HHS Public Access

Author manuscript

Int J Psychophysiol. Author manuscript; available in PMC 2018 May 01.

Published in final edited form as:

Int J Psychophysiol. 2017 May ; 115: 74–85. doi:10.1016/j.ijpsycho.2016.12.008.

A Genome Wide Association Study of Fast Beta EEG in Families of European Ancestry

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Abstract

BACKGROUND—Differences in fast beta (20–28 Hz) electroencephalogram (EEG) oscillatory activity distinguish some individuals with psychiatric and substance use disorders, suggesting that it may be a useful endophenotype for studying the genetics of disorders characterized by neural hyper-excitability. Despite the high heritability estimates provided by twin and family studies, there have been relatively few genetic studies of beta EEG, and to date only one genetic association finding has replicated (i.e., *GABRA2*).

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METHOD—In a sample of 1,564 individuals from 117 families of European Ancestry (EA) drawn from the Collaborative Study on the Genetics of Alcoholism (COGA), we performed a Genome-Wide Association Study (GWAS) on resting-state fronto-central fast beta EEG power, adjusting regression models for family relatedness, age, sex, and ancestry. To further characterize genetic findings, we examined the functional and behavioral significance of GWAS findings.

RESULTS—Three intronic variants located within *DSE* (dermatan sulfate epimerase) on 6q22 were associated with fast beta EEG at a genome wide significant level ($p<5\times10^{-8}$). The most significant SNP was rs2252790 ($p<2.6\times10^{-8}$; MAF= 0.36; β= 0.135). rs2252790 is an eQTL for *ROS1* expressed most robustly in the temporal cortex ($p= 1.2\times10^{-6}$) and for *DSE*/*TSPYL4* expressed most robustly in the hippocampus ($p=7.3\times10^{-4}$; β= 0.29). Previous studies have indicated that *DSE* is involved in a network of genes integral to membrane organization; gene-based tests indicated that several variants within this network (i.e., *DSE, ZEB2, RND3, MCTP1,* and *CTBP2*) were also associated with beta EEG (empirical $p<0.05$), and of these genes, *ZEB2* and *CTBP2* were associated with DSM-V Alcohol Use Disorder (AUD; empirical $p<0.05$).

DISCUSSION—In this sample of EA families enriched for AUDs, fast beta EEG is associated with variants within *DSE* on 6q22; the most significant SNP influences the mRNA expression of *DSE* and *ROS1* in hippocampus and temporal cortex, brain regions important for beta EEG activity. Gene-based tests suggest evidence of association with related genes, *ZEB2, RND3, MCTP1, CTBP2,* and beta EEG. Converging data from GWAS, gene expression, and gene-networks presented in this study provide support for the role of genetic variants within *DSE* and related genes in neural hyperexcitability, and has highlighted two potential candidate genes for AUD and/or related neurological conditions: *ZEB2* and *CTBP2*. However, results must be replicated in large, independent samples.

Keywords

Genome-wide Association Study (GWAS); Endophenotype; Electrophysiology; Resting EEG

Introduction

The resting-state human electroencephalography (EEG) represents the ongoing oscillations of spontaneous and continuous brain electrical activity, typically recorded while the participant is in a relaxed state (Niedermeyer, 1999). EEG is traditionally decomposed into the following frequency bands: delta (0–3 Hz), theta (4–7 Hz), alpha (8–12 Hz), beta (13–28 Hz), and gamma (>29 Hz), with each band reflecting different topography and brain activity (Niedermeyer, 1999). For example, alpha rhythm reflects a relaxed state and has a posterior occipital topography, while beta rhythm reflects an active brain state and is present all over the scalp but predominantly at fronto-central loci (Rangaswamy and Porjesz, 2014). Dynamic coordination of lower frequencies (theta or alpha rhythms from subcortical region) and higher frequencies (beta or gamma rhythms from cortical sites) through a mechanism of phase-amplitude coupling modulates thalamo-cortical and cortico-cortical activity (Canolty and Knight, 2010; Malekmohammadi et al., 2015). Further, coherence at the beta frequency may serve to establish transient physiological connections among neurons in the hippocampus and related brain structures (Leung, 1992a; Vecchio et al., 2016a). While local excitatory-inhibitory interactions underlying sensory, motor and perceptual functions involve

local gamma-band oscillations, more integrative cognitive functions mediated by long-range cortical interactions often involve the beta range (Donner and Siegel, 2011). Resting-state brain activity in the beta range (herein referred to as beta EEG) is associated with several behavioral traits, including alcohol use disorders (Bauer, 2001; Begleiter and Porjesz, 1999; Choi et al., 2013; Gilmore et al., 2010a; Lee et al., 2014; Rangaswamy et al., 2002). Given these associations, and the high degree of genetic influence observed (Malone et al., 2014; van Beijsterveldt et al., 1996), beta EEG has been proposed as a useful endo-phenotype (Gottesman and Gould, 2003) for identifying genetic risk factors for disorders characterized by disinhibitory traits (Edenberg et al., 2004; Porjesz et al., 2002). Despite the promise of the endo-phenotype concept however, the genetic complexity of resting-state EEG (Malone et al., 2014), coupled with the scant number of replicable and/or clinically useful genetic variants uncovered by this approach (Iacono et al., 2016), has necessitated large scale genetic association studies of beta EEG, utilizing best-practices in genetic epidemiology.

Previous studies report differences in the magnitude of beta EEG among individuals with alcohol use disorders (AUD) and related problems (Bauer, 2001; Begleiter and Porjesz, 1999; Gilmore et al., 2010b; Propping et al., 1981; Rangaswamy et al., 2002; Winterer et al., 1998), gambling addiction (Choi et al., 2013), and Attention Deficit Hyperactive Disorder (ADHD; (Lee et al., 2014)). Researchers have consistently reported that individuals affected with DSM (III-R and IV) Alcohol Dependence (AD) show higher beta EEG (Bauer, 2001; Propping et al., 1981; Rangaswamy et al., 2002; Winterer et al., 1998). Further decomposition of the beta frequency band demonstrates that increased fast beta power (>19 Hz) is of key importance in the association of beta EEG and AUD. For example, multiple studies have reported that fast beta EEG is superior to severity of illness, depression level, and childhood conduct problems in predicting relapse in abstinent individuals with AUD (Bauer, 2001; Saletu-Zyhlarz et al.). Since elevated beta EEG is present in the offspring of alcoholics prior to the onset of risky drinking (Begleiter and Porjesz, 1999; Deckel et al., 1996; Rangaswamy et al., 2002), it has been suggested that excess beta power precedes the development of AUDs and is likely related to an underlying genetic predisposition for developing AUD, rather than a consequence of heavy alcohol use. Begleiter and colleagues have suggested that this may be an electrophysiological index of an imbalance in the excitation–inhibition homeostasis in the cortex, which underlies a predisposition to develop AUD and related disorders (Begleiter and Porjesz, 1999; Porjesz et al., 2005). Further supporting this hypothesis is the association of beta EEG and other disorders characterized by behavioral disinhibition such as behavior problems and hyperactivity in children (Deckel et al., 1996), externalizing psychopathology (e.g., substance abuse symptoms) in a community sample of adolescents (Gilmore et al., 2010b), ADHD (Choi et al., 2013), and internet addiction with comorbid depression (Lee et al., 2014). Although the precise role of increased beta EEG in these behaviors and disorders remains unclear, this literature suggests that there is variation in fast beta EEG among individuals with AUD and related psychopathology.

