Family-based genome-wide association study of frontal theta oscillations identifies potassium channel gene *KCNJ6*

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Event-related oscillations (EROs) represent highly heritable neuroelectric correlates of cognitive processes that manifest deficits in alcoholics and in offspring at high risk to develop alcoholism. Theta ERO to targets in the visual oddball task has been shown to be an endophenotype for alcoholism. A family-based genome-wide association study was performed for the frontal theta ERO phenotype using 634 583 autosomal single nucleotide polymorphisms (SNPs) genotyped in 1560 family members from 117 families densely affected by alcohol use disorders, recruited in the Collaborative Study on the Genetics of Alcoholism. Genome-wide significant association was found with several SNPs on chromosome 21 in *KCNJ6* (a potassium inward rectifier channel; KIR3.2/GIRK2), with the most significant SNP at $P = 4.7 \times 10^{-10}$). The same SNPs were also associated with EROs from central and parietal electrodes, but with less significance, suggesting that the association is frontally focused. One imputed synonymous SNP in exon four, highly correlated with our top three SNPs, was significantly associated with the frontal theta ERO phenotype. These results suggest *KCNJ6* or its product GIRK2 account for some of the variations in frontal theta band oscillations. GIRK2 receptor activation contributes to slow inhibitory postsynaptic potentials that modulate neuronal excitability, and therefore influence neuronal networks.

Keywords: Alcoholism, EEG, GWAS, *KCNJ6*, oscillations

The electroencephalogram (EEG) recorded from the scalp during cognitive tasks contains oscillation patterns in specific frequency bands associated with task processing. These event-related oscillations (EROs) provide a versatile framework to generate coordination and communication during complex brain operations (Buzsaki 2010; Buzsaki & Draguhn 2004); they bind neural ensembles by providing windows of opportunity for neurons to fire enabling functional integration of networks (Fries 2005). EROs in specific frequency bands [δ (1.0–2.5 Hz); θ (3.0–7.0 Hz); α (7.5–12.0 Hz); β (12.5–29.0 Hz) and γ (>29.0 Hz)] have been attributed to specific cognitive processes during normal and pathological brain function (Babiloni *et al*. 2011; Basar *et al*. 2001; Klimesch *et al*. 2001; Rothenberger 2009). Delta EROs are associated with signal evaluation and decision making (Basar *et al*. 1999; Schurmann *et al*. 2001), while theta EROs are important for processes underlying frontal inhibitory control, conscious awareness, recognition memory and episodic retrieval (Gevins *et al*. 1998; Klimesch *et al*. 1994, 2001). Theta oscillations have been implicated in sensorimotor integration (Bland & Oddie 2001; O'Keefe & Recce 1993), evaluating loss and gain (Kamarajan *et al*. 2008) and several processes associated with memory (Jacobs *et al*. 2006; Klimesch *et al*. 2008; Vertes 2005). Theta rhythm plays a role in information processing using an attentional double-gating mechanism, 'filtering-in' signals for effective registration and encoding of selected information and 'filtering-out' interfering inputs (Vinogradova 1995). Brain oscillations have been shown to be stable, highly heritable (van Beijsterveldt & Boomsma 1994; van Beijsterveldt *et al*. 1996), and to be reliable endophenotypes that reflect a shared liability between alcoholism and related disorders (Porjesz

et al. 2005). Power estimates of oscillations are more heritable than event-related potential (ERP) components, giving them a slight edge as endophenotypes (de Geus 2010).

Alcoholism is part of a spectrum of disinhibitory disorders, which include externalizing and substance use disorders; a shared set of genetic factors influencing impulse control are postulated to underlie these cooccurring disinhibitory disorders (Kendler *et al.* 2003; Lahey *et al.* 2011). Hence, examining neuroelectric phenotypes that reflect shared liabilities provides a powerful strategy to investigate underlying risk for alcoholism and related disorders (Porjesz & Rangaswamy 2007; Rangaswamy & Porjesz 2008). Our earlier studies have shown that both delta and theta power are significantly reduced in alcoholics and adolescent offspring of alcoholics when compared with normal controls during target processing in a visual oddball paradigm (Jones *et al.* 2006b; Rangaswamy *et al.* 2007). Frontal theta ERO has successfully served as an endophenotype in family and case–control genetic studies in the Collaborative Study on the Genetics of Alcoholism (COGA) (Chen *et al.* 2009; Jones *et al.* 2004, 2006a; Zlojutro *et al.* 2011). This study examines the theta ERO endophenotype recorded at the midline frontal (Fz) electrode in response to targets in a visual oddball paradigm in a family-based genome-wide association study (GWAS). This study also evaluates the scalp topography of associations for theta ERO and single nucleotide polymorphisms (SNPs) in the most significant gene. The advantage of a family-based study design is robustness against population substructure and the availability of the genotypes of both parents, which enables a more correct evaluation of genotype errors. This is the first family-based GWAS of EROs.

Materials and methods

Participants
Alcoholic and community probands and their families were recruited and tested as part of the national multi-site COGA. Alcoholic probands were recruited from inpatient and outpatient treatment facilities; all participants were administered the Semi-Structured Assessment for the Genetics of Alcoholism (Bucholz *et al.* 1994; Hesselbrock *et al.* 1999), and alcoholic subjects met criteria for alcohol dependence (DSM-IV). Data from the six COGA collection sites were included in the analysis: SUNY Downstate Medical Center at Brooklyn, New York; University of Connecticut Health Science Center; Washington University School of Medicine in St. Louis; University of California at San Diego; University of Iowa, and Indiana University School of Medicine. Recruitment and assessment procedures have been outlined previously (Begleiter *et al.* 1995; Foroud *et al.* 2000; Nurnberger *et al.* 2004; Reich *et al.* 1998) and are available at zork.wustl.edu/niaaa/coga_instruments/resources.html. Institutional review boards at each site approved the research protocols in the COGA study and written consent was obtained from each individual before participation.

Prior to neurophysiological assessments, alcoholic subjects were required to abstain from alcohol for a minimum of 3 weeks and not exhibit withdrawal symptoms. Subjects were excluded from neurophysiological assessment if they had any of the following: (1) recent substance or alcohol use (i.e. positive breath-analyzer test and/or urine screen), (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, (6) history/symptoms of psychoses, (7) positive test for human immunodeficiency virus, (8) other acute/chronic medical illnesses that affects brain function and (9) and a score of less than 25 on the Mini Mental State Examination (Folstein *et al.* 1975).

