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Neurophysiologic Effect of GWAS Derived Schizophrenia and Bipolar Risk Variants

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Abstract

Genome-wide association studies (GWAS) have identified multiple single nucleotide polymorphisms (SNPs) as disease associated variants for schizophrenia (SCZ), bipolar disorder (BPD), or both. Although these results are statistically robust, the functional effects of these variants and their role in the pathophysiology of SCZ or BPD remain unclear. Dissecting the effects of risk genes on distinct domains of brain function can provide important biological insights into the mechanisms by which these genes may confer illness risk. This study used quantitative event related potentials to characterize the neurophysiological effects of well-documented GWAS-derived SCZ/BPD susceptibility variants in order to map gene effects onto important domains of brain function. We genotyped 199 patients with DSM-IV diagnoses of SCZ or BPD and 74 healthy control subjects for 19 risk SNPs derived from previous GWAS findings and tested their association with five neurophysiologic traits (P3 amplitude, P3 latency, N1 amplitude, P2 amplitude, and P50 sensory gating responses) known to be abnormal in psychosis. The *TCF4* SNP rs17512836 risk allele showed a significant association with reduced auditory P3 amplitude ($P = 0.00016$) after correction for multiple testing. The same allele was also associated with delayed P3 latency ($P = 0.005$). Our results suggest that a SCZ risk variant in *TCF4* is associated with neurophysiologic traits thought to index attention and working memory abnormalities in psychotic disorders. These findings suggest a mechanism by which *TCF4* may contribute to the neurobiological basis of psychotic illness.

Keywords

genetics; neurophysiology; ERP; TCF4; psychosis

INTRODUCTION

Schizophrenia (SCZ) and bipolar disorder (BPD) are serious mental disorders that are responsible for substantial personal, familial, economic and societal burdens. Genetic factors are known to be the strongest risk factors for these disorders, but their precise architecture remains poorly understood. The results of genome-wide association studies (GWAS) have begun to have a major impact on our understanding of the genetics of psychiatric diseases. Using this strategy, compelling results have emerged for BPD and SCZ [WTCCC, 2007; Stefansson et al., 2009; Sklar et al., 2011], with the identification of statistically robust and replicable associations with specific genetic variants. To date, more than a dozen single nucleotide polymorphisms (SNPs) have been identified by GWAS analyses involving tens of thousands of samples as disease risk variants for SCZ and/or for BPD [Stefansson et al., 2009; Ripke et al., 2011; Steinberg et al., 2011]. Growing evidence also suggests that the susceptibility to psychiatric disorders conferred by genes may transcend DSM categories, with particularly strong shared genetic risk factors for the two major psychotic disorders, SCZ and BPD [Purcell et al., 2009; Dragt et al., 2012; Hall et al., 2012].

Establishing a genetic association with a disorder is only the beginning of understanding the genotype and phenotype relationships. Available tools to measure brain structure and/or function, such as EEG or neuroimaging, provide powerful opportunities to characterize neurophysiologic effects of SCZ and BPD associated susceptibility variants [Hall and Smoller, 2010]. For example, investigators have used structural MRI techniques to characterize the effect of psychosis risk variant rs1347706 in *ZNF804a*, with results supporting an effect of the SNP in shaping brain structure [Lencz et al., 2010; Donohoe et al., 2011; Meijer et al., 2011]. Others have used functional MRI techniques to investigate BPD risk variant rs1006737 in *CACNA1C*, with results supporting an effect of the SNP in altering brain activation and circuitry [Bigos et al., 2010; Erk et al., 2010; Tesli et al., 2013]. Decoster and colleagues have used event related potential (ERP) to examine association between P300 and a range of candidate SCZ and BPD variants, selected from either candidate gene studies or GWAS. They found that P300 amplitude was associated with candidate SNP rs1625579 in *ABCB1* [Decoster et al., 2012].

Psychosis is a core feature of SCZ and is common in BPD [Coryell et al., 2001]. Schizoaffective disorder, which has prominent symptoms of both psychosis and mood disorder, occurs at similarly increased rates in families of probands with SCZ and BPD [Rice et al., 1987; Kendler et al., 1998]. These findings, together with genetic evidence, have led investigators to propose that SCZ and BPD share genes predisposing individuals to psychosis in general [Craddock et al., 2005, 2009]. This notion is supported by recent results from the Bipolar and Schizophrenia Network on Intermediate Phenotypes (B-SNIP) consortium [Ivleva et al., 2012; Hill et al., 2013; Tamminga et al., 2013]. Impaired P50 sensory-gating responses, reduced amplitude in auditory N100, P200, and P300 ERP, and delayed latency in P300 ERP are robust findings in patients with SCZ and BPD and in their unaffected biological relatives [Bramon et al., 2004; Hall et al., 2006b, 2009; Turetsky et al., 2007; Salisbury et al., 2010] and have been suggested as likely endophenotypes [Braff et al., 2007a]. These ERPs components appear to be uncorrelated with one another, suggesting each may be mediated by distinct mechanisms [Hall et al., 2006a]. The underlying mechanisms and brain circuits subserving each ERP activity have been extensively studied using drug challenge investigations and animal models [Turetsky et al., 2007; Braff et al., 2007a; Javitt et al., 2008]. Therefore, follow-up association analyses using these neurophysiological endophenotypes offer a powerful strategy to discover how risk variants impact brain functions, which may, in turn, implicate biologically plausible mechanisms linking these genes to illness [Rangaswamy and Porjesz, 2008].

