from any two of the three SNPs that are significantly associated with both theta band ERO and alcohol dependence (Table II), rs1361995, rs10487457, rs10487459, are all equal to 1.

Table II demonstrates *P*-values of the results of the FBAT with the theta band EROs as well as diagnoses of alcohol dependence. Multiple significant genetic associations ($P < 0.05$) were identified with theta band of ERO and the SNPs in the intron 6 and intron 7 of GRM8. Of note, 3 SNPs, rs1361995, rs10487457, rs10487459, in the intron 6 region were significantly associated with both the quantitative trait phenotype, theta band of EROs, and the ICD-10 diagnostic criteria of alcohol dependence.

False discovery rate (FDR) was calculated to correct for multiple comparisons. We found 74 significant tests, and 1 (1.115) false positives is expected under the traditional p-value cut-off criterion (0.05). If the *P*-value cutoff point is set to 0.01, it yields 60 significant tests, where we would expect 0.220 positive results by chance.

Figure 2 demonstrates that individuals with CC genotype for the rs1361995 SNP show significantly lower theta ERO power compared with those with CT or TT genotypes. Similar difference in the electrophysiological quantitative phenotypic variables in relation to different genotypes was also observed in the rs10487457 and rs10487459 SNPs. The same genotypes in these three SNPs that are associated with the reduction in the theta ERO power are also found to be associated with increased vulnerability to develop alcohol dependence (Table III).

DISCUSSION

GRM8 spans over 800 kb, and is composed of 10 exons [Shigemoto et al., 1997]. The present study demonstrated a significant association of target evoked theta ERO, and of alcohol dependence with SNPs from the intron 6 and intron 7 of the metabotropic glutamate receptor 8 gene (*GRM8*).

Intracellular actions of metabotropic glutamate receptors (mGluRs) are mediated by G-protein. mGluRs are divided into three groups, mGluI–mGluIII, based on signal transduction pathways and sequence homology [Schoepp, 2001]. Group I mGluRs (i.e., mGlu1/5 receptors) are predominantly postsynaptic, whereas group II (i.e., mGlu2/3 receptors) agonists have been shown to reduce glutamate release via a presynaptic mechanism [Schoepp, 2001]. GRM8 is a member of group III. Similar to group II agonists, group III agonists can negatively modulate glutamate transmission [Pothecary et al., 2002]. *GRM8* mRNA has been detected in the cerebral cortex, hippocampus, lateral reticular nucleus of the thalamus, and retina [Duvoisin et al., 1995; Saugstad et al., 1997]. Electrophysiological and morphological studies suggested that the mGluR8 receptor is localized at the presynaptic grid of glutamate synapses, and it functions as a presynaptic autoreceptor controlling glutamate release from the lateral perforant path terminals in the dentate gyrus [Shigemoto et al., 1997]. mGluR8-expressing nerve terminals have also been found to target subsets of GABAergic neurons in the hippocampus [Ferraguti et al., 2005]. Substances acting as agonists of group III mGlu receptors were shown to produce an anxiolytic-like effect after intrahippocampal administration to rats [Palucha et al., 2004]. Administration of the mGlu8 receptor agonist has also been shown to suppress alcohol self-administration and cue-induced reinstatement of alcohol seeking in preclinical study [Backstrom and Hyytia, 2005]. Taken together, these findings suggest that disturbance of a variety of neurotransmitter systems mediated by mGluR8 may be involved in the pathophysiology of alcohol dependence as well as developing symptoms associated with the course of alcoholism.