Prioritization of families for the COGA family-based GWAS was based on the number of alcohol-dependent family members, the number of individuals who supplied DNA and the number of family members with electrophysiological measurements. The family-based GWAS of the theta ERO phenotype only included families of primarily Caucasian descent (to reduce heterogeneity of the sample) and those with measurements of the theta ERO phenotype at the frontal (Fz) lead. Thus, the dataset for the family-based GWAS of theta ERO comprised 1560 individuals (male = 738, female = 822) ranging in age from 7 to 74 years (male: 7–74 years, average = 30.7 years; female: 7–72 years, average = 31.6 years) from 117 multi-generational families affected with alcoholism. Family sizes ranged from 4 to 39 individuals and had an average of 13.4 subjects per family (Fig. S1).

Visual oddball task
A three stimulus visual oddball task was employed with 280 visual stimuli of three different types: targets (rarely occurring letter X to which the subjects responded quickly and accurately with a button press, non-targets (frequently occurring white squares) and novels (rarely occurring random colored geometric figures). Stimuli subtended a visual angle of 2.5° with stimulus durations of 60 milliseconds and inter-stimulus intervals of 1.625 milliseconds. The task comprised 35 target, 35 novel and 210 non-target stimuli with a probability of occurrence of 0.125, 0.125 and 0.750, respectively. The stimuli were presented pseudo-randomly with the only constraint that the targets and novels always followed non-targets.

Event-related potential recording
All six collaborating sites used identical experimental procedures and EEG acquisition hardware and software programs. Subjects were seated comfortably 1 m from a monitor in a dimly lit sound-attenuated RF-shielded booth (Industrial Acoustics Company, Bronx, NY, USA), and wore a 19-channel electrode cap (Electro-Cap International, Inc., Eaton, OH, USA) as specified by the International 10–20 System for Electrode Placement (Fig. S2). The nose served as reference and the forehead served as ground. Electrode impedances were maintained below 5 kΩ. Electrical activity was amplified 10 000 times using Sensorium EPA-2 Electrophysiology amplifiers (Charlotte, VT, USA) was recorded continuously over a bandwidth of 0.02–100.0 Hz on a Neuroscan system (Version 4.1–4.5; Neurosoft, Inc., El Paso, TX, USA) at sampling rates of 256, 500 and 512 Hz, and stored for further analysis. During analysis all signals were re-sampled to 256 Hz and bandpass filtered between 0.05 and 55.0 Hz. Artifact rejection threshold was set at 100 µV. A minimum of 20 epochs of 100 milliseconds pre-stimulus to 750 milliseconds post-stimulus artifact free trials for each stimulus was required for analysis.

ERO energy estimation
Estimates of localized power of non-stationary evoked potential time series were obtained using the *S*-transform, a time-frequency representation method developed by Stockwell (2007) and Stockwell *et al.* (1996). This method has been previously described and implemented in our laboratory to evaluate event-related signals in the time-frequency domain (Jones *et al.* 2006b; Kamarajan *et al.* 2006; Rangaswamy *et al.* 2007).

Phenotype
Event-related electrophysiological data for the target stimulus from the visual oddball task were analyzed. The amplitude envelope of the *S*-transform time-frequency region was averaged across single trials, per individual, to obtain estimates of event-related total power. Mean power was calculated for each electrode within time-frequency regions of interest that were defined by frequency band ranges and time intervals (Lachaux *et al.* 2003). The primary phenotype used

for the family GWAS was the total power in the θ (3.0–7.0 Hz) oscillation at the frontal midline channel (Fz) extracted from the 300–700 ms time window, which corresponds to the window of the P300 component in the event-related waveforms. The total theta power measure was also extracted for the central (Cz) and parietal (Pz) channels for secondary analyses. As the theta ERO phenotype showed significant age and gender effects with amplitude, age and gender were included as covariates in the genetic analyses.

Genotyping and quality control
Samples from the COGA DNA and Cell Repository at Rutgers University were genotyped at the Genome Technology Access Center at Washington University School of Medicine in St. Louis using the Illumina Human OmniExpress array 12.VI (731 444 SNPs; Illumina, San Diego, CA, USA) on 2098 subjects selected from 118 densely affected families. An additional 224 subjects from these 118 families had been genotyped in a previous case–control GWAS by the Center for Inherited Disease Research (CIDR) at John Hopkins University using the Illumina Human 1M-Duo BeadChip Technology (1 041 465 SNPs; Illumina, San Diego, CA, USA; Edenberg *et al*. 2010). To insure quality control (QC), 51 subjects previously genotyped at CIDR were included among the 2098 subjects genotyped at Washington University.

The genotypic data set of 731 444 SNPs was carefully examined to ensure high quality standards (Turner *et al*. 2011). In this QC, each SNP was first examined for genotypic completeness. A genotype call score threshold of 0.15 was used, as recommended by Illumina Technical Support, which led to the removal of 1.7% of SNPs from further analysis. For those cases ($n = 162$, 0.33%) in which duplication or deletion was observed, the entire chromosome harboring this copy number variant was removed from further analysis. Inconsistencies (a non-missing, but different, genotype on the two arrays) of the 544 276 overlapping SNPs on the Illumina Human 1M-Duo and the Illumina Human OmniExpress arrays were tested using the 51 twice-genotyped subjects. We found 571 SNPs with more than one discrepancy and removed those SNPs in all 2322 subjects. Deviations from Hardy–Weinberg equilibrium (HWE) were evaluated using 442 genotyped founders, and SNPs that failed the HWE test at $P < 10^{-6}$ were removed. SNPs with minor allele frequency less than 0.01 were removed. In total, 634 627 autosomal SNPs were analyzed for association.

The software package PLINK v1.07 (Purcell *et al*. 2007) was used to calculate pairwise identity-by-descent (IBD) estimates of 100 000 SNPs, which were not in linkage disequilibrium with each other, to verify the reported pedigree structure of the 118 families. Based on these IBDs, some family structures were modified. Using the software PEDCHECK v1.1 (O'Connell & Weeks 1998) on the modified pedigrees, 2899 SNPs with two or more inconsistencies with Mendelian inheritance were identified and removed. Ethnic stratification was assessed with EIGENSTRAT (Price *et al*. 2006) using the 100 000 SNPs and the HapMap Caucasian reference samples, and no individual was excluded based on the EIGENSTRAT v3.0 results.