Resting state EEG is highly heritable (Malone et al., 2014; van Beijsterveldt et al., 1996), with studies reporting heritability (h^2) estimates of monopolar resting state EEG power ranging from 0.49 to 0.85 (Malone et al., 2014; Smit et al., 2005; van Beijsterveldt et al., 1996). Bipolar EEG derivations offer an improvement over monopolar EEG derivations in

the spatial resolution of the electrical sources, and reduce volume conduction effects (Ingber and Nunez, 1995; Nunez et al., 1997). In addition, the stability of EEG signals is excellent and under standardized conditions, there are high test-retest correlations. Studies that have examined the heritability of bipolar eyes-closed resting EEG power have shown comparable estimates to monopolar derivations (Tang et al., 2007b) and indicate that bipolar derivations are in greater accord with genetic findings in brain anatomy (Tang et al., 2007a). Despite the high heritability estimates provided by twin and family studies, there have been relatively few large (i.e., adequately powered) genetic studies of beta EEG (Iacono et al., 2016), and to date only one finding has replicated. An early analysis found linkage between beta EEG and a region of chromosome 4 (Porjesz et al., 2002) harboring variants in the gene that encodes the GABA α2 receptor subunit (*GABRA2*), which were subsequently associated with both beta EEG and AD (Edenberg et al., 2004). More recently, a study of 586 individuals of European ancestry (EA) with DSM-IV AD, and 603 ancestrally matched individuals without AD, replicated the association between beta activity and several *GABRA2* variants (Lydall et al., 2011). To date, only two genome-wide association studies (GWAS) of beta EEG have been conducted (Hodgkinson et al., 2010; Malone et al., 2014). In a study of 322 Native-American individuals, there were no genome-wide significant associations reported for beta EEG (Hodgkinson et al., 2010). We note that there were genome-wide significant findings for other EEG parameters; an association was observed among theta power (and AD) and several variants in *SGIP1* (Hodgkinson et al., 2010). A recent GWAS of several EEG measures (including monopolar beta EEG, assessed at the central electrode) in 4,026 adolescent twins and their parents (Malone et al., 2014) did not report any genome-wide significant variants, but replicated the previous associations observed between beta EEG and *GABRA2* and expanded our understanding of the genetic epidemiology of other EEG parameters (i.e., resting-state theta EEG).

Given that beta EEG is highly heritable and has been found to be related to several externalizing traits including AUD (Bauer, 2001; Begleiter and Porjesz, 1999; Choi et al., 2013; Gilmore et al., 2010b; Lee et al., 2014; Rangaswamy et al., 2002), genetic analysis of beta EEG may aid in our understanding basic brain functioning, and potentially differences and similarities among individuals with a range of behavioral and psychiatric disorders. As the elevation of beta power reported in individuals with AUD has a largely anterior topography, particularly in the higher frequency fast beta band (20–28 Hz; (Rangaswamy et al., 2002)), the primary aim of this study was to conduct a GWAS of fast beta (20–28 Hz) EEG power (bipolar derivation at fronto-central loci) in families from the Collaborative Study on the Genetics of Alcoholism (COGA); many of these families were densely affected with AD. In an effort to move beyond genotype-phenotype association and further characterize genetic association findings, the secondary aims of this study were to examine the functional and behavioral significance of GWAS findings. To this end, we explore the functional significance of GWAS variants using publically available gene expression data. In addition, we explore biological networks using publically available prediction programs.

SAMPLE & METHODS

Sample

COGA recruited DSM-III-R and DSM-IV AD probands from inpatient and outpatient treatment facilities through six participating sites: State University of New York Downstate Medical Center, University of Connecticut Health Science Center, Indiana University School of Medicine, University of Iowa College of Medicine, University of California School of Medicine, and Washington University School of Medicine. Recruitment and assessment procedures, including a clinical interview, neurophysiological assessments and DNA collection have been described previously (Begleiter et al., 1995; Foroud et al., 2000). Probands and family members were administered the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA), a poly-diagnostic interview (Bucholz et al. 1994; Hesselbrock et al. 1999). Individuals below the age of 18 were administered an adolescent version of the SSAGA. The laboratory and data-collection procedures were identical at each of the sites (Begleiter et al., 1998). Institutional review boards at all sites approved the study.

All COGA DNA samples were genotyped for a 96 SNP (single nucleotide polymorphism) array (Fluidigm SNPtrace, Rutgers University Cell and DNA Repository) that included 64 ancestry informative markers. The principal components derived from these SNPs were used to assign ancestry, and were the basis for the selection of the European Ancestry (EA) families. Prioritization of families was based on the most informative families, defined as those with the largest number of alcohol dependent family members with DNA and electrophysiological measurements. The analytic sample consisted of all family members with both resting state EEG and GWAS data available: 1,564 individuals (824 females and 740 males; average age: 31.6) from 117 multi-generational families affected with AD. Family sizes ranged from 4 to 39 individuals with an average of 13.4 individuals (with EEG data) per family.

EEG Recording & Processing

Prior to neurophysiological assessments, participants were required to have abstained from alcohol for a minimum of 3 weeks. Individual were excluded from neurophysiological assessment if they had any of the following: (1) recent substance or alcohol use (i.e., positive breath-analyzer test); (2) hepatic encephalopathy/cirrhosis of the liver; (3) significant history of head injury, seizures or neurosurgery; (4) uncorrected sensory deficits; (5) taking medication known to influence brain functioning; and (6) other acute/chronic medical illnesses that affect brain function.

Participants were seated comfortably in a dimly lit sound-attenuated temperature-regulated booth (Industrial Acoustics, Bronx, NY). They were instructed to keep their eyes closed and remain relaxed, but to not fall asleep. EEG data were collected in the awake, eyes-closed condition for 4.25 minutes. Each participant wore a fitted electrode cap (Electro-Cap International, Eaton, OH) using the 19-channel montage as specified according to the 10–20 international system (Supplementary Figure 1). The nose was used as a reference, and a forehead electrode served as the ground electrode. Both vertical and horizontal eye movements were monitored with electrodes that were placed supraorbitally and at the outer

canthus of the left eye to perform ocular artifact correction. Electrode impedances were maintained below 5 kΩ. Electrical activity was amplified 100,000 times by Sensorium (Charlotte, VT) EPA-2 electrophysiology amplifiers with either a bandpass between 0.02 and 50 Hz and digitized on a Concurrent (Atlanta, GA) 5550 computer at a sampling rate of 256 Hz or a band pass between 0.02 Hz and 100.0 Hz on a Neuroscan system (Version 4.1 to 4.5) (Neurosoft, Inc., El Paso, TX) at sampling rates of 500 Hz or 512 Hz. All six collection sites used identical experimental procedures and EEG acquisition hardware and software programs.

A continuous interval comprising 256 seconds of eyes-closed resting EEG data was analyzed. The raw data were subjected to wavelet filtering and reconstruction to eliminate very high and low frequencies (Bruce & Gao, 1994; Strang & Nguyen, 1996). The s12 wavelet was used to perform a six-level analysis, and the output signal was reconstructed with levels d6–d3, roughly equivalent to applying a bandpass filter with a range of 2–64 Hz to the data. Subsequently, eye movements were removed by using the method developed by Gasser *et al.* (Gasser and Laemmli, 1987; Gasser et al., 1986). The filtered artifact-free data were transformed into bipolar derivations. Bipolar derivations were used in preference to monopolar derivations to improve the spatial resolution of the electrical sources (Ingber and Nunez, 1995; Nunez et al., 1997). Bipolar derivations were analyzed in 254 overlapping 2-second epochs by use of a Fourier transform. After windowing effects were minimized by application of a Hamming function (Hamming, 1983), the resulting spectral densities, sampled at 0.5 Hz intervals, were aggregated into bands, divided by the bandwidth, and then averaged across epochs. As the elevation of beta power reported in individuals with alcohol use problems has a largely anterior topography, particularly in the higher frequency fast beta band (20–28 Hz; (Rangaswamy et al., 2002)), the current study examines fast beta EEG (20–28 Hz) at fronto-central pairs: Fz-Cz, F3-C3, and F4-C4. Given the high degree of correlation observed among these phenotypes, GWAS results are presented for F3-C3, for which the most robust effects were observed.

