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ORIGINAL ARTICLE

Analysis of schizophrenia-related genes and electrophysiological measures reveals ZNF804A association with amplitude of P300b elicited by novel sounds

EC del Re[1,2,12], SE Bergen[2,3,4,12], RI Mesholam-Gately[2,5], MA Niznikiewicz[1,2], JM Goldstein[6,7,8], TU Woo[2,9,10], ME Shenton[1,11], LJ Seidman[2,5,8], RW McCarley[1,2] and TL Petryshen[2,3]

Several genes have recently been identified as risk factors for schizophrenia (SZ) by genome-wide association studies (GWAS), including ZNF804A which is thought to function in transcriptional regulation. However, the downstream pathophysiological changes that these genes confer remain to be elucidated. In 143 subjects (68 clinical high risk, first episode or chronic cases; 75 controls), we examined the association between 21 genetic markers previously identified by SZ GWAS or associated with putative intermediate phenotypes of SZ against three event-related potential (ERP) measures: mismatch negativity (MMN), amplitude of P300 during an auditory oddball task, and P300 amplitude during an auditory novelty oddball task. Controlling for age and sex, significant genetic association surpassing Bonferroni correction was detected between ZNF804A marker rs1344706 and P300 amplitude elicited by novel sounds (beta = 4.38, $P = 1.03 \times 10^{-4}$), which is thought to index orienting of attention to unexpected, salient stimuli. Subsequent analyses revealed that the association was driven by the control subjects (beta = 6.35, $P = 9.08 \times 10^{-5}$), and that the risk allele was correlated with higher novel P300b amplitude, in contrast to the significantly lower amplitude observed in cases compared to controls. Novel P300b amplitude was significantly correlated with a neurocognitive measure of auditory attention under interference conditions, suggesting a relationship between novel P300b amplitude and higher-order attentional processes. Our results suggest pleiotropic effects of ZNF804A on risk for SZ and neural mechanisms that are indexed by the novel P300b ERP component.

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INTRODUCTION

The genetic contribution to developing SZ is relatively high, with heritability estimates of 81% by meta-analysis of twin studies[1] and 64% by a large family-based study.[2] Recent efforts to identify genetic risk loci in order to gain insight into SZ pathophysiology have involved GWAS with increasing success, and nearly 20 loci have now been associated with SZ.[3] One of the first genes to be identified with genome-wide significant evidence was ZNF804A, a brain-expressed gene that encodes a zinc finger domain indicating a transcriptional regulatory function. ZNF804A was initially implicated in risk of SZ and psychosis by a GWAS, and subsequently replicated by several targeted association studies.[4–10] A report of stronger association in SZ cases with higher IQ suggests that variation in ZNF804A may confer risk of a disease subtype with relatively preserved cognition.[11]

Impaired cognitive functioning based on neuropsychological tests, alterations in neuroanatomy and brain dysfunction as measured by structural and functional neuroimaging, and electrophysiological changes are all associated with SZ. In addition, impairments are also frequently observed in unaffected relatives, suggesting that these traits may represent heritable intermediate phenotypes that could be used to decipher disease pathology.[12,13] Event-related potentials (ERPs) reflect pre- and post-synaptic activity in neurons, and index normal and pathological brain processes in real time.[14] Several ERPs are considered to be intermediate phenotypes of SZ, with amplitude or latency abnormalities observed in affected individuals and unaffected relatives.[15–17] This includes the novel P300 component elicited ~300 ms after complex, 'novel' sounds in an oddball sequence,[18] which is reported to have abnormal amplitude in SZ and high-risk subjects.[19,20] Further, evidence of P300 deficits in healthy siblings of affected subjects[21,22] provides support for the novel P300 ERP component being an intermediate phenotype of SZ.

The novel P300 is thought to represent a brain mechanism involved in the rapid orienting of attention to events that are unexpected, salient and contextually novel.[23–25] Detection of novel stimuli involves, among other areas, the anterior cingulate,[26] followed by engagement of other brain regions important for