This study applies ERP measures to characterize genetic loci recently associated with SCZ and BPD in GWAS reports. We focus on ERP brain neurophysiology previously shown to be heritable and associated with disease liability. Importantly our approach is not to use neurophysiological endophenotypes for risk allele *discovery*, but rather to *characterize* the functional impact of risk alleles previously established with clinical disorders by GWAS. To accomplish this and to minimize multiple testing, we take a highly focused approach by examining specific risk variants that achieved the strongest statistical evidence in the largest GWAS study published at the time we initiated this study [Ripke et al., 2011; Sklar et al., 2011]. The goal was to examine whether GWAS-validated risk variants are associated with single or multiple neurophysiologic properties that are robustly linked to psychotic disorders.

METHODS

Sample

The sample consisted of 199 patients with psychotic illness and 74 healthy controls. Cases were clinically stable patients with a DSM-IV diagnosis of SCZ (n =48), schizoaffective disorder, depressed type (n =22), schizoaffective disorder, bipolar type (n =36), or BPD type I (n =93). Based on their clinical features, patients with a diagnosis of schizoaffective depressed type were included in the SCZ group (n =70) and those with a diagnosis of schizoaffective bipolar type were included in the BPD group (n =129). All but three BPD patients had a history of psychosis. Inclusion criteria were age between 18 and 65, no substance abuse (excluding nicotine) in the preceding 6 months or dependence in the preceding 12 months, no prior head injury with loss of consciousness, no history of seizures or ECT treatment in the preceding 12 months, and no hearing impairment as confirmed by audiometry (> 30 dB sound pressure level between 750 Hz and 2,000 Hz). Diagnoses were made by trained diagnosticians (with inter-rater reliability estimates of 0.95) using the SCID for DSM-IV and all available hospital records. Additional sample characteristics ascertained at the time of the neurophysiological assessment included family history; medications and dosages; and smoking history. Fifty four of the 70 SCZ patients were taking antipsychotic medication (mean CPZ equivalent =632.8 mg [SD =521]) with or without adjunctive antidepressants or mood stabilizers. Three SCZ patients were not taking any psychotropic medication. Thirteen SCZ patients were taking antidepressants or mood stabilizers only. Ninety-four of the 129 BPD patients were taking antipsychotic medication (mean CPZ equivalent =481.2 mg [SD =489]) in combination with mood stabilizers and/or antidepressants. Twenty-four BPD patients were taking mood stabilizers only. Eleven BPD patients were not taking any psychotropic medications. Table I presents demographic and clinical information about the sample.

The healthy control (HC) sample was recruited through local advertisements. Control participants were assessed using the SCID and were included only if they met the following criteria: age between 18 and 65, no current or past history of psychotic disorder, BPD, or a SCZ spectrum disorder, no affective disorder in the preceding 12 months, no substance abuse in the preceding 12 months or previous chronic dependence, no known neurological disorder, no prior head injury with loss of consciousness, and no first-degree relative with a history of psychosis or BPD.

All participants in the study had normal intellectual ability based on the North American Adult Reading Test (NAART) or years of education (high school level or higher). Recruitment was restricted to individuals of self-reported European ancestry. This study was approved by the McLean Hospital Institutional Review Board. After a complete description of the study, written informed consent was obtained from each subject.

SCZ and BPD patients did not differ in mean age or mean age of illness onset; SCZ patients showed a trend toward higher mean CPZ equivalent daily dose compared with BPD patients. Control subjects were significantly younger than patients (see Table I). The control and BPD groups comprised a significantly larger proportion of females and had significantly more years of education than the SCZ group. A significantly larger proportion of SCZ (46%, $P < 0.001$) and BPD patients (29%, $P = 0.003$) were smokers compared with controls (10%). Among smokers, patient groups did not differ from each other, but smoked significantly more cigarettes per day than HCs ($P < 0.01$).