Genotyping, Imputation and Quality Review

Genotyping of 1,564 individuals from 117 EA families was performed at the Center for Inherited Disease Research (CIDR) using the Illumina 2.5M array (Illlumina, San Diego, CA, USA). COGA's quality control (QC) approach has been previously reported (Wetherill et al., 2015). Briefly, individuals with a genotype rate <98% were excluded from analysis, and SNPs with a genotyping rate <98% were excluded from analysis. The 795 genotyped founders were used to remove SNPs which violated Hardy-Weinberg equilibrium (HWE; $p<10^{-6}$). SNPs with minor allele frequency (MAF) less than 3% in the founders were also removed from further analysis. The reported pedigree structure was assessed using a pruned set of 1,519,440 SNPs. Pairwise identity by descent estimates were computed in PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) to detect pairs of individuals whose allele sharing was not consistent with the reported family relationship. Family structures were altered as needed, and then SNP genotypes were tested for Mendelian inconsistencies (Pedcheck; O'Connell & Weeks, 1998) with the revised family structure. The cleaned genotype data were imputed to 1000 genomes (EUR and AFR, Phase 3, b37, October 2014) with build hg19 using SHAPEIT (https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/

shapeit.html) and IMPUTE2 (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html). To avoid ambiguities in strand designation, SNPs with A/T or C/G alleles were removed. After imputation, genotype probabilities ≥ 0.90 were changed to genotypes. Mendelian errors in the imputed SNPs were reviewed and resolved as described in Wetherill et al., 2015 (Wetherill et al., 2015). All SNPs with imputation genotyping rate < 98% and MAF < 0.03 were excluded from association analyses.

Association Analysis

Primary analyses were conducted in GWAF (Genome-Wide Association analyses with Family) on 12,972,748 SNPs, of which 1,519,440 were genotyped directly, using a generalized estimating equation (GEE) framework to control for the relatedness in the family sample (Chen and Yang, 2010). Sex and log-transformed age (at the time of EEG recording) were included as covariates in the model, as each of these variables were associated with fast beta EEG ($p<0.0001$). The first three principal components (PC1-PC3) computed from SNPRelate (Zheng et al, 2012) were also included as covariates to reduce the risk of false-positive associations owing to population stratification. Established thresholds for genome-wide significance ($p<5\times10^{-8}$) were utilized. In an effort to assess the influence of alcohol use problems on the genetic associations observed for beta EEG, given the association of AUD and beta EEG in this and previous studies, we conducted a secondary analysis in which we repeated the initial GWAS as described above, with the addition of DSM-V AUD severity as a covariate.

Functional Analyses

We utilized publicly available data from the UK Brain Expression Consortium (BRAINEAC; http://www.braineac.org/) to examine whether the most significant GWAS variant for fast beta EEG was an expression quantitative trait locus (eQTL) for any known gene. BRAINEAC draws on data from 134 neuropathologically normal individuals of EA and assesses 10 different regions of the brain, including: cerebellar cortex, frontal cortex, hippocampus, medulla (inferior olivary nucleus), occipital cortex (primary visual cortex), putamen, substantia nigra, thalamus, temporal cortex, and intralobular white matter (Trabzuni et al., 2011). All p-values presented are Bonferonni corrected for multiple-testing, based on the ten brain regions examined. Due to the large number of brain regions examined, only the SNPs genome-wide associated with fast beta EEG were examined in BRAINEAC to minimize multiple-testing. All associations that withstood multiple testing were examined in the Genotype-Tissue Expression Project (GTex) database (www.gtexportal.org) to confirm eQTL findings (For brain eQTLs, sample sizes ranged from 70–127). GeneMANIA Cytoscape 3.0.0 plugin (Mostafavi et al., 2008), a multiple association network integration algorithm for predicting gene function, was employed to identify genes in related gene networks; physical, co-expression, co-localization and pathway gene-gene interactions were evaluated. Once a gene network was identified (via GeneMANIA as described above), DAVID (Database for Annotation, Visualization, and Integrated Discovery; (Dennis et al., 2003) bioinformatics resource was used to assess gene functions common to this network. Functional Categories and Gene Ontologies were evaluated based on enrichment scores (Fisher Exact Probability Value or “EASE Score”) and

tests of statistical significance, including p-values adjusted for multiple testing (Bonferonni correction).

Post-Hoc Analyses

Following the identification of gene networks of relevance to GWAS findings (as described above via GeneMANIA), gene-based tests of association with fast beta EEG were conducted in PLINK (Purcell et al., 2007), using set-based analyses ("--set-test"). Gene-based tests included all available SNPs in a given gene, corrected for the number of independent signals (i.e., linkage disequilibrium blocks) within that gene set. In addition, association models were adjusted for the relatedness in the family sample, sex, log-transformed age, and ancestry. Tests of association were accepted as significant if the 100,000 permutations of the set-based regression analysis (Bonferroni corrected for the number of independent signals within the set) produced an empirical *p*-value <0.05. Subsequently, these procedures were repeated for tests of gene-based association with DSM-V AUD severity only among genes that *were* associated with beta EEG. In addition, *GABRA2* variants previously shown to be associated with aspects of beta EEG (Edenberg et al., 2004; Lydall et al., 2011; Malone et al., 2014) were tested for association with fast beta EEG (20–28 Hz) at fronto-central pairs: Fz-Cz, F3-C3, and F4-C4.

Results

Association Analysis

Three highly correlated (i.e., in high linkage disequilibrium; r^2 =1.0; D-prime=1.0 based on hg19 1000 Genomes from the CEU sample) intronic SNPs (rs10456907, rs13214667, rs2252790) located within *DSE* (dermatan sulfate epimerase) on 6q22 were associated with fast beta EEG at a genome wide significant level ($p<5\times10^{-8}$). All variants associated with beta EEG at $p<5\times10^{-7}$ are detailed in Table 1 and depicted in Figures 1 and 2. Conditional analyses, and the high degree of linkage disequilibrium observed among the most significant SNPs (Figure 2), suggest that a single genome-wide signal is implicated. The most significant SNP was rs2252790 ($p<2.6\times10^{-8}$; MAF: 0.36; β: 0.135; Table 1). GWAS results adjusted for DSM-V AUD severity (i.e., GWAS model included DSM-V AUD severity as a covariate) yielded similar results as the primary analyses: intronic *DSE* variant rs2252790 remained the most significantly associated SNP. However, p-values were slightly less robust (Supplemental Table 1) with only one of three *DSE* variants remaining genome-wide significant (rs2252790). Three additional sub-threshold ($5\times10^{-7}>p>5\times10^{-8}$; Table 1) signals were also detected, including a signal on the long arm of Chromosome 3 (3q11.2; *UROC1*, *FRMD4B*; Supplemental Figure 2), an intergenic signal on the long arm of Chromosome 12 (12q14; Supplemental Figure 3), and an intergenic signal on the long arm of Chromosome 21 (21q21; Supplemental Figure 4).