Statistical genetic analyses
Genome-wide association tests with the frontal theta ERO phenotype to target stimuli were performed on 1560 samples from 117 of the 118 families genotyped; one of the 118 families was excluded because there were no theta ERO measurements available. Association testing was carried out assuming an additive model using the generalized disequilibrium test. Phenotype data was derived from multivariate linear regression models which were constructed from log-transformed theta power recorded at Fz, controlling for log-transformed age and stratified by gender and the residual values were fit to a standard normal distribution to create z-scores. Secondary analysis examined the topographic distribution of association signals in the *KCNJ6* region using total theta power at Cz and Pz scalp locations.

Imputation of SNPs in the *KCNJ6* region was performed using the program BEAGLE version 3.3.1 (Browning & Browning 2009) (http://www.sph.umich.edu/csg/abecasis/MaCH/). We used reference data from the European population in the August 2010 release of the 1000 Genomes Project provided with the Beagle release for our European American sample. SNPs with a final r^2, the estimated squared correlation between the estimated allele dosage and the true allele dosage, >0.30 were used. For individual-level genotype data, we retained genotypes having a probability $\geq 80\%$ (from the gprob metric in Beagle); otherwise that genotype was set to missing. To account for uncertainty, we used the mean of the distribution of imputed genotypes, which corresponds to an expected allelic or genotypic count (dosage) for each individual. Subsequently, association tests of the Fz theta ERO phenotype were performed.

Results

Event-related electrophysiological data for the target stimulus from the visual oddball task yielded measures of total power in the θ (3.0–7.0 Hz) oscillation at the frontal midline channel (Fz). This measure was estimated for 1560 individuals from the signals in the 300–700 milliseconds time window, which corresponded to the time window of the P300 component in the event-related waveforms. The mean power in the theta band for the frontal midline channel was $30.2 \pm 18.7 \, \mu V^2$ (males = $28.2 \, \mu V^2$; females = $32.0 \, \mu V^2$).

The quantile–quantile (QQ) plot of predicted and observed association results is presented in Fig. S3. The genomic inflation factor λ is 1.00, indicating that there is no bias of the test statistic. Figure 1 displays the *P*-values of association tests for the θ Fz phenotype in a Manhattan plot. The most significant results were located on chromosome 21, with seven SNPs reaching genome-wide significant *P*-values (see Table 1 for SNPs with $P < 1 \times 10^{-5}$). The SNP with the
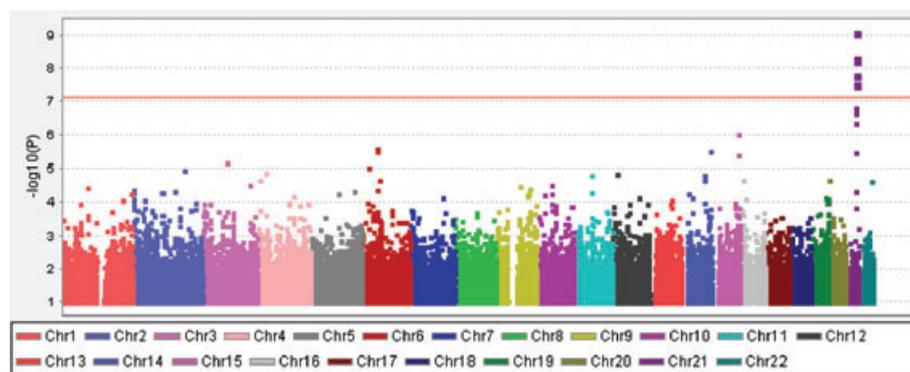
Figure 1: Manhattan plot of genome-wide association results for theta ERO at Fz. Negative log-transformed *P*-values for SNPs are plotted against position on each chromosome. One genomic region on chromosome 21 contains SNPs that exceed the genome-wide significance threshold of 7.88×10^{-8} (indicated by red line).

Table 1: Top SNPs with $P < 1 \times 10^{-5}$ associated with theta power at Fz in family GWAS

Chromosome	Gene	Function	SNP	bp	A1*	A2	Freq.[†]	Effect[‡]	P
21	*KCNJ6*	Intron	rs2835872	39 027 272	G	A	0.681	−0.145	4.70×10^{-10}
21	*KCNJ6*	Intron	rs702860	39 008 629	A	G	0.699	−0.139	3.10×10^{-9}
21	*KCNJ6*	Intron	rs2835850	39 012 977	T	C	0.700	−0.139	3.50×10^{-9}
21	*KCNJ6*	Intron	rs857975	39 001 613	C	A	0.699	−0.135	9.20×10^{-9}
21	*KCNJ6*	Intron	rs857978	38 998 126	C	T	0.678	−0.129	1.90×10^{-8}
21			rs2835831	38 987 233	T	C	0.684	−0.129	2.00×10^{-8}
21			rs2835837	38 990 443	G	A	0.681	−0.129	2.10×10^{-8}
21	*KCNJ6*	Intron	rs2154553	39 004 501	A	G	0.696	−0.130	1.00×10^{-7}
21	*KCNJ6*	Intron	rs12482570	39 077 777	A	G	0.671	−0.123	1.10×10^{-7}
21	*KCNJ6*	Intron	rs2835893	39 060 893	G	A	0.672	−0.126	1.50×10^{-7}
21	*KCNJ6*	Intron	rs1787422	39 076 961	T	C	0.610	−0.115	1.90×10^{-7}
21			rs2835833	38 987 897	G	A	0.683	−0.123	2.90×10^{-7}
14			rs2766692	100 684 192	G	A	0.691	−0.110	2.10×10^{-6}
22	*PRR5-ARHGAP8*	Intron	rs16992796	45 183 014	G	A	0.050	−0.236	3.00×10^{-6}
15			rs7181753	96 844 727	T	C	0.199	0.128	3.00×10^{-6}
3			rs9860340	87 783 976	A	G	0.749	−0.122	3.60×10^{-6}
21	*KCNJ6*	Intron	rs858008	39 065 630	C	T	0.512	0.099	4.30×10^{-6}
21	*KCNJ6*	Intron	rs2835886	39 040 342	C	T	0.586	0.101	4.60×10^{-6}
6			rs9395865	53 307 694	T	C	0.279	0.106	6.10×10^{-6}
11	*C11orf84*	Intron	rs10897449	63 592 621	T	C	0.453	−0.099	7.20×10^{-6}
6	*FAM65B*	Intron	rs4256430	24 863 075	A	G	0.465	−0.099	8.40×10^{-6}
6			rs4712029	53 317 012	G	A	0.238	0.114	8.40×10^{-6}