Electrophysiological Recording and Analysis

For 251 of the subjects, EEG was recorded using the BioSemi Active Two system. The EEG was acquired in a continuous mode at a digitization rate of 512 Hz, with a bandpass of DC–104 Hz and a Common Mode Sense (CMS) as the reference (PO2 site), and stored for later analysis. Blinks and eye movements were monitored through electrodes placed on the left and right temples and above and below the left eye. For the other 25 individuals (5 HCs, 10 SCZ, 10 BPD) EEG was recorded using Neuroscan Synamp amplifiers (0.01–100 Hz, 500 Hz digitization rate) with the nose tip and referenced to the left mastoid. The forehead (AFz) served as ground. Bipolar vertical and horizontal electrooculograms were recorded from electrodes above and below the right eye (VEOG) and at the left and right outer canthi (HEOG). Electrode impedances were below 5 k Ω . EEGs were acquired in a continuous mode and stored for later analysis. The EEG data were processed offline using the Brain Vision Analyzer package (Brain Products, Gilching, Germany) and re-referenced offline to the averaged mastoid. To avoid acute nicotine effects on brain EEG, subjects were not allowed to smoke for a minimum of 40 min prior to the recording [Adler et al., 1992]. All participants completed the following tasks (in a fixed order): an auditory dual-click paradigm for eliciting P50 sensory gating responses and an auditory “odd-ball” paradigm for eliciting the N100, P200, and P300 ERP components.

Dual-Click paradigm—160 pairs of identical click stimuli (5-ms duration; 2-ms rise/fall; 500-ms inter-click interval; 10-s inter-trial interval) were presented in 4 blocks (40 pairs per block). Stimulus intensity was adjusted to 50 dB above each individual’s hearing threshold, producing a stimulus at a sound level of 80 dB. Signal processing was performed off-line using NEUROSCAN software (4.3). P50 ERPs were processed using the same procedures described previously [Hall et al., 2006b, 2011]. EEG signals were segmented into epochs (–100 to 400 ms), filtered (1-Hz high-pass filter), and corrected for baseline values using the 100-ms pre-stimulus interval. Epochs with activity exceeding 50 μ V in the Cz, Fz, or electro-oculography channel between 0 and 75 ms post-stimulus were automatically rejected. Epochs were averaged separately for the S1 and S2 waveforms, digitally filtered (10-Hz high-pass filter), and smoothed (by using a 7-point moving average applied twice). P50 ERPs are reported at the Cz site. For the S1 response, the most prominent peak 40–80 ms post-stimulus was selected as the P50 peak. The preceding negative trough was used to calculate the amplitude. For the S2 response, the positive peak with a latency closest to that of the S1 P50 peak was selected, and its amplitude was determined as for the S1 wave. P50 sensory gating was calculated as a ratio $(S2/S1) \times 100$. A higher ratio reflects less gating.

Auditory Oddball paradigm—400 binaural tones (80 dB; 50-msec duration, 5 ms rise/fall times); 15% target tones (1500 Hz) and 85% standard tones (1,000 Hz) were presented. Participants were instructed to count target tones silently. All participants had >90% accuracy. Signal processing was performed off-line using Brain Vision Analyzer software (Brain Products, Inc., 2000). For P300 ERP components, the EEG data were segmented into epochs (–100 to 1,000 ms) relative to stimulus onset, zero phase-shift digital low-pass filtered at 8.5 Hz (24 dB/Oct) and baseline corrected using the 100-ms pre-stimulus interval.

Eye-blink artifacts were corrected by using the default method provided by the software [Gratton et al., 1983]. Epochs containing artifact $>100 \mu\text{V}$ at the Fz, Cz, or Pz site were removed. Separate average waves for target and standard tones were calculated. P3 amplitude and latency components were automatically detected from the average wave for target tones at the Pz site between 280 and 650 ms [Salisbury et al., 1999; Hall et al., 2009].

For the N100 and P200 ERP components, EEG signals were digitally low-pass filtered at 20 Hz (24 dB/Oct). Eye-blink artifacts were corrected by using the default method provided by the software [Gratton et al., 1983]. The EEG data were segmented into epochs from -100 to $1,000$ ms relative to stimulus onset and baseline corrected using the 100-ms pre-stimulus interval. Epochs containing artifact $>100 \mu\text{V}$ at Fz, Cz, or Pz site were removed. Peak N100 amplitude was automatically detected from the average wave for standard tones as the most negative point from 50 to 200 ms at Cz. Peak P200 amplitude was automatically detected as the most positive point from 150 to 300 ms at Cz [Salisbury et al., 2010].