Functional Analyses

Braineac indicates that rs2252790 is nominally associated with the mRNA expression of *DSE/TSPYL1* (TSPY like 4), and *ROS1* (ROS proto-oncogene 1, receptor tyrosine kinase) in several brain regions. Two of these findings survived a Bonferroni multiple test correction ($p<5\times10^{-3}$): rs2252790 is an eQTL for *DSE/TSPYL1* expression in hippocampus tissue

($p=1.26\times10^{-4}$) and for *ROS1* expression in temporal cortex tissue ($p=1.20\times10^{-6}$). In the GTEx database, rs2252790 is associated with the expression of *DSE/TSPYL1, ROS1*, *NT5DC1*, and *FRK*, with the most robust effects observed for *DSE* expression ($p=8.0\times10^{-20}$). GeneMANIA indicated that *DSE* is involved in the following network of genes: *DSEL, ACSL4, CPSF3L, HFE, ZEB2, PXN, AHR, MCTP1, TGFBI, FCGR1A, TNS3, TRPM2, SLC27A3, EMR2, TAX1BP3, MRC2, F11R, RND3, MARCKS, CTBP2*. These genes are detailed in Table 2 and Figure 3. DAVID indicated that 11/21 of these genes were integral to membrane organization (enrichment score: 1.33, p-value=0.03); however, this enrichment score did not survive a Bonferonni correction (p-value= 0.09).

Post-Hoc Analyses

Following the identification of gene networks of relevance to GWAS findings (i.e., genes shown to interact with *DSE* via physical, co-expression, co-localization and/or pathway analyses in previous studies, curated using GeneMANIA as described above), gene-based analyses indicated that when considered as a set (all available SNPs in a given gene, corrected for the number of independent signals within that gene set), variants within the following genes are associated with fast beta EEG (empirical p-value<0.05): *DSE*, *ZEB2, MCTP1, RND3 and CTBP2* (Table 3). In addition, gene-based analyses indicated that *ZEB2* and *CTBP2* were also associated with DSM-V AUD (empirical p-value<0.05; Table 3). Table 3 details the number of variants examined in each gene-set, the number of variants nominally associated with beta EEG and/or AUD ($p<0.05$), the number of independent signals represented among each of the SNPs tested, and the empirical p-value based on 100,000 permutations. In addition, 20/26 *GABRA2* variants previously shown to be associated with aspects of beta EEG were modestly (p<0.05) associated with fast beta EEG at specific fronto-central pairs (Table 4). Of these 20 variants, 5 survive a multiple-test correction (0.05/3 LD Blocks: p<0.017).

Discussion

Although previous studies have reported variation in beta EEG among individuals diagnosed with AD and related conditions, there have been relatively few studies examining genetic variants in relation to beta EEG and only one finding that has been replicated to date (*GABRA2* (Porjesz *et al.* 2002b; Edenberg *et al.* 2004; Lydall *et al.* 2011; Ittiwut *et al.* 2012; Malone *et al.* 2014)). Subsequently, associations between *GABRA2,* AD (Edenberg *et al.* 2004; Lappalainen et al. 2005; Covault *et al*, 2004; Drgon *et al.*, 2006; Fehr *et al.*, 2006; Soyka *et al.* 2008; Enoch *et al.* 2006, 2010; Roh *et al.* 2011), drug dependence (Agrawal *et al.* 2006; Enoch *et al.* 2010; Ehlers & Gizer 2013), and externalizing behavior (Dick et al., 2013; Salvatore et al., 2015; Trucco et al., 2016; Wang et al., 2016) have been observed, indicating the potential of genetic studies of beta EEG to facilitate discovery of genes underlying disinhibitory behavior. However, the number of replicable genetic variants uncovered by this approach has been limited (Iacono et al., 2016), necessitating large scale genetic association studies of beta EEG utilizing the best-practices of genetic epidemiology.

In a GWAS of fronto-central fast beta EEG in families of EA, we report a genome-wide significant signal in an intronic region of *DSE* (dermatin sulfate) on 6q22. The most

significant SNP, rs2252790 ($p<2.6\times10^{-8}$; MAF: 0.36), was positively associated with fast beta EEG (β: 0.135). Taken together, data from Brainiac and GTEx suggest that rs2252790 is associated with the expression of *DSE/TSPYL1* (note, that *DSE* and *TSPYL1* have overlapping regions) and *ROS1* in several brain tissues. Notably, rs2252790 is an eQTL for *DSE/TSPYL1* mRNA expression in hippocampus tissue and for *ROS1* expression in temporal cortex tissue. Both of these brain regions may be particularly relevant to beta EEG. It has been suggested that the beta rhythm may serve to establish transient physiological connections, reflected in coherence at the beta frequency among neurons in the hippocampus and related structures (Leung, 1992b; Vecchio et al., 2016b). Further, the high-frequency (i.e., fast beta) oscillations, often referred to as 'rapid discharges', have often been associated with seizure generation. Evidence from hippocampal slices shows that neuroelectric bursts in CA1 pyramidal cells are caused by highly synchronized β-band activity (Netoff and Schiff, 2002). Further, impairments observed in temporal lobe epilepsy (TLE) and Alzheimer's disease have links to these brain structures and fast beta EEG. For example, lesions due to TLE typically involve mesial structures of the temporal lobe, particularly the amygdala and hippocampus (Kiernan, 2012). These structures play a central role in learning and memory (Leritz et al., 2006), while additionally involving sub-domains of working memory and executive functions (Stretton and Thompson, 2012; Zhao et al., 2014). Seizures caused by mesial TLE often involve fast frequency oscillations in the range of EEG fast-beta (Spencer et al., 1992; Bartolomei et al., 2004; Bartolomei et al., 2008). Further, researchers have hypothesized that cognitive impairments observed in Alzheimer's disease may involve disrupted functional connectivity between frontotemporal and frontoparietal regions related to beta (and alpha) frequency EEG (Hsiao et al., 2013). These findings highlight that the brain regions implicated by the mRNA expression associated with *DSE* variant rs2252790 (hippocampus, temporal cortex) are related to beta band activity as well as higher cognitive functions, increasing the biological plausibility of this study's findings. However, further studies are needed to understand the relationship of the *DSE* variant rs2252790, mRNA expression in hippocampus and temporal cortex, and beta EEG.

Variation within *DSE* has been associated with several cancers (Gouignard et al., 2016a; Thelin et al., 2013, 2012), Heschl's Gyrus thickness (Cai et al., 2014), and is also a notable risk factor for Ehlers-Danlos syndrome, with a subtype specifically linked to dysfunction of *DSE* (Müller et al., 2013). Recent work by Gouignard et al. (Gouignard et al., 2016b) demonstrates a functional role for *dse* (the protein encoded by *DSE*) in cranial neural crest cell migration and in cell adhesion providing a potential biological mechanism linking *DSE* dysfunction to Ehlers-Danlos syndrome and other neural crest related disorders (i.e., neurocristopathies); the knockdown of *dse* impaired the correct activation of transcription factors involved in the epithelial-mesenchymal transition and reduced the extent of neural crest cell migration, subsequently leading to a decrease in neural crest-derived craniofacial skeleton, melanocytes and dorsal fin structures.

Given the association observed between AUDs and beta EEG in this and previous studies (Rangaswamy et al., 2004), we conducted a secondary analysis in which we repeated the initial GWAS of beta EEG, adjusting for DSM-V AUD severity. Results produced similar findings as the primary analyses; however, all p-values were slightly less significant. This suggests that the association of rs2252790 and beta EEG is not explained entirely by AUD;

however, there may be an interaction among *DSE* variants, DSM-V AUD severity, and beta EEG. To comment on this more conclusively, future studies employing longitudinal data should assess the interaction of DSM-V AUD symptoms, *DSE* variants, and beta EEG.