*A1 is the reference allele.
[†]Freq. is the allele frequency of the reference allele A1.
[‡]Effect is the effect of the reference allele A1 on the phenotype theta power recorded at Fz.

lowest P-value (4.70×10^{-10}) was rs2835872 in *KCNJ6* on chromosome 21. The G allele of rs2835872 was associated with lower theta power at Fz. This marker, along with 10 other SNPs listed in Table 1, were located within the introns of *KCNJ6*.

Given the significant findings of *KCNJ6* SNPs, we imputed additional SNPs in this region. Our imputed data show that the top three highly correlated SNPs ($r^2 > 0.99$) in introns of *KCNJ6* are associated with theta Fz at a genome-wide significance level (rs2835880, imputed, $P = 2.80 \times 10^{-10}$; rs2835872, genotyped, $P = 4.70 \times 10^{-10}$; rs10483038, imputed, $P = 6.60 \times 10^{-10}$). A synonymous SNP, rs702859 (imputed) in *KCNJ6* that is highly correlated with the top three SNPs, rs2835880, rs2835872, and rs10483038 ($r^2 > 0.8$) is associated with theta Fz at P-value 1.60×10^{-8} (Table S1). Figure 2 displays *KCNJ6* in the chromosome 21q22 association with theta Fz for both genotyped and imputed SNPs.

To evaluate the topographic specificity of the significant findings of *KCNJ6* SNPs with the theta ERO phenotype at Fz, we investigated the association of SNPs in *KCNJ6* with theta ERO power at the central midline (Cz) and parietal midline (Pz) channels. The association analysis results for Cz and Pz for *KCNJ6* SNPs did not reach genome-wide significance levels, nor yield as many P-values $<1 \times 10^{-4}$ as Fz. There are 15 SNPs with P-values $<1 \times 10^{-4}$ for Fz while there is only one for Cz and none for Pz. The most significant SNP for Fz, rs2835872, was also most significant for Cz (6.00×10^{-5}). The SNP with the lowest P-value for Pz, rs2835850 ($P = 1.10 \times 10^{-4}$), reached genome-wide significance for Fz.

This suggests that the theta oscillations in all three regions shared a genetic determinant in *KCNJ6*, and that the frontal channel shows the strongest signal.

In addition to *KCNJ6*, some evidence of association ($P < 1.0 \times 10^{-5}$) was found with SNPs in or near other genes (Table 1). These other findings were in *PRR5-ARHGAP8* on chromosome 22, *C11orf84* on chromosome 11, and *FAM65B* on chromosome 6.

Discussion

The results from this family GWAS have provided the first genome-wide significant association of theta oscillations with three SNPs in *KCNJ6*. These SNPs were associated with theta oscillations across the scalp; however, the strongest associations were frontal (Fz). One imputed synonymous SNP in exon four, highly correlated with our top three SNPs, was significantly associated with the frontal theta ERO phenotype. These results suggest a role for *KCNJ6* or its product GIRK2 in variations of frontal theta band oscillations.

The protein encoded by *KCNJ6* is known as GIRK2, GIRK2a and KIR 3.2, and is part of a superfamily of inward rectifier channels. GIRK2 is widely distributed in the brain and is an important functional element in dopaminergic, cholinergic, GABAergic and glutamatergic synapses (Saenz del Burgo *et al.* 2008). Studies have suggested that three different splice variants of GIRK2 (GIRK2a, GIRK2b and GIRK2c) channels may exist in neurons (Isomoto *et al.* 1997). Of the four GIRK channels (GIRK1–4) expressed in mammals,
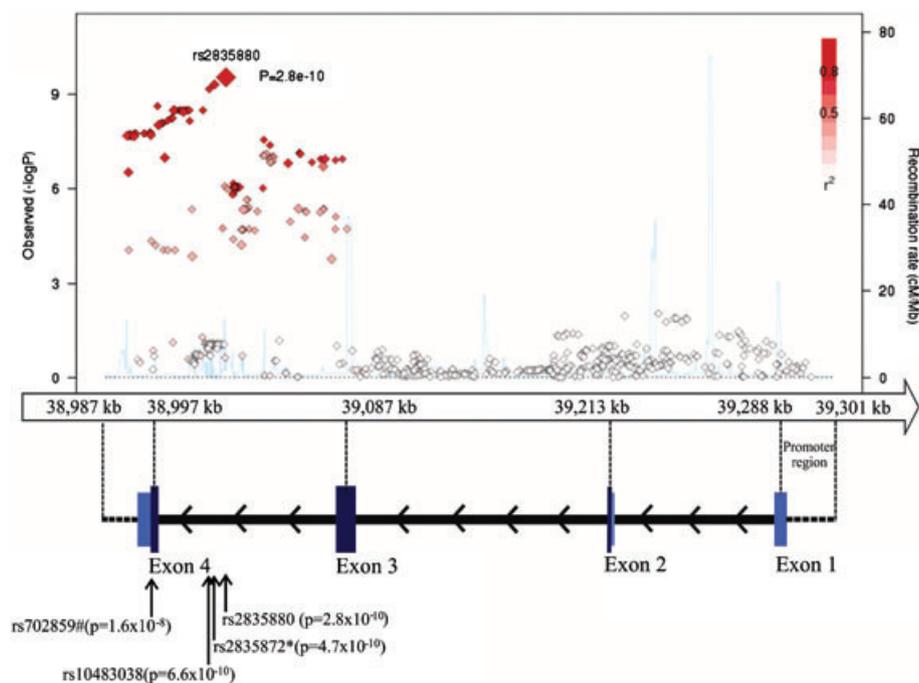
GIRK1–3 are abundantly expressed in the brain (Wickman *et al*. 2000). Of these, only GIRK2 is capable of forming both heterotetrameric (with GIRK1/3) and homotetrameric functional channels (Liao *et al*. 1996). By virtue of its channel properties, GIRK2 contributes to the slow inhibitory postsynaptic potentials as a result of $GABA_B$ action (Luscher *et al*. 1997; Nicoll 2004). Activity of GIRK receptors results in hyperpolarization that decreases neuronal excitability and this in turn directly influences activity levels in neurons. There are several lines of evidence that highlight the role of inhibition in tuning responses and pacing oscillations and establishing synchrony during cognitive processing in the brain (Isaacson & Scanziani 2011;review). A simulation study examining decision time and theta rhythm suggests that a mixture of slow and fast inhibition can affect the power in the theta band and speed up the reaction times in a decision-making network (Smerieri *et al*. 2010).