Genotyping of Samples

Genomic DNA from blood was extracted following the Gentra Puregene protocol. DNA quantification was performed using picogreen analysis. Genotyping was performed by the Sequencing, Genotyping, Microarray Laboratory at the Partners Center for Personalized Genetic Medicine using a Sequenom MassArray system and an iPLEX assay design. A total of 63 SNPs were genotyped in each individual. Forty of these were used in a custom AIM [Ancestry Informative Markers] SNP array designed to assess individuals' genetic ancestry. Seventeen SNPs were selected from statistically significant SCZ and/or BPD GWAS association results reported by the Psychiatric Genomics Consortium with P -value $<1.00 \times 10^{-6}$ [Ripke et al., 2011; Sklar et al., 2011]. Two additional SNPs were selected from GWAS of nicotine dependence [Bousman et al., 2012], based on prior evidence that P50 sensory gating ERP is specifically related to nicotine use and nicotinic receptor function [Adler et al., 1993; Quednow et al., 2012]. In addition, four SNPs at the *GLS1* locus were selected based on our prior finding of a significant association with brain measures of the glutamine/glutamate ratio using magnetic resonance spectroscopy [Ongur et al., 2011]. *GLS1* encodes glutaminase, which catalyzes the formation of glutamate from glutamine, and glutamatergic neuro-transmission has consistently been observed to be altered in psychotic disorders.

Genotyping was performed in multiplex reactions in 96-well plates with a total of three assays. For each assay, five duplicate samples were included for quality control purposes (concordance rate = 100%). All individuals included in the analyses had sample pass-rate of $>94\%$. Affected and unaffected individuals were spread across genotyping plates to avoid bias due to plate-specific genotyping error. SNPs were included for association analyses only if they met the following quality control criteria: (1) minimum call rate $>90\%$ by assay group, (2) alleles in Hardy–Weinberg equilibrium in controls ($P > 0.03$), and (3) 1% minor allele frequency. After these quality control checks, two SNPs (one from the *GLS1* gene and one from the SCZ GWAS list) were removed, resulting in 21 SNPs to be included in the association analyses (Table II). Of those, 19 SNPs were included in the association analyses with each ERP endophenotype. The two nicotinic receptor SNPs rs16969968 and rs6495308 were included only in the association analyses with the P50 sensory gating measure. The minor allele frequencies observed in this dataset for each SNP were comparable to those observed in the HapMap CEU population. European ancestry was confirmed by performing an MDS analysis on the 40 AIMs SNPs in PLINK [Purcell et al., 2007] and generating MDS plots using the first two components from the analysis.

Statistical Analyses

Behavioral data—Logistic or linear regression analyses were used to compare the groups on demographic characteristics and each ERP phenotype using STATA (STATA version 12; Stata Corp., College Station, TX). Gender, age, and EEG equipment system were included as covariates in the analyses of the ERP variables. A Bonferroni corrected P value ($P < 0.017$, correction for 3 post-hoc comparisons) was used as the threshold for statistical significance.

Genetic analyses—Associations between the investigated SNPs and each of the five quantitative ERP phenotypes were tested using additive linear regression models in PLINK [Purcell et al., 2007]. The primary analyses examined the effects of each SNP genotype on the various ERP measures in the whole sample. Five separate association analyses, one for each ERP phenotype (P50 ratio, P300 amplitude and latency, N100 amplitude, P200 amplitude), were performed. Age, gender and smoking status were included as covariates. A bonferroni-corrected significance level for genotype effects was set at $P < 0.0005$ (19 SNPs by 5 phenotypes). We also performed secondary analyses to explore the specificity of genotype effects by diagnosis. We selected SNPs in the primary analyses with P values < 0.1 to be included in the secondary analyses and ran models that included diagnosis (coded as affected and non-affected) and genotype by diagnosis interaction to check for possible interactions, controlling for age, gender, and smoking status. In addition, we examined the effects of genotype on ERP phenotype in cases and controls separately. The statistical significance threshold for exploratory analyses was set at $P < 0.05$.

RESULTS

Analyses of Neurophysiological Variables

Table III shows summary statistics for each ERP phenotype. Compared with HC, patients with SCZ or BPD showed a highly significant impairment of sensory gating (both $P < 0.0001$), reduced P300 amplitude (both $P < 0.0001$), and reduced P200 amplitude (SCZ: $P = 0.004$, BPD $P < 0.0001$; Table III). Patients with SCZ also showed significantly delayed P300 latency ($P = 0.006$) and reduced N100 amplitude ($P = 0.003$) compared with controls; the p values for BPD patients compared with HC were at the trend level, and BPD patients were consistently intermediate in values between SCZ and HC. SCZ and BPD patients did not differ from one another significantly on any ERP variable. Effect of EEG equipment on each ERP variable was not significant ($P > 0.1$ all). Medication dose in CPZ equivalents was not significantly associated with any ERP variable ($P > 0.09$ all). The dose of Lithium, Lamotrigine, Lorazepam, or Divalproex sodium, the most prescribed mood stabilizers in our sample, were not significantly associated with any ERP variable ($P = 0.06$ all).