Based on previous physical, co-expression, co-localization and pathway gene-gene interactions observed in the literature, GeneMANIA indicated that *DSE* is involved in a network of genes integral to membrane organization (Table 2). Given that scalp electrodes record potential differences that are caused by postsynaptic potentials in the cell membrane of cortical neurons, the relation between genes critical in cellular membrane organization and EEG seems broadly plausible. Several genes in this network have been previously linked to phenotypes of relevance to beta EEG and related traits (i.e., cognitive performance, bipolar disorder, AUD). For example, variants within *DSEL* (Dermatin Sulfate epimerase-like) have been associated with cognitive performance (Need et al., 2009), depression (Shi et al., 2011), and bipolar disorder (Goosens et al., 2003). Each of these phenotypes have been linked to variation in beta rhythms (cognitive performance (Klimesch, 1999); depression (Zotev et al., 2014); bipolar disorder (Andersson et al., 2008). In the present study, we find evidence of association among many variants within this *DSE* gene network and beta EEG, with the most robust associations (empirical $p<0.05$) observed for *ZEB2, MCTP1, RND3*, and *CTBP2*. Of these genes, *ZEB2* and *CTBP2* were also associated (empirical p-value>0.05) with DSM-V AUD (Table 3). Both *ZEB2* and *CTBP2* have been shown to influence gene expression in the brain, particularly during brain development, and have been previously correlated with traits of relevance to beta EEG and/or AUD.

CTBP2 is from a family of COOH-terminal binding proteins (CtBPs), which are widely expressed during several developmental processes, and have been linked to various complex traits, including cancers and Alzheimer's disease (Liu et al., 2014; Zhang et al., 2014; Zheng et al., 2015). Importantly, two previous studies have found associations among *CTBP2* and alcohol related phenotypes. A linkage analysis of alcohol and cigarette consumption (maximum cigarettes/grams of alcohol consumed per day) conducted in 1,390 individuals from 41 extended Mexican American families indicated a linkage peak on Chromosome 10. Subsequently, an expression profile analyses of 342 RNA transcripts under the linkage peak pointed to two genes, one of which was *CTBP2*. Further, *CTBP2* was shown to influence RNA levels, which was negatively correlated with smoking and/or drinking (Viel et al., 2008). In addition, an early GWAS of alcohol and nicotine dependence reported an association of *CTBP2* and DSM-IV Alcohol Dependence in an Australian cohort of 1,224 cases and 1,162 controls, although the association did not meet genome-wide significant criteria (p-value: 3.91×10^{-7};(Lind et al., 2010)). Further support for the role of *CTBP2* in alcohol related behavior comes from model organism work (Grotewiel and Bettinger, 2015); a genetic screen in nematode *Caenorhabditis elegans* identifies *ctbp-1* (the ortholog of *CTBP2*) as a key regulator required for the development of acute functional tolerance to ethanol (Bettinger et al., 2012; Reid et al., 2015). While this previous literature lends corroborating support for the association of *CTBP2* and AUD reported here, this finding should clearly be replicated in independent, larger samples.

ZEB2 (zinc finger E-box binding homeobox 2) encodes the Smad Interacting Protein 1, which is involved in the *TGF*-β/*BMP*/*Smad* signaling cascade (Babkina et al., 2016). *ZEB2*

mRNA is expressed during early embryogenesis in brain tissue, and is thought to play an important role in neural crest cell migration (Van de Putte et al., 2003) and in the regulation of corticogenesis (Seuntjens *et al.* 2009). Mutations in *ZEB2* have been linked with epilepsy (EPICURE Consortium et al., 2012) and related disorders, such as Hirschsprung disease/ Mowat-Wilson syndrome (MWS; (Cordelli et al., 2013)). MWS is caused by heterozygous mutations or deletions of *ZEB2* and is characterized by epilepsy, moderate to severe intellectual disability, corpus callosum abnormalities and other congenital malformations. Recent studies (Cordelli et al., 2013) suggest that a distinct "electroclinical" phenotype, characterized by age-dependent EEG changes, can be recognized in most patients with MWS. While the mechanism underlying epilepsy in individuals with *ZEB2* mutations is not well understood, studies by McKinsey and Van den Berghe (McKinsey et al., 2013; van den Berghe et al., 2013) show the influence of *ZEB2* on the neurogenesis of cortical γ-aminobutyric acid (GABA)ergic interneurons. Further, lack of *ZEB2* prevents the repression of *NKX2-1* homeobox transcription factor, the expression of which induces the differentiation of progenitor cells into striatal interneurons rather than cortical neurons (McKinsey et al., 2013; van den Berghe et al., 2013). Subsequently, deficit of GABAergic inhibition is thought to result in seizures (Yalçın, 2012). A recent exome sequencing study identified a de novo missense variant in *ZEB2* and early infant epileptic encephalopathy characterized by burst-suppression EEG (Babkina et al., 2016). In addition, independent genetic studies have also found an association of *ZEB2* variants, obesity related traits (Comuzzie et al., 2012), depression, bipolar disorder, and schizophrenia (Ripke et al., 2014). Taken together, this may suggest that variation in *ZEB2* may have broader neurobiological implications, beyond epilepsy. While no previous genetic association studies have provided a clear association of either *ZEB2* or *CTBP2* and beta EEG, mRNA expression in key brain regions for beta EEG, along with associations with potentially related traits (i.e., AUD, alcohol and cigarette use frequency, epilepsy) could provide a potential link for the association of *ZEB2*, *CTBP2* and beta EEG. These conclusions however, are beyond the scope of the present study and must be explored in future, independent studies.

Since the three genome-wide associated variants were located within an intron of *DSE*, this discussion has primarily focused on *DSE* and related gene networks. However, it should be noted that the pattern of association observed for beta EEG could implicate several nearby genes (see Figure 2); several variants in high linkage disequilbirum with rs2252790 are located within *TSPYL4*. For example, rs910391, which is in perfect linkage disequilbirum with rs2252790, is located in the promoter region of *TSPYL4*. Further, rs2252790 is nominally associated with the mRNA expression of several neighboring genes including *TSPYL1, TSPYL4, ROS1*, *NT5DC1*, and *FRK*, although the most robust effects were observed for *DSE* expression. Therefore, it is possible that associations observed in this study are due to the influence of these (or other) genes on beta EEG.

This study also confirmed that several variants within *GABRA2* were modestly ($p<0.05$) associated with fast beta EEG in the fronto-central region (Table 4). The association of *GABRA2* and beta EEG was initially reported with linkage and association analysis in COGA AD families using the first spatial/spectral component of 11 bipolar electrode pairs and 3 beta frequency bands ranging from 12.5–28 Hz (Porjesz et al., 2002; Edenberg et al., 2004). Subsequently, an association between *GABRA2* variants and bipolar beta (13.5–27

Hz) EEG, assessed at fronto-central electrodes, was also reported in a case-control study of AD (Lydall et al., 2011). More recently, Malone et al., (2014) reported an association of *GABRA2* and monopolar beta EEG, assessed at the central electrode (i.e., CZ) using GWAS data on a community sample of adolescent twins and their parents (Malone et al., 2014). In the present study, we build upon this prior literature in providing additional support for the association of *GABRA2* variants and bipolar fast beta (20–28 Hz) EEG, assessed at fronto-central electrodes, in a family sample enriched for AD.

Limitations

Most notable is the relatively small sample size and related lack of statistical power to detect subtle genotypic effects. A recent article described the large projected sample sizes needed for a well powered genetic study of EEG, and highlighted the concerns that statistically underpowered genetic studies raise (Iacono et al., 2016). However, GWAS results seem reliable based on corroborating information (i.e., multiple genome-wide significant SNPs in high LD, biological plausibility). Nevertheless, genetic associations reported in this study must be replicated in a large, independent sample. Furthermore, given the nominal associations observed in eQTL analyses, these findings must also be replicated in larger samples. In addition, the current study includes participants with a wide age range (ages 7–74), which introduces potential for unmeasured confounding effects due to age-related changes in beta EEG; GWAS analyses were adjusted for age and age^2 in an effort to minimize age related differences in beta EEG genetic association findings. However, future studies should examine the effects of genetic variants on trajectories of beta EEG during development in order to delineate age-specific effects, and the links between these effects and/or the onset of psychopathology, such as AUD. Finally, the analytic software employed for this genome-wide analysis of family based samples (GWAF) does not currently allow for the analysis of sex chromosomes.