Very few studies have examined electrophysiology and genetics in humans. Small effects from several genes associated with neurotransmitters may contribute to the variation in P300 and theta and delta oscillations during cognitive tasks. The neurochemical basis of the target stimulus response and P300 component has been suggested to be triggered by glutamatergic activity and modulated by influences from both cholinergic and GABAergic sources (Frodl-Bauch *et al*. 1999; Kenemans & Kahkonen 2011). Previous studies conducted in COGA examining the genetics of EROs have employed whole genome linkage followed by candidate gene association methods on family and case control data. Based on the regions identified in whole genome linkage scans with theta EROs, we identified candidate genes, namely, *GRM8* (metabotropic glutamate receptor) and *CHRM2* (muscarinic cholinergic receptor), and subsequently reported that SNPs in both candidate genes

are associated with theta EROs to targets (Chen *et al*. 2009; Jones *et al*. 2006a; Jones *et al*. 2004). In this study, we also found several SNPs in both *CHRM2* and *GRM8* genes were significantly associated with frontal theta EROs; however, not quite at the level of genome-wide significance (Table S2). Another study endorsing a role for the thalamus in cognitively relevant brain rhythms has suggested both metabotropic glutamate receptor and muscarinic cholinergic receptor activation can cause rhythmic bursting at alpha/theta frequencies (Hughes *et al*. 2008). Since the GIRK2 is an important functional element in dopaminergic, cholinergic, GABAergic and glutamatergic synapses (Saenz del Burgo *et al*. 2008), a functional involvement of the GIRK2 channels in the oscillatory dynamics of theta band can be speculated.

The activation of GIRK channels can influence neuronal networks at different levels in the brain via different mechanisms: (1) neuronal self-inhibition – where the transmitter released from the dendrites activates the GIRK channels on the same cell (Bacci *et al*. 2004), (2) neuron-to-neuron inhibition – a result of activation of post synaptic receptors such as $GABA_B$ (Newberry & Nicoll 1985), D2 (Beckstead & Williams 2007) and group II metabotropic glutamate receptors (Dutar *et al*. 1999) by the relevant transmitters released from presynaptic neurons and (3) network level inhibition – a result of ambient levels of neuromodulators like adenosine and somatostatin, which are endogenous G protein coupled receptor agonists. Somatostatin may alter the oscillatory behavior of thalamic networks through postsynaptic activation of GIRK channels together with presynaptic inhibition (Sun *et al*. 2002); in addition, endogenous adenosine may suppress gamma oscillations in the hippocampus, which also activate GIRK channels (Pietersen *et al*. 2009).

Animal models have shown that GIRK channels are important effectors in both opioid- and ethanol-induced

analgesia (Ikeda *et al*. 2002), as well as being directly activated by ethanol with the involvement of a discrete alcohol pocket (Aryal *et al*. 2009). Another study on rat midbrain dopaminergic neurons suggests that the action of ethanol occurs on activated GIRK channels downstream of the GABA$_B$ receptors (Federici *et al*. 2009). The authors suggest that the enhancing effects of ethanol on GABA$_B$ responses could modulate alcohol intake and the mental and motor performance in an acute intoxicative phase. These receptors are the focus for developing new analgesics (Lotsch & Geisslinger 2011) or therapeutic compounds that could mitigate the effects of alcohol. Hence, this may also be a pathway by which alcohol can modulate oscillatory brain activity.

A candidate gene study looking for genetic variants of risk for nicotine dependence identified a marker in *KCNJ6* gene as one of their top signals (Saccone *et al*. 2007). Recently, another study examined a few SNPs in the promoter region of *KCNJ6* and found some association with these SNPs for alcohol dependence in adults and for hazardous drinking behavior in adolescents who were exposed to early life stress (Clarke *et al*. 2011). Another recent study has suggested epistatic interaction of *KCNJ6* with CREB1 (cyclic adenosine 5′-phosphate (adenosine monophosphate)-response element binding protein) may influence rumination, which is a core cognitive feature of depression (Lazary *et al*. 2011).

A polymorphism in this gene has been associated with opioid effects on analgesia and methadone replacement dose (Lotsch *et al*. 2010). A study examining dopamine-dependent phenotypes in GIRK2 knockout mice noted the presence of dopamine-dependent hyperactivity and enhanced responses to drugs that stimulate dopamine neurotransmission (Arora *et al*. 2010). However, these phenotypes were not solely attributable to the loss of GIRK (1/2) signaling in dopamine neurons, but could be due to adaptations in the mesolimbic dopamine system that facilitated excitatory glutamatergic neurotransmission. Therefore the authors suggest that drugs of abuse may evoke adaptations to promote chronic use through a regulation of GIRK signaling strength in dopaminergic neurons or their input neurons.

In conclusion, the results of the present study suggesting a role for the *KCNJ6* gene in variations of frontal theta band oscillations are very compelling. Although all the neurotransmitter interactions and the functional role of GIRK2 channels on brain electrophysiology and behavior is yet to be completely defined, there is robust evidence (as reviewed above) for an important role of this channel in regulating the excitability of neuronal networks. Our findings underscore the potential for identifying meaningful genetic correlates of brain oscillations associated with pathophysiology of neuropsychiatric conditions.

Despite these strong findings, there are some limitations and caveats to bear in mind. As this method is designed for common variants, studies are planned to examine rare variants (through sequencing). Furthermore, increasing the sample size and meta-analysis with other data sets will improve power to identify associations. Future studies using an independent data sample are needed to confirm this association and examine the functional importance of these associations.