Neural oscillatory responses have been attributed to various cognitive processes in the literature. Delta responses are considered to mediate signal detection and decision-making [Basar et al., 1999], while theta rhythms have been attributed with attention, recognition memory, and episodic retrieval [Basar et al., 2001; Klimesch et al., 2001]. The theta component of the P3 response

TABLE II. Data Represent the *P*-Values of the Results of the FBAT With the Theta Band ERO and Diagnoses (DSM-IV and ICD-10) of Alcohol Dependence

				Frontal			Central			Parietal				
***GRM8* SNP**	**b.p. position**	**Location**	**MAF**	**F3**	**Fz**	**F4**	**C3**	**Cz**	**C4**	**P3**	**Pz**	**P4**	**DSM-IV**	**ICD-10**
RS2402816	125978412	intron7	0.4253	0.093351	0.252568	0.093892	0.118173	0.145693	**0.024175**	0.379564	0.149178	0.15882	0.09698	0.12249
RS2299459	125982604	intron7	0.19989	**0.000242**	**0.000858**	**0.000135**	**0.000225**	**0.000272**	**0.00005**	**0.000658**	**0.000318**	**0.00115**	0.72322	0.93887
RS1158720	125995542	intron7	0.21721	**0.000553**	**0.0016**	**0.000389**	**0.000356**	**0.000679**	**0.000191**	**0.001271**	**0.002041**	**0.00659**	0.71321	0.94042
RS7797602	126075063	intron6	0.2004	**0.000151**	**0.000318**	**0.000087**	**0.000275**	**0.000363**	**0.000103**	**0.00096**	**0.001005**	**0.00302**	0.60524	0.78174
RS2402820	126084934	intron6	0.23282	**0.025128**	**0.049439**	**0.012641**	0.074566	0.140728	**0.041059**	0.058175	0.077618	0.08446	0.68316	0.46549
RS1074728	126100080	intron6	0.47656	0.096584	0.216603	0.114742	0.091394	0.094091	**0.021838**	0.134333	0.074237	0.15928	0.13374	0.20081
RS4731323	126111266	intron6	0.21172	**0.005013**	**0.012996**	**0.006684**	**0.005948**	**0.009776**	**0.002702**	**0.011203**	**0.006601**	**0.01558**	0.14905	0.53272
RS1361991	126125878	intron6	0.14926	0.214853	0.360647	0.380487	0.319222	0.210134	0.127477	0.579042	0.29104	0.27828	**0.0174**	**0.03222**
RS2299495	126144203	intron6	0.15501	0.085667	0.112744	0.1531	**0.026541**	**0.040644**	0.145957	**0.011396**	**0.028879**	**0.03238**	0.48952	0.77779
RS2299498	126145163	intron6	0.21187	0.486164	0.767491	0.647005	0.797503	0.808164	0.418746	0.785061	0.556374	0.37939	**0.02424**	**0.04608**
RS10256873	126155528	intron6	0.33062	0.935112	0.735431	0.971051	0.51099	0.473886	0.981567	0.269095	0.385705	0.58687	**0.03838**	0.20383
RS1361995	126159293	intron6	0.36852	**0.00035**	**0.000996**	**0.000603**	**0.000133**	**0.000141**	**0.000057**	**0.000532**	**0.000298**	**0.00077**	0.05841	**0.03678**
RS10487457	126164126	intron6	0.36784	**0.001119**	**0.002741**	**0.001726**	**0.000218**	**0.00042**	**0.000121**	**0.001015**	**0.000857**	**0.00211**	0.07036	**0.03734**
RS10487459	126165491	intron6	0.36627	**0.000718**	**0.002283**	**0.001359**	**0.000228**	**0.000297**	**0.000098**	**0.001097**	**0.000693**	**0.00194**	0.07276	**0.04096**

P-values in bold font and shading denote significance ($P < 0.05$). *MAF: Minor allele frequency. Note that three SNPs in bold font are significantly associated with both theta band ERO and alcohol dependence.