Conclusions

To date, there have been relatively few genetic studies examining beta EEG, and only one finding that has been replicated. This study reports association between intronic SNPs located within *DSE* on 6q22 and fronto-central fast beta EEG in a sample of related individuals of EA. The most significant SNP is an eQTL for *DSE,* a gene encoding a protein important in cranial neural crest development, previously implicated in several complex traits (e.g., Ehlers Danlos syndrome, bipolar disorder, brain morphology) and expressed in hippocampus and temporal cortex, brain regions of importance to beta EEG. Further, GeneMANIA has indicated that *DSE* interacts with a network of genes integral to membrane organization. In the present study, gene-based tests of association suggest that several variants within this network (i.e., variants within *DSE, ZEB2, MCTP1, RND3,* and *CTBP2)* were associated with beta EEG, and *ZEB2 and CTBP2* were also associated with DSM-V AUD. Converging data from GWAS, gene expression, and gene-networks presented in this study provide support for the role of genetic variants within *DSE* and related genes in neural hyperexcitability, and has highlighted two genes potentially related to AUD. While results presented in this study are intriguing, findings clearly need additional support including replication in larger, independent studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The Collaborative Study on the Genetics of Alcoholism (COGA), Principal Investigators B. Porjesz, V. Hesselbrock, H. Edenberg, L. Bierut, includes eleven different centers: University of Connecticut (V. Hesselbrock); Indiana University (H.J. Edenberg, J. Nurnberger Jr., T. Foroud); University of Iowa (S. Kuperman, J. Kramer); SUNY Downstate (B. Porjesz); Washington University in St. Louis (L. Bierut, J. Rice, K. Bucholz, A. Agrawal); University of California at San Diego (M. Schuckit); Rutgers University (J. Tischfield, A. Brooks); University of Texas Health Science Center at San Antonio (L. Almasy), Virginia Commonwealth University (D. Dick), Icahn School of Medicine at Mount Sinai (A. Goate), and Howard University (R. Taylor). Other COGA collaborators include: L. Bauer (University of Connecticut); D. Koller, J. McClintick, S. O'Connor, L. Wetherill, X. Xuei, Y. Liu (Indiana University); G. Chan (University of Iowa; University of Connecticut); D. Chorlian, N. Manz, C. Kamarajan, A. Pandey (SUNY Downstate); J.-C. Wang, M. Kapoor (Icahn School of Medicine at Mount Sinai) and F. Aliev (Virginia Commonwealth University). A. Parsian and M. Reilly are the NIAAA Staff Collaborators.

We continue to be inspired by our memories of Henri Begleiter and Theodore Reich, founding PI and Co-PI of COGA, and also owe a debt of gratitude to other past organizers of COGA, including Ting-Kai Li, currently a consultant with COGA, P. Michael Conneally, Raymond Crowe, and Wendy Reich, for their critical contributions. This national collaborative study is supported by NIH Grant U10AA008401 from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and the National Institute on Drug Abuse (NIDA). We thank the Genome Technology Access Center in the Department of Genetics at Washington University School of Medicine for help with genomic analysis. The Center is partially supported by NCI Cancer Center Support Grant #P30 CA91842 to the Siteman Cancer Center and by ICTS/CTSA Grant# UL1RR024992 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical Research. This publication is solely the responsibility of the authors and does not necessarily represent the official view of NCRR or NIH.

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Highlights

- GWAS of resting state fast beta (20–28Hz) EEG (F3-C3) in COGA families of European Ancestry.
- Fast beta EEG is genome-wide associated with three variants within *DSE* (6q22).
- rs2252790 influences mRNA expression in brain tissue.
- Related genes *ZEB2, RND3, MCTP1,* and *CTBP2* are associated with beta EEG

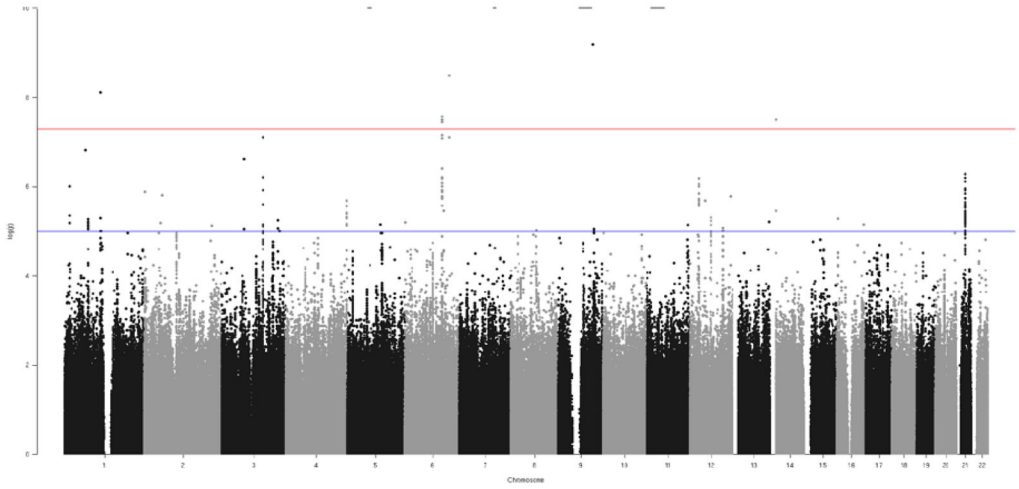

Fig. 1.

Manhattan plot of GWAS results for the fast beta EEG (20–28 Hz; bipolar derivation at the fronto-central electrode pair (F3-C3)).

Negative log-transformed *p*-values for SNPs are plotted against base-pair position for each chromosome. Three intronic *DSE* variants on chromosome 6 exceeded the genome-wide significance threshold of 5×10^{-8}, indicated by the red line.

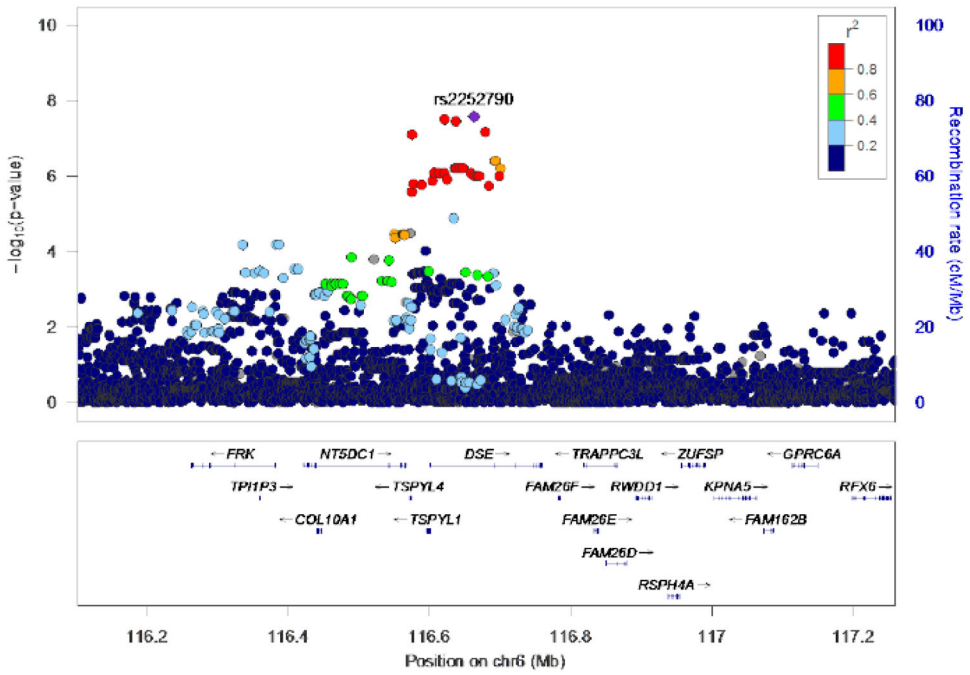

Figure 2. Association results for fast beta EEG on Chromosome 6

Y-axis denotes the −log10(p-value) for association. X-axis is the physical position on the chromosome (Mb). The most significantly associated SNP (rs2252790) is shown in purple. The extent of linkage disequilibrium (as measured by r^2) between each SNP and the most significantly associated SNP is indicated by the color scale at top left. Larger values of r^2 indicate greater linkage disequilibrium (LD). LD is based on hg19 1000 Genomes from the CEU sample.