Effect of SNP Genotype on Brain Neurophysiology

Association analyses revealed that the SCZ risk allele (G) at the *TCF4* SNP rs17512836 was significantly associated with reduced P300 amplitudes ($P = 0.00017$; Table IV). This association survived correction for multiple testing. This association signal seems to have been driven primarily by the patient group ($P = 0.00083$ vs. $P = 0.4$ in HC), but the genotype by diagnosis interaction was not significant. The risk allele at rs17512836 also exhibited delayed latency ($P = 0.0051$), but this association was not statistically significant after correction for multiple comparisons. Excluding the three non-psychotic BPD patients from analysis did not change these results. Secondary analyses showed that the association signal was present primarily in the patient group ($P = 0.0023$ vs. $P = 0.7$ in HC) but no significant genotype by diagnosis interaction was found.

In the analyses of N100, P200, and sensory gating phenotypes, no statistically significant associations were found in the primary analyses. In the secondary analyses, a significant genotype by diagnosis interaction was found between SNP rs4765913 of the *CACNA1C* gene and the sensory gating phenotype ($P = 0.017$). The minor allele of this SNP (which has previously been associated with BPD and SCZ) was associated with better sensory gating in patients ($P = 0.0012$) but not in controls. Also, a significant genotype by diagnosis interaction was found between SNP rs13000464 of the *GLS* gene and the P300 amplitude ($P = 0.0083$). The risk allele of this SNP was associated with reduced P300 amplitude in patients ($P = 0.012$) but not in controls.

DISCUSSION

In this study, we explored the functional effects of genetic variants identified by SCZ and BPD GWAS studies by examining relationships between quantitative ERP measures and SCZ/BPD risk variants. Sixteen confirmed SCZ and/or BPD associated SNPs and three *GLS* related SNPs were examined for associations with five ERP endophenotypes observed in psychotic disorders (auditory P300 amplitude, P300 latency, N100 amplitude, P200 amplitude, and sensory gating). Two GWAS derived nicotinic dependence SNPs were investigated for specific associations with the sensory gating measure. We found that risk allele of the intronic *TCF4* SNP rs17512836 was significantly associated with reduced auditory P300 amplitude, with an effect that appeared to be driven primarily by the patient group. The same SNP was also associated with delayed P300 latency although to a lesser degree. This SNP was associated with SCZ ($P = 2.35 \times 10^{-8}$) in the PGC GWAS mega-analysis [Ripke et al., 2011]. In the exploratory analyses, we found genotype by diagnosis interactions between SNP rs4765913 of the *CACNA1C* gene and the sensory gating phenotype and between SNP rs13000464 of the *GLS* gene and P300 amplitude.

P3 amplitude is thought to be proportional to the amount of attentional resources devoted to the task [Polich and Kok, 1995]. The latency reflects stimulus processing speed [McCarthy and Donchin, 1981]. The results of the present study suggest that the impact of the *TCF4* SNP rs17512836 risk allele on brain functions may be related to compromised attention and working memory (WM) capacities. Since P3 amplitude and latency were negatively correlated (partial correlation $= 0.35$, $P < 0.001$), it is possible that the observed associations with both phenotypes were due to shared variance. An alternative explanation could be a direct pleiotropic effect of the SNP on both ERP measures.

Current theory suggests that P300 activity is generated by a distributed neural network system that synchronously impacts the temporal-parietal junction and lateral prefrontal regions to inhibit ongoing extraneous brain activity and to facilitate transmission of task relevant information [Polich, 2007]. That is, ability to focus attention on task-relevant information and ignore irrelevant stimuli is achieved by goal-directed top-down control of differential enhancement and suppression of neural activity in the brain. Such selective, goal-directed modulation of brain activity is intimately related to WM performance [Zanto and Gazzaley, 2009]. The association between P300 activity and *TCF4* SNP rs17512836 suggests a link to cognitive processes of attention and WM.