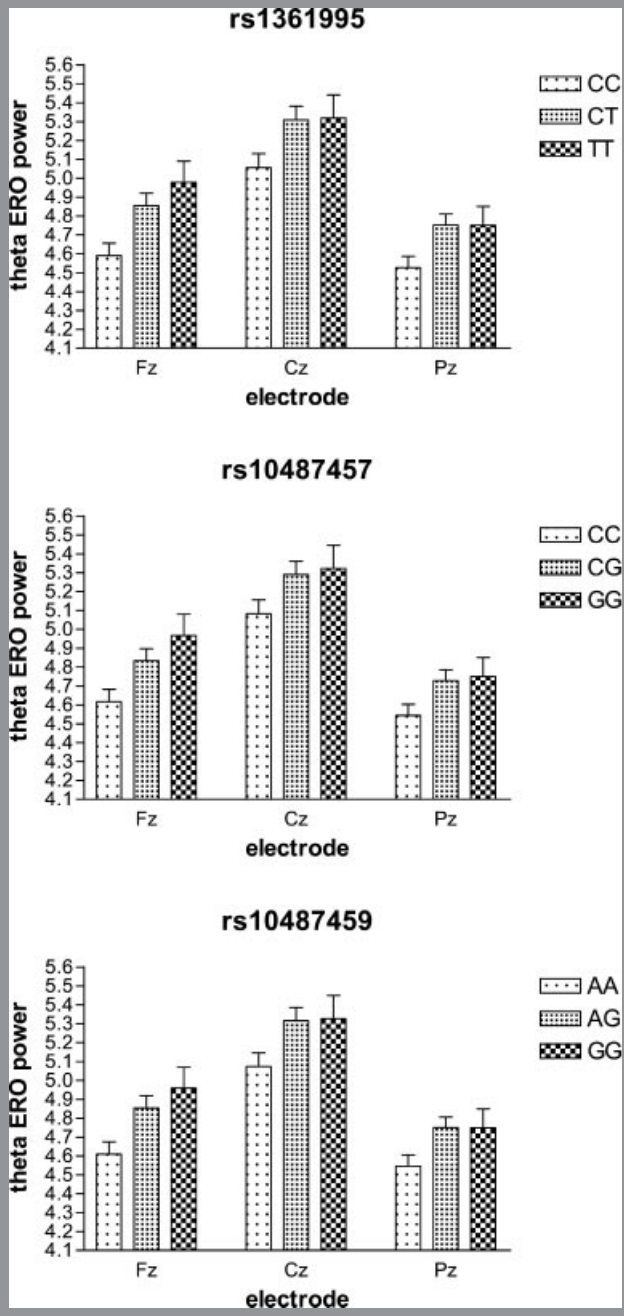

FIG. 2. Mean Theta ERO power at the representative electrodes in the midline frontal (Fz), central (Cz), and parietal (Pz) leads grouped by each of the genotypes in the three SNPs, rs1361995, rs10487457, rs10487459. The bars represent the mean value; the vertical lines issuing from the bars represent the standard error of mean (SEM). Individual *P*-values ($P < 0.01$ at Fz; $P < 0.001$ at Cz, Pz) are shown in Table II.

may be of particular relevance in relation to the interaction of cholinergic and glutamatergic systems in light of recent neurophysiological data acquired from experiments on rat brains. For example, cholinergic agonists induced oscillations in the delta, and theta frequency range in the rat hippocampal [Fellous and Sejnow-

TABLE III. Phenotypic Variables of Diagnoses (Alcohol Dependence by ICD-10) and Genotype Distributions With the Three SNPs that are Significantly Associated With Both Theta Band ERO and Alcohol Dependence

	rs1361995							
	CC		CT		TT		Total	
ICD-10								
Unaffected	231	36.33%	291	45.75%	114	17.92%	636	100%
Alcohol dependence	143	44.00%	143	44.00%	39	12.00%	325	100%
Total	374		434		153		961	91.6% genotyped
		38.91%		45.16%		15.93%		100%
	rs10487457							
	CC		CG		GG		Total	
ICD-10								
Unaffected	244	35.83%	322	47.28%	115	16.89%	681	100%
Alcohol dependence	147	43.62%	150	44.51%	40	11.87%	337	100%
Total	391		472		155		1018	97.0% genotyped
		38.41%		46.66%		15.23%		100%
	rs10487459							
	AA		AG		GG		Total	
ICD-10								
Unaffected	243	36.16%	316	47.02%	113	16.82%	672	100%
Alcohol dependence	146	42.94%	154	45.29%	40	11.77%	340	100%
Total	389		470		153		1012	96.5% genotyped
		38.44%		46.44%		15.12%		100%