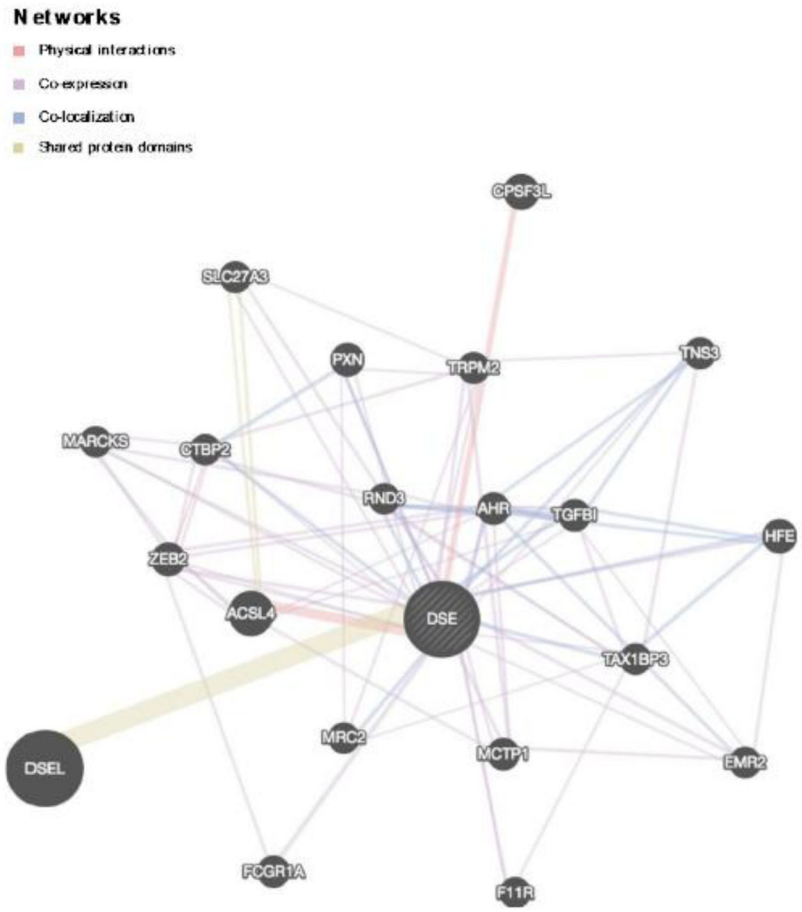

Figure 3.

DSE related gene network identified via GeneMANIA, a multiple association network integration algorithm utiilizing physical, co-expression, and co-localization gene-gene interactions as well as genes with shared protein domains observed in previous studies.

Table 1

Variants associated with Fast beta EEG (20–28 Hz; bipolar derivation at the fronto-central electrode pair (F3-C3)) at p-value<5×10^{-7}

SNP	CHR	BASE PAIR	AL1	AL2	MAF	BETA	SE	*P-VALUE*	GENE
rs112196420	3	69499803	T	G	0.03	−0.339	0.066	2.30E-07	*FRMD4B*
rs16837279	3	125932261	G	T	0.216	−0.131	0.026	6.00E-07	intergenic
rs1687482	3	126215694	T	G	0.151	−0.162	0.03	7.60E-08	*UROC1*
rs910391	6	116575344	T	G	0.362	0.128	0.024	7.90E-08	*TSPYL4, DSE*
rs2982842	6	116606807	G	A	0.359	0.114	0.023	8.00E-07	*DSE*
rs12212556	6	116613907	G	A	0.359	0.114	0.023	8.30E-07	*DSE*
rs10456906	6	116621035	A	C	0.359	0.114	0.023	8.30E-07	*DSE*
rs10456907	**6**	**116621036**	**C**	**T**	**0.362**	**0.133**	**0.024**	**3.10E-08**	***DSE***
rs13209267	6	116635819	C	A	0.358	0.116	0.023	6.10E-07	*DSE*
rs12213442	6	116636907	A	G	0.358	0.116	0.023	6.10E-07	*DSE*
rs13214667	**6**	**116637440**	**A**	**G**	**0.364**	**0.134**	**0.024**	**3.50E-08**	***DSE***
rs12203516	6	116638619	G	A	0.357	0.116	0.023	6.10E-07	*DSE*
rs12214926	6	116643292	T	C	0.357	0.116	0.023	6.00E-07	*DSE*
rs12204234	6	116647587	C	T	0.358	0.116	0.023	6.00E-07	*DSE*
rs2501053	6	116658732	A	G	0.359	0.114	0.023	8.20E-07	*DSE*
rs2501052	6	116658955	A	G	0.358	0.114	0.023	8.20E-07	*DSE*
rs2501050	6	116663206	G	A	0.358	0.114	0.023	9.80E-07	*DSE*
rs2252790	**6**	**116663323**	**G**	**A**	**0.36**	**0.135**	**0.024**	**2.60E-08**	***DSE***
rs4946160	6	116667472	C	A	0.358	0.114	0.023	9.80E-07	*DSE*
rs3021301	6	116670364	A	G	0.358	0.114	0.023	9.80E-07	*DSE*
rs2498710	6	116670636	G	A	0.358	0.114	0.023	9.80E-07	*DSE*
rs2858848	6	116679275	A	G	0.364	0.131	0.024	6.70E-08	*DSE*
rs2640873	6	116692795	A	G	0.403	0.115	0.023	3.90E-07	*DSE*
rs2640872	6	116693384	T	C	0.403	0.115	0.023	3.90E-07	*DSE*
rs2213563	6	116698931	A	G	0.374	0.112	0.023	9.90E-07	*DSE*
rs574871	6	116700809	C	T	0.403	0.113	0.023	6.20E-07	*DSE*
rs258430	12	28033502	G	A	0.573	−0.111	0.022	6.30E-07	intergenic
rs258442	12	28045607	T	C	0.567	−0.109	0.022	9.90E-07	intergenic

SNP	CHR	BASE PAIR	AL1	AL2	MAF	BETA	SE	*P-VALUE*	GENE
rs258444	12	28046102	G	A	0.574	−0.109	0.022	8.60E-07	intergenic
rs10843021	12	28050947	C	T	0.568	−0.109	0.022	9.90E-07	intergenic
rs399384	21	27968714	T	C	0.365	−0.111	0.023	7.40E-07	intergenic
rs384901	21	27970165	G	T	0.385	−0.11	0.022	7.40E-07	intergenic
rs222957	21	27977823	A	G	0.376	−0.111	0.022	6.10E-07	intergenic
rs376583780	21	28003040	T	C	0.328	−0.118	0.023	5.10E-07	intergenic
rs370690781	21	28003042	T	C	0.328	−0.118	0.023	5.10E-07	intergenic
rs372255102	21	28003047	T	C	0.328	−0.118	0.023	5.10E-07	intergenic
rs376311445	21	28003049	A	C	0.328	−0.118	0.023	5.10E-07	intergenic
rs56387300	21	28003051	T	C	0.328	−0.118	0.023	5.10E-07	intergenic

Note: Genome-wide significant (p-value<5×10^{-8}) variants are bolded.