References

Arora, D., Haluk, D.M., Kourrich, S., Pravetoni, M., Fernandez-Alacid, L., Nicolau, J.C., Lujan, R. & Wickman, K. (2010) Altered neurotransmission in the mesolimbic reward system of Girk mice. *J Neurochem* **114**, 1487–1497.

Aryal, P., Dvir, H., Choe, S. & Slesinger, P.A. (2009) A discrete alcohol pocket involved in GIRK channel activation. *Nat Neurosci* **12**, 988–995.

Babiloni, C., Vecchio, F., Lizio, R., Ferri, R., Rodriguez, G., Marzano, N., Frisoni, G.B. & Rossini, P.M. (2011) Resting state cortical rhythms in mild cognitive impairment and Alzheimer's disease: electroencephalographic evidence. *J Alzheimers Dis* **26** (Suppl. 3), 201–214.

Bacci, A., Huguenard, J.R. & Prince, D.A. (2004) Long-lasting self-inhibition of neocortical interneurons mediated by endocannabinoids. *Nature* **431**, 312–316.

Basar, E., Basar-Eroglu, C., Karakas, S. & Schurmann, M. (1999) Are cognitive processes manifested in event-related gamma, alpha, theta and delta oscillations in the EEG? *Neurosci Lett* **259**, 165–168.

Basar, E., Basar-Eroglu, C., Karakas, S. & Schurmann, M. (2001) Gamma, alpha, delta, and theta oscillations govern cognitive processes. *Int J Psychophysiol* **39**, 241–248.

Beckstead, M.J. & Williams, J.T. (2007) Long-term depression of a dopamine IPSC. *J Neurosci* **27**, 2074–2080.

Begleiter, H., Reich, T., Hesselbrock, V.M., Porjesz, B., Li, T.K., Schuckit, M.A., Edenberg, H.J. & Rice, J.P. (1995) The collaborative study on the genetics of alcoholism. *Alcohol Health Res World* **19**, 228–236.

van Beijsterveldt, C.E. & Boomsma, D.I. (1994) Genetics of the human electroencephalogram (EEG) and event-related brain potentials (ERPs): a review. *Hum Genet* **94**, 319–330.

van Beijsterveldt, C.E., Molenaar, P.C., de Geus, E.J. & Boomsma, D.I. (1996) Heritability of human brain functioning as assessed by electroencephalography. *Am J Hum Genet* **58**, 562–573.

Bland, B.H. & Oddie, S.D. (2001) Theta band oscillation and synchrony in the hippocampal formation and associated structures: the case for its role in sensorimotor integration. *Behav Brain Res* **127**, 119–136.

Browning, B.L. & Browning, S.R. (2009) A unified approach to genotype imputation and haplotype-phase inference for large data sets of trios and unrelated individuals. *Am J Hum Genet* **84**, 210–223.

Bucholz, K.K., Cadoret, R., Cloninger, C.R., Dinwiddie, S.H., Hesselbrock, V.M., Nurnberger, J.I. Jr, Reich, T., Schmidt, I. & Schuckit, M.A. (1994) A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. *J Stud Alcohol* **55**, 149–158.

Buzsaki, G. (2010) Neural syntax: cell assemblies, synapsembles, and readers. *Neuron* **68**, 362–385.

Buzsaki, G. & Draguhn, A. (2004) Neuronal oscillations in cortical networks. *Science* **304**, 1926–1929.

Chen, A.C., Tang, Y., Rangaswamy, M., Wang, J.C., Almasy, L., Foroud, T., Edenberg, H.J., Hesselbrock, V., Nurnberger, J. Jr, Kuperman, S., O'Connor, S.J., Schuckit, M.A., Bauer, L.O., Tischfield, J., Rice, J.P., Bierut, L., Goate, A. & Porjesz, B. (2009) Association of single nucleotide polymorphisms in a glutamate receptor gene (GRM8) with theta power of event-related oscillations and alcohol dependence. *Am J Med Genet B Neuropsychiatr Gene* **150B**, 359–368.

Clarke, T.K., Laucht, M., Ridinger, M., Wodarz, N., Rietschel, M., Maier, W., Lathrop, M., Lourdusamy, A., Zimmermann, U.S., Desrivieres, S. & Schumann, G. (2011) KCNJ6 is associated with adult alcohol dependence and involved in gene × early life stress interactions in adolescent alcohol drinking. *Neuropsychopharmacology* **36**, 1142–1148.

Dutar, P., Vu, H.M. & Perkel, D.J. (1999) Pharmacological characterization of an unusual mGluR-evoked neuronal hyperpolarization

mediated by activation of GIRK channels. *Neuropharmacology* **38**, 467–475.

Edenberg, H.J., Koller, D.L., Xuei, X. *et al*. (2010) Genome-wide association study of alcohol dependence implicates a region on chromosome 11. *Alcohol Clin Exp Res* **34**, 840–852.

Federici, M., Nistico, R., Giustizieri, M., Bernardi, G. & Mercuri, N.B. (2009) Ethanol enhances GABAB-mediated inhibitory postsynaptic transmission on rat midbrain dopaminergic neurons by facilitating GIRK currents. *Eur J Neurosci* **29**, 1369–1377.

Folstein, M.F., Folstein, S.E. & McHugh, P.R. (1975) ''Mini-mental state''. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* **12**, 189–198.

Foroud, T., Edenberg, H.J., Goate, A., Rice, J., Flury, L., Koller, D.L., Bierut, L.J., Conneally, P.M., Nurnberger, J.I., Bucholz, K.K., Li, T.K., Hesselbrock, V., Crowe, R., Schuckit, M., Porjesz, B., Begleiter, H. & Reich, T. (2000) Alcoholism susceptibility loci: confirmation studies in a replicate sample and further mapping. *Alcohol Clin Exp Res* **24**, 933–945.

Fries, P. (2005) A mechanism for cognitive dynamics: neuronal communication through neuronal coherence. *Trends Cogn Sci* **9**, 474–480.

Frodl-Bauch, T., Bottlender, R. & Hegerl, U. (1999) Neurochemical substrates and neuroanatomical generators of the event-related P300. *Neuropsychobiology* **40**, 86–94.

Gevins, A., Smith, M.E., Leong, H., McEvoy, L., Whitfield, S., Du, R. & Rush, G. (1998) Monitoring working memory load during computer-based tasks with EEG pattern recognition methods. *Hum Factors* **40**, 79–91.

de Geus, E.J. (2010) From genotype to EEG endophenotype: a route for post-genomic understanding of complex psychiatric disease? *Genome Med* **2**, 63.