Individual *P*-values for each SNPs ($P < 0.05$) are shown in Table II.

ski, 2000] and neocortical [Lukatch and MacIver, 1997] slices. Recently, a human study using a 3-T proton magnetic resonance spectroscopy (1H-MRS) and EEG theta activity during an auditory target detection paradigm demonstrated a robust relationship between hippocampal glutamate concentration and frontal theta activity during stimulus processing [Gallinat et al., 2006]. The results suggest a functional coupling between the frontal cortex and hippocampal region during stimulus processing and support the idea of the hippocampus as a neural rhythm generator driven by glutamatergic neurotransmission [Gallinat et al., 2006]. It is possible that modulation of glutamate release has a role in inhibiting cortical and sub-cortical glutamatergic sub-systems which are irrelevant to the processing of the target condition and thereby facilitating the information processing in the relevant sub-systems.

It is an interesting finding that three SNPs in the intron 6 region of *GRM8* were significantly associated with not only the theta band of EROs but also the ICD-10 diagnosis of alcohol dependence. There is a trend that these three SNPs may also be associated with the DSM-IV diagnostic criteria of alcohol dependence ($P = 0.058$, 0.070, 0.073 with rs1361995, rs10487457, rs10487459, respectively) although it did not reach a statistically significant level ($P < 0.05$) in the dataset we examined. The ICD-10 criteria for alcohol dependence suggest more biological and somewhat more restrictive set compared to those for DSM-IV, since the former are focused on a cluster of the consequences after repeated use of alcohol. For example, the ICD-10 criteria require "a cluster of behavioral, cognitive, and physiological phenomena that develop after repeated alcohol use and that typically include a strong desire to take the substance, difficulties in controlling its use, persisting in its use despite harmful consequences, a higher priority given to drug use than to other activities and obligations, increased tolerance, and sometimes a physical withdrawal state", while the DSM-IV requires only three or more of similar criteria to make such diagnosis.

We did not find any significant difference in distribution of comorbid psychiatric diagnoses between the unaffected and alcohol dependent groups in this dataset except cannabis dependence (Table I). Comorbid psychotic disorders (e.g., schizophrenia or schizoaffective disorder) do not appear in the dataset because they are one of the exclusion criteria for subject recruitment. To test whether comorbid psychiatric disorders contribute to the

association, we performed additional FBATs with these SNPs in *GRM8* using comorbid psychiatric diagnoses as phenotypes. The results were all not significant ($P > 0.05$) except some weak association ($P < 0.05$) of major depressive disorder, cannabis dependence, and social phobia with only 1 SNP, rs2299495. The results suggest that the association we found among the three SNPs in the intron 6 region of *GRM8*, rs1361995, rs10487457, rs10487459, is rather specific to alcohol dependence.

In conclusion, our findings underscore the great potential to utilize the brain oscillations evoked under cognitive conditions as phenotypes in combination with neurochemical and neuroanatomical information in deciphering the interaction of the subsystems involved in the pathophysiology of complex neuropsychiatric diseases. We expect that the identification of genes that regulate cognitive processes will be of enormous benefit to the field of psychiatric genetics. These data support the notion that carefully chosen brain oscillations may be adopted as endophenotypes. Nevertheless, further studies using an independent data sample are warranted, and as with all genetic studies of complex phenotypes, caution must be advised until the findings are replicated.

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