Table 2

DSE -related gene network identified via GeneMANIA, a multiple association network integration algorithm utilizing physical, co-expression, co-localization, and pathway gene-gene interactions observed in previous studies.

GENE SYMBOL	CHROMOSOME LOCATION	OFFICIAL GENE NAME
ACSL4	X	acyl-CoA synthetase long-chain family member 4
CPSF3L	1	cleavage and polyadenylation specific factor 3-like
FCGR1A	1	Fc fragment of IgG receptor Ia
SLC27A3	1	solute carrier family 27 member 3
F11R	1	F11 receptor
ZEB2	2	zinc finger E-box binding homeobox 2
RND3	2	Rho family GTPase 3
MCTP1	5	multiple C2 and transmembrane domain containing 1
TGFBI	5	transforming growth factor beta induced
DSE	6	dermatan sulfate epimerase
HFE	6	hemochromatosis
MARCKS	6	myristoylated alanine rich protein kinase C substrate
AHR	7	aryl hydrocarbon receptor
TNS3	7	tensin 3
CTBP2	10	C-terminal binding protein 2
PXN	12	paxillin
TAX1BP3	17	Tax1 binding protein 3
MRC2	17	mannose receptor C type 2
DSEL	18	dermatan sulfate epimerase-like
ADGRE2	19	adhesion G protein-coupled receptor E2
TRPM2	21	transient receptor potential cation channel subfamily M member 2

Note: *ACSL4* was not assessed in the current study

Table 3

Gene-based association of *DSE*-related genes, fast beta EEG, and DSM-V AUD

GENE SYMBOL	# OF SNPS TESTED		# OF SIGNIFICANT SNPS	# OF INDEPENDENT SIGNALS	EMPIRICAL P-VALUE	
	Beta EEG	*DSM-V AUD*		*Beta EEG*	*DSM-V AUD*	
DSE	602	163	89	5	**<0.000**	0.095
CTBP2	780	53	64	5	**0.019**	**0.044**
ZEB2	218	23	43	5	**0.032**	**0.022**
MCTP1	1651	140	110	5	**0.044**	0.068
RND3	90	4	3	1	**0.046**	0.210
TNS3	936	52	Not tested	5	0.065	Not tested
MRC2	167	15	Not tested	5	0.082	Not tested
F11R	180	16	Not tested	5	0.125	Not tested
MARCKS	61	20	Not tested	5	0.176	Not tested
CPSF3L	103	10	Not tested	5	0.204	Not tested
TRPM2	270	7	Not tested	5	0.267	Not tested
ADGRE2	317	19	Not tested	5	0.319	Not tested
TGFBI	142	4	Not tested	4	0.345	Not tested
DSEL	77	3	Not tested	5	0.374	Not tested
PXN	143	6	Not tested	5	0.404	Not tested
HFE	115	22	Not tested	5	0.583	Not tested
SLC27A3	46	4	Not tested	3	0.591	Not tested
AHR	117	11	Not tested	5	0.608	Not tested
FCGR1A	4	0	Not tested	0	1.000	Not tested
TAX1BP3	115	0	Not tested	1	1.000	Not tested

Note: Following the identification of *DSE* related genes identified via GeneMANIA, gene-based tests of association with fast beta EEG were conducted in PLINK (Purcell et al., 2003), using set-based analyses ("--set-test"). Gene-based tests included all available SNPs in a given gene, corrected for the number of independent signals (i.e., linkage disequilibrium blocks) within that gene set. In addition, all association models were adjusted for the relatedness in the family sample, sex, log-transformed age, and ancestry. Tests of association were accepted as significant if the 100,000 permutations of the set-based regression analysis produced an empirical *p*-value <0.05. Subsequently, these procedures were repeated for tests of gene-based association with DSM-V AUD severity only among genes that were associated with beta EEG. Table 3 details the number of SNPs examined in each gene-set, the number of SNPs within each gene-set associated with beta EEG and AUD ($p<0.05$), the number of independent signals represented among each of the SNPs tested, and the empirical p-value based on 100,000 permutations.

Table 4

Associations of previously reported *GABRA2* variants and fast beta EEG (20–28 Hz; bipolar derivation at the fronto-central electrode pairs (FZ-CZ, F3-C3, F4-C4))

SNP	Base Pair Location	Major Allele	Minor Allele	MAF	β	p-value	β	p-value	β	p-value
	FZ-CZ				*F3-C3*			*F4-C4*		
rs561779	46238778	G	A	0.59	−0.04	**0.046**	0.01	0.680	0.00	0.980
rs572227	46251393	C	T	0.59	−0.05	**0.014***	0.00	0.940	−0.01	0.620
rs573400	46252066	T	C	0.59	−0.04	**0.047**	0.01	0.690	0.00	0.980
rs532780	46261366	T	C	0.58	−0.04	**0.035**	0.01	0.560	0.00	0.920
rs548583	46263344	G	A	0.58	−0.06	**0.011***	0.01	0.820	−0.01	0.720
rs496650	46264385	A	C	0.58	−0.04	**0.050**	0.01	0.600	0.00	0.940
rs540363	46274246	A	G	0.58	−0.04	**0.039**	0.01	0.610	0.00	0.950
rs526752	46276629	A	G	0.58	−0.05	**0.031**	0.01	0.580	0.00	0.950
rs530329	46281119	T	C	0.58	−0.05	**0.031**	0.01	0.580	0.00	0.950
rs483160	46287075	G	T	0.58	−0.05	**0.025**	0.01	0.640	0.00	0.950
rs279871	46305733	T	C	0.57	−0.06	**0.009***	0.00	0.860	−0.01	0.640
rs279867	46308303	A	C	0.58	−0.05	**0.029**	0.01	0.590	0.00	0.990
rs279866	46309764	A	G	0.58	−0.04	**0.036**	0.01	0.550	0.00	0.960
rs279863	46313022	C	A	0.58	−0.04	**0.032**	0.01	0.580	0.00	0.950
rs279858	46314593	T	C	0.57	−0.06	**0.011***	0.01	0.830	−0.01	0.700
rs279843	46325204	C	T	0.57	−0.05	**0.028**	0.00	0.980	−0.01	0.680
rs279846	46329886	C	T	0.55	−0.04	0.066	0.01	0.590	0.01	0.700
rs279826	46334209	A	G	0.55	−0.04	0.059	0.01	0.590	0.01	0.770
rs279828	46334810	A	C	0.55	−0.04	**0.045**	0.01	0.660	0.01	0.770
rs279837	46339323	A	G	0.57	−0.04	**0.031**	0.01	0.770	0.00	0.830
rs279841	46340763	G	A	0.57	−0.05	**0.017***	0.00	0.980	−0.01	0.830
rs189957	46346679	A	G	0.55	−0.04	**0.051**	0.01	0.600	0.01	0.790
rs1442062	46377076	A	G	0.25	0.03	0.280	0.02	0.460	0.03	0.280
rs3756007	46391064	C	T	0.04	−0.02	0.770	−0.02	0.710	−0.03	0.620
rs894269	46393612	C	T	0.78	−0.01	0.720	0.03	0.200	0.01	0.580
rs1545234	46404413	A	G	0.70	0.01	0.630	0.03	0.230	0.02	0.340

* Withstands a multiple-test correction