Several recent studies have investigated the effects of *TCF4* polymorphisms on brain functions. Quednow and colleagues examined associations of 21 *TCF4* SNPs with the sensory gating response in a large population-based sample [Quednow et al., 2012]. They found that risk allele carriers of four *TCF4* SNPs (rs9960767, rs17512836, rs17597926, and rs10401120) had significantly reduced auditory sensory gating. Interestingly, the genetic effects of each SNP were strongly modulated by smoking behavior, with only smokers showing reliable associations between *TCF4* and P50 suppression, whereas the genetic

effect was not present in never-smokers. In our sample, we did not find significant associations between sensory gating and *TCF4* SNPs in the primary analyses when controlling for smoking status. However, we did find a significant correlation between smoking status and sensory gating response (partial $r = 0.17$, $P = 0.0069$) such that smokers had significantly worse sensory gating responses (75.04, $SD = 57.7$) than non-smokers (59.18, $SD = 34.5$; $P < 0.01$). We further examined whether smoking status modulated genotypic effects as reported in the Quednow et al. study. For SNP rs9960767, the sensory gating ratio was 63.18 ($SD = 42.2$) in non-smoker major allele carriers ($n = 246$) and 126.56 ($SD = 45.2$) in smoker risk allele G carriers ($n = 4$). For rs17512836, the sensory gating ratio was 64.14 ($SD = 43.0$) in non-smoker major allele carriers ($n = 254$) and 147.72 ($SD = 19.5$) in smoker risk allele G carriers ($n = 3$) [Note that we genotyped the opposite strands of alleles reported in the Quednow et al. study]. Genotype-by-smoking interactions were significant for both for rs9960767 ($P = 0.02$) and rs17512836 ($P = 0.03$). These results are consistent with the report of Quednow and colleagues, despite the fact that our sample of risk allele carriers who were smokers is quite small.

In another study Quednow and colleagues found that SNP rs9960767 of *TCF4* was associated with prepulse inhibition (PPI), a measure of sensorimotor gating and also an established SCZ endophenotype [Quednow et al., 2011]. PPI was strongly decreased in risk allele carriers of *TCF4* SNP rs9960767. However, no modifying influence of smoking on the effect of rs9960767 on PPI was found. We also found no modifying effect of smoking on the effect of *TCF4* SNPs on P3 ERPs in the present sample. Smoking was not correlated with either P3 amplitude (partial $r = -0.07$, $P = .41$) or latency (partial $r = 0.10$, $P = .21$). Taken together, these results suggest that smoking as a mediating factor on the *TCF4* gene may be specific to early sensory gating processes via the cholinergic pathway and alpha-7 nicotine receptors [Freedman et al., 1994]. PPI, sensory gating, and P3 ERPs appear to be uncorrelated and each is thought to be regulated by a distinct neuronal mechanism [Hall et al., 2006a; Braff et al., 2007b]. Moreover, our results were consistent with the report of Decoster et al. [2012], which found that association between rs9960767 and P3 amplitude was not significant [Decoster et al., 2012].

Our exploratory analyses showed a genotype by diagnosis interaction between SNP rs13000464 of the *GLS* gene and the P300 amplitude. The risk allele of this SNP was associated with reduced P300 amplitude in patients but this effect was not observed in healthy controls. Although preliminary, this result could be viewed as consistent with the glutamatergic NMDA receptor hypofunction hypothesis in the pathophysiology of schizophrenia [Lisman et al., 2008]. NMDA hypofunction is thought to lead to pyramidal neuron disinhibition resulting in an imbalance between excitatory and inhibitory feedback input, which, in turn, may lead to a dysregulated information processing and, ultimately, behavior deficits, such as abnormal P3 ERP. Our previous report of a relationship between the *GLS1* genotype and cortical glutamine/glutamate ratios measured in vivo also suggests that *GLS* genotype plays an important role in regulating glutamatergic neurotransmission and, possibly, in the pathophysiology of SCZ and BPD [Ongur et al., 2011]. We also found a genotype by diagnosis interaction between SNP rs4765913 (an intronic SNP in *CACNA1C*) and the sensory gating phenotype. In patients, but not in healthy controls, the BPD risk allele was associated with better sensory gating. Interpretation of this interaction is problematic as the allele previously associated with BPD was associated here with less impairment in sensory gating, a phenotype related to psychotic BPD. Further studies will be needed to clarify whether the effect we observed can be replicated and how it is mediated.

Our study has several limitations. First, while larger than many genetic studies of neurophysiologic phenotypes, our sample was small. We had 80% power to detect a variant with a locus-specific heritability of 7.5% at an alpha level of 0.0005, raising the possibility

of Type II error in our results. Second, we examined variants that had been associated with SCZ or BPD at genomewide significant levels in the largest published GWAS studies (by the PGC), but given the highly polygenic nature of these disorders, there are undoubtedly many more variants that confer risk. Third, a small proportion of EEG data was collected using a different EEG recording system. However, no significant equipment effect on each ERP phenotype was found. Finally, we cannot rule out whether medication effects or the toxic effects of having a chronic psychiatric illness contributed to our findings in the genotype by diagnosis interaction analyses.