Hesselbrock, M., Easton, C., Bucholz, K.K., Schuckit, M. & Hesselbrock, V. (1999) A validity study of the SSAGA – a comparison with the SCAN. *Addiction* **94**, 1361–1370.

Hughes, S.W., Errington, A., Lorincz, M.L., Kekesi, K.A., Juhasz, G., Orban, G., Cope, D.W. & Crunelli, V. (2008) Novel modes of rhythmic burst firing at cognitively-relevant frequencies in thalamocortical neurons. *Brain Res* **1235**, 12–20.

Ikeda, K., Kobayashi, T., Kumanishi, T., Yano, R., Sora, I. & Niki, H. (2002) Molecular mechanisms of analgesia induced by opioids and ethanol: is the GIRK channel one of the keys? *Neurosci Res* **44**, 121–131.

Isaacson, J.S. & Scanziani, M. (2011) How inhibition shapes cortical activity. *Neuron* **72**, 231–243.

Isomoto, S., Kondo, C. & Kurachi, Y. (1997) Inwardly rectifying potassium channels: their molecular heterogeneity and function. *Jpn J Physiol* **47**, 11–39.

Jacobs, J., Hwang, G., Curran, T. & Kahana, M.J. (2006) EEG oscillations and recognition memory: theta correlates of memory retrieval and decision making. *NeuroImage* **32**, 978–987.

Jones, K.A., Porjesz, B., Almasy, L. *et al*. (2004) Linkage and linkage disequilibrium of evoked EEG oscillations with CHRM2 receptor gene polymorphisms: implications for human brain dynamics and cognition. *Int J Psychophysiol* **53**, 75–90.

Jones, K.A., Porjesz, B., Almasy, L., Bierut, L., Goate, A., Hinrichs, A., Rice, J.P., Rohrbaugh, J., Bauer, L.O., Crowe, R.R., Edenberg, H.J., Foroud, T., Hesselbrock, V., Kuperman, S., Nurnberger Jr, J., O'Connor, S.J., Schuckit, M.A., Tischfield, J.A. & Begleiter, H. (2006a) A cholinergic receptor gene (CHRM2) affects event-related oscillations. *Behav Genet* **36**, 627–639.

Jones, K.A., Porjesz, B., Chorlian, D., Rangaswamy, M., Kamarajan, C., Padmanabhapillai, A., Stimus, A. & Begleiter, H. (2006b) S-transform time-frequency analysis of P300 reveals deficits in individuals diagnosed with alcoholism. *Clin Neurophysiol* **117**, 2128–2143.

Kamarajan, C., Porjesz, B., Jones, K., Chorlian, D., Padmanabhapillai, A., Rangaswamy, M., Stimus, A. & Begleiter, H. (2006) Event-related oscillations in offspring of alcoholics: neurocognitive disinhibition as a risk for alcoholism. *Biol Psychiatry* **59**, 625–634.

Kamarajan, C., Rangaswamy, M., Chorlian, D.B., Manz, N., Tang, Y., Pandey, A.K., Roopesh, B.N., Stimus, A.T. & Porjesz, B. (2008) Theta oscillations during the processing of monetary loss and gain: a perspective on gender and impulsivity. *Brain Res* **1235**, 45–62.

Kendler, K.S., Prescott, C.A., Myers, J. & Neale, M.C. (2003) The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. *Arch Gen Psychiatry* **60**, 929–937.

Kenemans, J.L. & Kahkonen, S. (2011) How human electrophysiology informs psychopharmacology: from bottom-up driven processing to top-down control. *Neuropsychopharmacology* **36**, 26–51.

Klimesch, W., Schimke, H. & Schwaiger, J. (1994) Episodic and semantic memory: an analysis in the EEG theta and alpha band. *Electroencephalogr Clin Neurophysiol* **91**, 428–441.

Klimesch, W., Doppelmayr, M., Yonelinas, A., Kroll, N.E., Lazzara, M., Rohm, D. & Gruber, W. (2001) Theta synchronization during episodic retrieval: neural correlates of conscious awareness. *Brain Res Cogn Brain Res* **12**, 33–38.

Klimesch, W., Freunberger, R., Sauseng, P. & Gruber, W. (2008) A short review of slow phase synchronization and memory: evidence for control processes in different memory systems? *Brain Res* **1235**, 31–44.

Lachaux, J.P., Chavez, M. & Lutz, A. (2003) A simple measure of correlation across time, frequency and space between continuous brain signals. *J Neurosci Methods* **123**, 175–188.

Lahey, B.B., Van Hulle, C.A., Singh, A.L., Waldman, I.D. & Rathouz, P.J. (2011) Higher-order genetic and environmental structure of prevalent forms of child and adolescent psychopathology. *Arch Gen Psychiatry* **68**, 181–189.

Lazary, J., Juhasz, G., Anderson, I.M., Jacob, C.P., Nguyen, T.T., Lesch, K.P., Reif, A., Deakin, J.F. & Bagdy, G. (2011) Epistatic interaction of CREB1 and KCNJ6 on rumination and negative emotionality. *Eur Neuropsychopharmacol* **21**, 63–70.

Liao, Y.J., Jan, Y.N. & Jan, L.Y. (1996) Heteromultimerization of G-protein-gated inwardly rectifying K$^+$ channel proteins GIRK1 and GIRK2 and their altered expression in weaver brain. *J Neurosci* **16**, 7137–7150.

Lotsch, J. & Geisslinger, G. (2011) Pharmacogenetics of new analgesics. *Br J Pharmacol* **163**, 447–460.

Lotsch, J., Pruss, H., Veh, R.W. & Doehring, A. (2010) A KCNJ6 (Kir3.2, GIRK2) gene polymorphism modulates opioid effects on analgesia and addiction but not on pupil size. *Pharmacogenet Genomics* **20**, 291–297.

Luscher, C., Jan, L.Y., Stoffel, M., Malenka, R.C. & Nicoll, R.A. (1997) G protein-coupled inwardly rectifying K$^+$ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron* **19**, 687–695.

Newberry, N.R. & Nicoll, R.A. (1985) Comparison of the action of baclofen with gamma-aminobutyric acid on rat hippocampal pyramidal cells in vitro. *J Physiol* **360**, 161–185.