[1]VA Boston Healthcare System, Brockton, MA, USA; [2]Department of Psychiatry, Harvard Medical School, Boston, MA, USA; [3]Psychiatric and Neurodevelopmental Genetics Unit, Center for Human Genetic Research and Department of Psychiatry, Massachusetts General Hospital, Boston, MA, USA; [4]Medical Epidemiology and Biostatistics Department, Karolinska Institute, Stockholm, Sweden; [5]Massachusetts Mental Health Center Public Psychiatry Division, Department of Psychiatry, Beth Israel Deaconess Medical Center, Boston, MA, USA; [6]Brigham and Women's Hospital, Connors Center for Women's Health and Gender Biology, Boston, MA, USA; [7]Departments of Psychiatry and Medicine, Harvard Medical School, Boston, MA, USA; [8]Department of Psychiatry, Massachusetts General Hospital, Boston, MA, USA; [9]Program in Cellular Neuropathology, McLean Hospital, Belmont, MA, USA; [10]Department of Psychiatry, Beth Israel Deaconess Medical Center, Boston, MA, USA and [11]Departments of Psychiatry and Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. Correspondence: Dr TL Petryshen, Center for Human Genetic Research, Massachusetts General Hospital, 185 Cambridge Street, Boston, MA 02114, USA.
E-mail: petryshen@chgr.mgh.harvard.edu
[12]These authors contributed equally to this work.
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processing and habituation to the stimulus,[27] notably the dorsolateral prefrontal cortex and the hippocampus.[18,28–30] Menon and Uddin[31] have proposed a model whereby the anterior cingulate response to a novel stimulus is preceded by amplification of the salient event by the insula. Both brain regions define the 'salience network' that is important in dynamic switching between central-executive and default mode networks in processes mediating detection of salient events and capturing of attention.[32]

Several candidate genes have been studied within the context of association with ERP components and other putative intermediate phenotypes of SZ. Catechol-O-methyltransferase (COMT) has been associated with auditory oddball P300 amplitude in SZ subjects, unaffected relatives and healthy individuals.[33,34] Neuregulin 1 (NRG1) is reported to be associated with latency of the auditory oddball P300 component,[35,36] while its receptor, ERBB4, has been related to reaction time in a visual oddball task.[37] Dysbindin 1 (DTNBP1) has been associated with amplitude of the P1 component in a visual ERP task.[38] Other genes have been associated with cognitive domains that are impaired in SZ, including GRIN2B,[39] KIBRA,[40] MTHFR[41] and DISC1.[42] Several genes identified in recent GWAS of SZ, including ZNF804A, have also been investigated for relationships with cognitive performance, neuroanatomical features and neural connectivity that are abnormal in SZ.[11,43–45]

In the present study, we sought to examine the genetic contribution to ERP abnormalities as putative intermediate phenotypes of SZ to gain insight into the biological pathways that mediate brain dysfunction in this disorder. We explored associations between well-characterized electrophysiological components and genes with strong prior evidence of contributing to risk of SZ by GWAS or to variation of putative intermediate phenotypes. The primary focus of this article is P300b peak amplitude evoked during an auditory novelty oddball task (denoted hereafter as novel P300b), because it was the only ERP component displaying a genetic association surpassing statistical significance after correction for multiple tests. We also explored the relationship between the novel P300b component and experimental neurocognitive measures of auditory attention and working memory as a means of linking novel P300 to cognitive and brain functions important in SZ. Our analyses indicate that the ZNF804A gene previously implicated in risk for SZ is significantly associated with novel P300b amplitude in a pleiotropic manner that may be independent from its contribution to SZ.

MATERIALS AND METHODS

Subjects

Subjects were recruited through the Boston CIDAR study (www.bostoncidar.org). The sample ($N = 143$) utilized in this study consisted of a total of 68 cases, including 27 individuals with clinical high risk (CHR) or putatively prodromal symptoms, 25 persons within the first episode of schizophrenia (FESZ), and 16 people with chronic schizophrenia (CSZ), as well as 75 healthy control participants. Patients were recruited by referrals from clinicians or through local hospitals and clinics, and controls were recruited through newspaper and website advertisements. The study was approved by the local IRB committees at Harvard Medical School, Beth Israel Deaconess Medical Center, Massachusetts General Hospital, Brigham and Women's Hospital and the Veteran Affairs Boston Healthcare System (Brockton campus). All study participants, or legal guardians for those under 18 years of age, gave written informed consent and received payment for participation.

DSM-IV diagnoses were based on interviews with the Structured Clinical Interview for DSM-IV-TR (SCID), Research Version (First *et al.*, 2002) and information from patient medical records. All FESZ participants met DSM-IV-TR criteria for SZ, schizoaffective disorder or schizophreniform disorder, and none had more than one year of continuous antipsychotic treatment. CSZ participants met DSM-IV-TR criteria for SZ or schizoaffective disorder and had an illness duration of at least five years. Inclusion criteria for CHR subjects incorporated different approaches. The majority ($N = 23$) were categorized with 'late prodromal syndromes' as classified by the Criteria of Prodromal States[46] based on the Scale of Prodromal Symptoms.[47] The other four CHR participants were categorized with 'early prodromal syndromes', which are more subtle and require wider criteria for definition,[48] and include the so-called basic symptoms[49,50] and schizotypal personality disorder.[48,51] Antipsychotic medication use was estimated using chlorpromazine equivalents calculated according to Stoll.[52]