In conclusion, we examined a set of variants that have been convincingly associated with psychotic illness in GWAS and our results suggest that the SCZ risk allele of *TCF4* rs17512836 variant could play a role in brain neurophysiology relevant to attention and working memory capacity. We also replicate the report of Quednow and colleagues regarding smoking as a mediating factor on the effects of the *TCF4* gene via sensory gating measures. The neurobiological basis of brain P300 and P50 sensory gating activities and their functional role may serve as a pointer to the underlying biological mechanisms for which this gene increases risk for psychotic disorder.

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TABLE I

Demographic Characteristics of the Sample

	SCZ (n =70)	BPD (n =129)	Controls (n =74)	Statistic	P-value
Age, years	42.4 (13.9)	41.7 (12.5)	35.78 (14.0)	$F_{2,269} = 7.0$	$P = 0.001$
Female sex, N (%)	21 (30)	65 (50)	42(57)	$F_{2,269} = 5.9$	$P = 0.003$
Education, years	14.15 (2.1)	15.34 (2.3)	15.38 (2.5)	$F_{2,158} = 4.1$	$P = 0.02$
Current smoker ^b , N (%)	32 (46)	37 (29)	7 (10)	$F_{2,264} = 12.4$	$P < 0.001$
Nr cigarettes/day, if smoker	19.3 (16.3)	15.3 (9.1)	5.7 (5.7)	$F_{2,67} = 3.4$	$P = 0.03$
Age of illness onset	23.55 (7.8)	21.00 (7.2)	N/A	$t = 2.12$	$P = 0.16$
CPZ ^a	632.76 (521.6)	481.17 (489)	N/A	$t = 1.72$	$P = 0.09$

Note: Data are presented as mean (SD) unless otherwise indicated. (SCZ schizophrenia, BPD bipolar disorder).

^a SCZ n =54, BPD n =94.

^b SCZ n = 69, BPD n =127, controls n =71.

TABLE II

SNPs Selected for Association Analysis

CHR	SNP	Location (build hg18)	MinorAllele	ObsMAF	MAF-HapMap-CEU	HWE P-value	Nearest gene	Prior associated phenotype (reference)
1	rs1625579	98502934	C	0.16	0.16	0.24	<i>MIR137</i>	SCZ Ripke et al. [2011]
2	rs17662626	193984621	G	0.08	0.12	1	<i>PCGEM1</i>	SCZ Ripke et al. [2011]
2	rs1344706	185778428	C	0.39	0.39	0.37	<i>ZNF804A</i>	SCZ O'Donovan et al. [2008]; Ripke et al. [2011]
2	rs12185688	191814608	C	0.13	0.15	0.79	<i>GLS</i>	POC Glutamine/Glutamate ratio Ongur et al. [2011]
2	rs13000464	191786868	T	0.48	0.42	0.33	<i>GLS</i>	POC Glutamine/Glutamate ratio Ongur et al. [2011]
2	rs17748089	191789907	A	0.05	0.06	1	<i>GLS</i>	POC Glutamine/Glutamate ratio Ongur et al. [2011]
4	rs4279178	47068580	A	0.46	0.47	1	<i>GABRB1</i>	SA/BPD Craddock et al. [2010]; Porjesz et al. [2002]
6	rs9371601	152790573	A	0.38	0.33	0.03	<i>SYNE1</i>	BPD Sklar et al. [2011]
8	rs7004633	89760311	G	0.23	0.17	1	<i>MMP16</i>	SCZ Ripke et al. [2011]
10	rs10994397	62279124	T	0.06	0.08	0.19	<i>ANK3</i>	BPD Sklar et al. [2011]
10	rs7914558	104775908	A	0.39	0.39	0.16	<i>CNNM2</i>	SCZ Ripke et al. [2011]
10	rs11191580	104906211	G	0.08	0.08	0.22	<i>NT5C2</i>	SCZ Ripke et al. [2011]
11	rs12576775	79077193	C	0.17	0.17	1	<i>ODZ4</i>	BPD Sklar et al. [2011]
11	rs548181	125461709	A	0.12	0.11	1	<i>STT3A</i>	SCZ Ripke et al. [2011]
12	rs4765905	2349584	C	0.33	0.35	0.27	<i>CACNA1C</i>	BPD/SCZ Sklar et al. [2011]
12	rs4765913	2419896	A	0.21	0.18	0.47	<i>CACNA1C</i>	BPD/SCZ Ripke et al. [2011]; Sklar et al. [2011]
15	rs16969968	78882925	A	0.37	0.38	1	<i>CHRNA5</i>	Nicotine Dependence Bousman et al. [2012]
15	rs6495308	78907656	G	0.22	0.21	1	<i>CHRNA5</i>	Nicotine Dependence Bousman et al. [2012]
18	rs12966547	52752017	A	0.40	0.42	0.44	<i>CCDC68</i>	SCZ [Ripke et al., 2011]
18	rs17512836	53194961	G	0.03	0.03	1	<i>TCF4</i>	SCZ Quednow et al. [2012a]; Quednow et al. [2012b]; Ripke et al. [2011]
18	rs9960767	53155002	G	0.04	0.07	1	<i>TCF4</i>	SCZ Quednow et al. [2012a]; Quednow et al. [2012b]; Ripke et al. [2011]