Nicoll, R.A. (2004) My close encounter with GABA(B) receptors. *Biochem Pharmacol* **68**, 1667–1674.

Nurnberger, J.I. Jr, Wiegand, R., Bucholz, K., O'Connor, S., Meyer, E.T., Reich, T., Rice, J., Schuckit, M., King, L., Petti, T., Bierut, L., Hinrichs, A.L., Kuperman, S., Hesselbrock, V. & Porjesz, B. (2004) A family study of alcohol dependence: coaggregation of multiple disorders in relatives of alcohol-dependent probands. *Arch Gen Psychiatry* **61**, 1246–1256.

O'Connell, J.R. & Weeks, D.E. (1998) PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* **63**, 259–266.

O'Keefe, J. & Recce, M.L. (1993) Phase relationship between hippocampal place units and the EEG theta rhythm. *Hippocampus* **3**, 317–330.

Pietersen, A.N., Lancaster, D.M., Patel, N., Hamilton, J.B. & Vreugdenhil, M. (2009) Modulation of gamma oscillations by endogenous adenosine through A1 and A2A receptors in the mouse hippocampus. *Neuropharmacology* **56**, 481–492.

Porjesz, B. & Rangaswamy, M. (2007) Neurophysiological endophenotypes, CNS disinhibition, and risk for alcohol dependence and related disorders. *Scientific World J* **7**, 131–141.

Porjesz, B., Rangaswamy, M., Kamarajan, C., Jones, K.A., Padmanabhapillai, A. & Begleiter, H. (2005) The utility of neurophysiological markers in the study of alcoholism. *Clin Neurophysiol* **116**, 993–1018.

Price, A.L., Patterson, N.J., Plenge, R.M., Weinblatt, M.E., Shadick, N.A. & Reich, D. (2006) Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* **38**, 904–909.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J. & Sham, P.C. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81**, 559–575.

Rangaswamy, M., Jones, K.A., Porjesz, B., Chorlian, D.B., Padmanabhapillai, A., Kamarajan, C., Kuperman, S., Rohrbaugh, J., O'Connor, S.J., Bauer, L.O., Schuckit, M.A. & Begleiter, H. (2007) Delta and theta oscillations as risk markers in adolescent offspring of alcoholics. *Int J Psychophysiol* **63**, 3–15.

Rangaswamy, M. & Porjesz, B. (2008) Uncovering genes for cognitive (dys)function and predisposition for alcoholism spectrum disorders: a review of human brain oscillations as effective endophenotypes. *Brain Res* **1235**, 153–171.

Reich, T., Edenberg, H.J., Goate, A. *et al*. (1998) Genome-wide search for genes affecting the risk for alcohol dependence. *Am J Med Genet* **81**, 207–215.

Rothenberger, A. (2009) Brain oscillations forever – neurophysiology in future research of child psychiatric problems. *J Child Psycho Psychiatry* **50**, 79–86.

Saccone, S.F., Hinrichs, A.L., Saccone, N.L. *et al*. (2007) Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. *Hum Mol Genet* **16**, 36–49.

Saenz del Burgo, L., Cortes, R., Mengod, G., Zarate, J., Echevarria, E. & Salles, J. (2008) Distribution and neurochemical characterization of neurons expressing GIRK channels in the rat brain. *J Comp Neurol* **510**, 581–606.

Schurmann, M., Basar-Eroglu, C., Kolev, V. & Basar, E. (2001) Delta responses and cognitive processing: single-trial evaluations of human visual P300. *Int J Psychophysiol* **39**, 229–239.

Smerieri, A., Rolls, E.T. & Feng, J. (2010) Decision time, slow inhibition, and theta rhythm. *J Neurosci* **30**, 14173–14181.

Stockwell, R.G. (2007) Why use the S-transform? *Fields Inst Commun* **52**, 279–309; 414.

Stockwell, R.G., Mansinha, L. & Lowe, R.P. (1996) Localization of the complex spectrum: The S transform. *IEEE Trans Signal Process* **44**, 998–1001.

Sun, Q.Q., Huguenard, J.R. & Prince, D.A. (2002) Somatostatin inhibits thalamic network oscillations in vitro: actions on the GABAergic neurons of the reticular nucleus. *J Neurosci* **22**, 5374–5386.

Turner, S., Armstrong, L.L., Bradford, Y. *et al*. (2011) Quality Control Procedures for Genome-Wide Association Studies. *Curr Protoc Hum Genet* **68**, 1.19.1–1.19.18.

Vertes, R.P. (2005) Hippocampal theta rhythm: a tag for short-term memory. *Hippocampus* **15**, 923–935.

Vinogradova, O.S. (1995) Expression, control, and probable functional significance of the neuronal theta-rhythm. *Prog Neurobiol* **45**, 523–583.

Wickman, K., Karschin, C., Karschin, A., Picciotto, M.R. & Clapham, D.E. (2000) Brain localization and behavioral impact of the G-protein-gated K+ channel subunit GIRK4. *J Neurosci* **20**, 5608–5615.

Zlojutro, M., Manz, N., Rangaswamy, M., Xuei, X., Flury-Wetherill, L., Koller, D., Bierut, L.J., Goate, A., Hesselbrock, V., Kuperman, S., Nurnberger, J. Jr, Rice, J.P., Schuckit, M.A., Foroud, T., Edenberg, H.J., Porjesz, B. & Almasy, L. (2011) Genome-wide association study of theta band event-related oscillations identifies serotonin receptor gene HTR7 influencing risk of alcohol dependence. *Am J Med Genet B Neuropsychiatr Genet* **156B**, 44–58.

Acknowledgments

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1: Distribution of family sizes in the GWAS sample.

Figure S2: 10–20 International system electrode montage used to record ERO. Frontal midline location (Fz in red) was used for primary analysis; Central (Cz) and parietal (Pz) locations in blue - used in secondary analysis.

Figure S3: QQ plot of GWAS results for theta power at Fz. The genomic control inflation factor λ is 1.00.

Table S1: Imputed SNPs in *KCNJ6* with $P < 1 \times 10^{-5}$ associated with theta power at Fz.

Table S2: SNPs in *CHRM2* and *GRM8* with $p < 0.05$ associated with theta power at Fz.

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