Controls were drawn from the same geographic bases with comparable age, gender, race and ethnicity, handedness, and parental socioeconomic status evaluated using the Hollingshead two-factor index.[53] Controls were ascertained to be comparable to each of the CHR, FESZ and CSZ groups. Exclusion criteria for controls included any current or past major DSM-IV-TR Axis I disorder, developmental disorders, psychiatric hospitalizations, prodromal symptoms, schizotypal or other Cluster A personality disorders, a first degree relative with psychosis, or current or past use of antipsychotics. Sleeping aids or anxiolytic agents for occasional use were acceptable, as well as other past psychotropic medication use if not within the prior 6 months.

Exclusion criteria for all subjects included sensory-motor handicaps, neurological disorders, medical illnesses that significantly impair neurocognitive function, diagnosis of intellectual disability ($IQ < 70$), not fluent in English, DSM-IV substance abuse within the past month or dependence within the past 3 months, current suicidality, or history of electroconvulsive therapy for controls or electroconvulsive therapy within the past 5 years for cases.

ERP tasks

Three different auditory tasks were employed: the novelty oddball, the classical oddball and the mismatch negativity (MMN). The latter two tasks were performed as detailed in McCarley *et al.*[54] and Niznikiewicz *et al.*,[55] respectively, and are not described here. As explained above, we have focused on the novel P300b given its association with genetic data.

Novelty oddball task

Subjects performed a novelty, 3-stimulus auditory oddball task over 4 minutes. Stimuli ($N = 180$) included brief tones (82 ms duration, 75 dB sound pressure level), with 20% (36) infrequent target tones (1.5 kHz) and 60% (108) frequent standard tones (1 kHz), as well as 20% (36) infrequent non-target novel stimuli (300–320 ms duration, 75 dB sound pressure level) consisting of six different complex environmental sounds (for example, dog bark, door slamming).[18] The inter-stimulus interval was 976 ms (onset-to-onset). Subjects were instructed to silently count the target tones.

EEG recording and processing

The EEG was recorded with a Biosemi Active-Two system using sintered Ag/AgCl electrodes in an electrode cap at 64 standard scalp sites (DC-100 Hz bandpass filter, 512 Hz digitization rate) with DC offsets kept below 25 mV. During data acquisition, all channels were referred to the system's internal loop (CMS/DRL sensors located in the parietal region). The bipolar vertical electro-oculogram was derived from electrode Fp1 and an electrode below the left eye. The horizontal electro-oculogram was derived from electrodes on the left and right outer canthi.

The EEG data were processed using the BrainVision Analyzer package, version 2.0 (Brain Products GmbH, Munich, Germany). The continuous EEG recordings were segmented from −100 to 900 ms relative to stimulus onset and re-referenced to the algebraic sum of the left and right mastoids. Ocular correction was performed using the method of Gratton *et al.*[56] Epochs with artifacts exceeding $+/-100\,\mu V$ with a maximal allowed difference of $200\,\mu V$ were excluded. Average ERPs were computed from the artifact-free epochs for each subject separately for each type of stimulus and baseline corrected with a pre-stimulus baseline of −100 to 0 ms following digital filtering (0–16 Hz). The number of epochs included in the average novel P300b ERP across all subjects was 33.6 ± 2.99 (cases, 33.4 ± 3.2; controls, 34.1 ± 2.5; $F(1,141) = 2.1$, $P = 0.147$).

ERP analyses

In the novelty oddball task, the peak amplitude of the novel P300 peak elicited by novel sounds was measured at electrode sites F1/Fz/F2 (frontal), C1/Cz/C2 (central), and P1/Pz/P2 (parietal). The latency range for selecting the peak in individual subject data was determined by visual inspection of the grand average ERPs, and all electrodes of interest were inspected to ensure that the same component was selected at each site. The novel P300 waveform in these data was biphasic (Figure 1), with an early peak, novel