CHR, chromosome; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; MAF-HapMap-CEU, minor allele frequency in the HapMap-CEU sample; ObsMAF, observed minor allele frequency; POC, parieto-occipital cortex; SCZ, schizophrenia; BPD, bipolar disorder.

TABLE III

Summary Statistics for ERP Phenotypes

Phenotype	SCZ	BPD	Controls	SCZ vs. Controls	BPD vs. Controls	SCZ vs. BPD
P50 ratio (%)	73.58 (42.3)	71.3 0(48.5)	45.29 (27.4)	$P < 0.0001$	$P < 0.0001$	$P = 0.80$
P3 amplitude	8.59 (4.4)	8.97 (5.1)	12.65 (5.35)	$P < 0.0001$	$P < 0.0001$	$P = 0.90$
P3 latency	414.04 (86.8)	398.28 (68.2)	374.59 (49.5)	$P = 0.006$	$P = 0.06$	$P = 0.16$
N1 amplitude	-3.46 (2.5)	-4.15 (2.7)	-4.92 (3.2)	$P = 0.003$	$P = 0.09$	$P = 0.13$
P2 amplitude	4.94 (3.2)	4.98 (3.0)	7.02 (4.2)	$P = 0.004$	$P < 0.0001$	$P = 0.68$

Note: age, sex, EEG equipment system were included as covariates. Significant level was set at $P < 0.017$, correction for 3 post-hoc comparisons, mean (SD).

TABLE IV

SNPs With P -Value < 0.1 in the Whole Sample and P -Values in Cases and Controls

Phenotype	Gene	SNP	All		Cases		Controls	
			BETA	P -value	BETA	P -value	BETA	P -value
P3 amplitude	<i>TCF4</i>	rs17512836	-5.665	0.00017	-5.396	0.000826	-3.449	0.3998
	<i>ANK3</i>	rs10994397	-2.198	0.02012	-2.257	0.02476	-1.234	0.6029
	<i>GABRB1</i>	rs4279178	0.898	0.05099	0.814	0.1096	1.077	0.2623
	<i>GLS</i>	rs13000464	-0.881	0.05473	-1.242	0.0128	1.153	0.2554
P3 latency	<i>TCF4</i>	rs9960767	-2.182	0.06468	-2.871	0.03951	-0.4752	0.8197
	<i>TCF4</i>	rs17512836	56.34	0.00514	77.26	0.00231	-12.91	0.7321
	<i>ODZ4</i>	rs12576775	20.42	0.01622	20.28	0.06141	18.66	0.1508
	<i>TCF4</i>	rs9960767	34.42	0.03115	63.86	0.00362	-7.912	0.7096
	<i>MIR137</i>	rs1625579	15.93	0.05684	14.74	0.1679	10.44	0.401
	<i>GLS</i>	rs13000464	10.74	0.08435	18.41	0.02029	-14.84	0.1141
P2 amplitude	<i>ANK3</i>	rs10994397	-1.476	0.02206	-1.455	0.02023	-1.398	0.4478
	<i>TCF4</i>	rs9960767	-1.635	0.04105	-0.973	0.2593	-2.895	0.0985
	<i>TCF4</i>	rs17512836	-2.061	0.05103	-1.065	0.3182	-3.392	0.2868
	<i>CNNM2</i>	rs7914558	-0.574	0.09731	-0.163	0.6397	-1.323	0.1231
N1 amplitude	<i>MIR137</i>	rs1625579	-0.801	0.0218	-1.002	0.00877	-0.1329	0.8621
	<i>CNNM2</i>	rs7914558	0.523	0.06784	0.333	0.2908	1.339	0.0323
Sensory gating	<i>CACNA1C</i>	rs4765913	-10.7	0.02594	-19.19	0.00121	8.475	0.1926
	<i>CNNM2</i>	rs7914558	7.967	0.05355	7.928	0.1335	7.281	0.18
	<i>GABRB1</i>	rs4279178	-6.617	0.07895	-7.627	0.1175	-0.5587	0.9062
	<i>CACNA1C</i>	rs4765905	-6.642	0.08947	-11.78	0.01711	2.854	0.1926

Bold indicates statistically significant results after correction for multiple comparisons. Italic indicates $P < 0.05$.