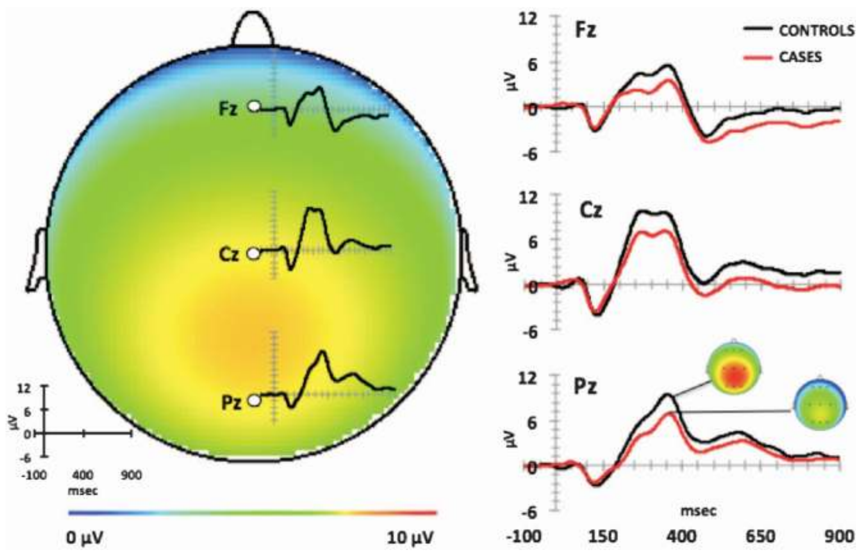

Figure 1. Novel P300 waveforms. Left: topographic map illustrating the central – parietal scalp distribution of novel P300b amplitude measured at latency between 315 – 390 ms and waveforms at the midline electrodes (Fz, Cz and Pz) in controls and cases combined ($N = 143$). Right: novelty P300b oddball task grand average waveforms in controls ($N = 75$) and in cases ($N = 68$) at the midline electrodes (Fz, Cz and Pz). Color distribution of topographical maps corresponds to novel P300b amplitude between 0 and 10 μV.

P300a, occurring 215–290 ms post stimulus, and a later peak, that we designate as novel P300b, between 315–390 ms. Amplitude of the P300a peak was highest at central sites, while the P300b peak was highest at central and parietal sites (Figure 1). Thus, novel P300b amplitude at the Pz electrode was chosen for genetic analyses to avoid overlap with the scalp distribution of novel P300a amplitude.

Neuropsychological tests

Subjects were administered a large neurocognitive test battery including tests estimating premorbid IQ (the Reading subtest from the Wide Range Achievement Test-4 (WRAT-4);[57] and current IQ (the Vocabulary and Block Design T-scores of the Wechsler Abbreviated Scale of Intelligence (WASI).[58] The 'vigilance', 'working memory' and 'working memory plus interference' measures from the Seidman auditory continuous performance test battery (ACPT)[59,60] were utilized to assess different components of auditory attention. All three auditory measures utilize letters of the alphabet presented on an audiotape at a rate of one per second for 90 seconds, with three blocks for vigilance and two blocks each for the two working memory conditions (10.5 min total). The vigilance measure taps sustained auditory attention/vigilance by requiring a rapid response (tapping a pencil) each time a letter 'A' immediately follows a letter 'Q' (QA vigilance). The auditory working memory measure involves rapidly responding each time a letter 'A' follows four letters after a letter 'Q' (Q3A memory). The more complex and higher load working memory plus interference condition (Q3A memory-interference) places increased demands on working memory by invoking auditory working memory when confronted with distracting/interfering information (that is, embedding distracting combinations of 'Q', 'A' or 'QA' in between the cue 'Q' letters and the target 'A's that follow four letters after, thus dividing attention, preventing counting and stressing the capacity to perform dual tracking). The hit rate (% of correctly responding to the target letter 'A's) was determined for each of the three conditions.[59,60]

Statistical analyses of ERP data

Statistical analyses of ERP data were performed with SPSS v.19 (IBM, Armonk, NY, USA). Novel P300b peak amplitude was analyzed using MANOVA with group (all cases versus all controls) as a between factor, and within factors of region (frontal/central/parietal) and site (F1/Fz/F2, C1/Cz/C2, P1/Pz/P2). We also assessed differences between diagnostic subgroups by comparing CHR, FESZ, and their matched controls, and comparing CSZ and their matched controls, using the MANOVA design described above. As IQ and pSES were significantly different between cases versus controls, the MANOVA was carried out with and without IQ and pSES as covariates; however, results did not differ, therefore only results without covarying for IQ and pSES are reported. Bonferroni-corrected *P*-values are reported.

Spearman's rho correlations were calculated between novel P300b amplitude at the Pz electrode and three measures from the Seidman ACPT, 'vigilance', 'working memory' and 'working memory plus interference'. The contributions of IQ and higher-order cognitive processes to novel P300b amplitude were further examined by entering current IQ and 'working memory with interference' performance as independent variables in a multiple regression model with novel P300b amplitude as the dependent variable.

Genotyping and quality control

Blood or, in some instances, saliva was collected from all subjects at the time of recruitment. DNA was extracted, quantified using Invitrogen's Quant-iT (TM) dsDNA Assay Kit (Life Technologies, Carlsbad, CA, USA) and stored at −80°C. Genotyping was conducted at the Broad Institute of Massachusetts Institute of Technology and Harvard using the Illumina OmniExpress array (Illumina, Inc., San Diego, CA, USA) containing 733 202 single-nucleotide polymorphisms (SNPs). Quality control steps were performed on the full data using PLINK[61] as follows: SNPs were removed if they had $< 1\%$ minor allele frequency, $< 95\%$ genotyping rate, deviation from Hardy – Weinberg equilibrium ($P < 1 \times 10^{-6}$), or differential missingness between cases and controls at the SNP or haplotype level. Three subjects with low genotyping rates ($< 90\%$) and/or high heterozygosity values (>34%) were removed. SNPs not directly genotyped were imputed with Beagle software[62] using HapMap3 reference panels containing >1.2 million markers.[63] All imputed SNPs included in association analyses were imputed with high confidence (Info >0.85).

Genetic markers

Thirteen SNPs conferring risk for SZ identified by published GWAS[64–67] and surpassing a genome-wide significance threshold[68] of $P < 5 \times 10^{-8}$ were selected. SNPs meeting this criterion but with minor allele frequencies $< 10\%$ were excluded due to too few minor allele carriers in this sample. In addition, eight SNPs in candidate genes were included that have prior evidence from at least two studies for association with SZ intermediate phenotypes, including ERP abnormalities (COMT[33]), neuropsychological impairments (ERBB4,[37,69,70] MTHFR,[71,72] DISC1,[42] COMT,[69,73] KIBRA,[40] NRG1,[69] GRIN2B[69]), and structural and functional neuroimaging measures (DTNBP1,[74,75] DISC1,[42,76] NRG1,[76] GRIN2B,[77] ERBB4,[37] COMT,[78] KIBRA[79]). We identified a total of 21 SNPs meeting these selection criteria (Supplementary Table 1).

Genetic association analyses

Genetic association analyses were performed using linear regression in PLINK with covariates accounting for sex and age, as well as population substructure captured by the first five multidimensional scaling components. Diagnostic subgroup (CHR, FESZ, CSZ and control) likely impacts true performance differences, but it is also a potential confounding variable since medication and other environmental conditions may differ by group. Accordingly, main analyses were conducted without diagnostic subgroup as a covariate, and results were subsequently confirmed in analyses with diagnostic subgroup as a covariate in the model. The following ERP components were tested: MMN at the Fz electrode, oddball P300 amplitude at the Cz electrode, and novel P300b amplitude at the Pz electrode. Bonferroni correction was applied to the genetic association results, not accounting for potential correlations between the ERP variables, to yield a study-wide significance threshold of $P < 7.9 \times 10^{-4}$ for the three ERP variables and 21 SNPs tested. As current IQ and pSES differed significantly by ZNF804A genotype, novel P300b amplitude was analyzed using MANCOVA with genotype as a between factor and pSES and current IQ as covariates. *Post hoc* analyses exploring the strength of ZNF804A association were conducted separately by diagnostic subgroup, sex and genetically defined Caucasian ancestry (the largest ancestral population within this sample, $N = 87$). Genetic association tests between ZNF804A and current IQ and a measure of auditory attention (Seidman ACPT 'working memory plus interference') were also conducted.

RESULTS

Subject characteristics

Sample demographic data are summarized in Table 1. Most variables did not differ between controls and cases (combined CHR, FESZ and CSZ), with the exception of current IQ ($F(1,141) = 9.5$; $P < 0.01$), years of formal education ($F(1,141) = 4.6$; $P < 0.05$), parental socioeconomic status ($F(1,141) = 4.35$, $P < 0.05$) and global assessment of functioning (GAF) score ($F(1,136) = 485$, $P < 0.01$). As the cases were a composite of three diagnostic groups, Supplementary Table 2 reports demographic data for CHR and FESZ cases both matched to their control group, and for CSZ cases matched to their control group.

Novel P300b amplitude is significantly reduced in SZ cases

MANOVA revealed a main effect of group (all cases versus all controls) on novel P300b amplitude ($F(1,141) = 26.2$; $P < 0.001$), which was significantly lower in cases than controls (Figure 1; Supplementary Table 3). There was also a main effect of brain region ($F(2,140) = 74.0$, $P < 0.001$), but no interaction of region by group ($F(2,140) = 0.02$, $P = 0.98$). *Post hoc* analysis revealed higher P300b amplitude at parietal and central regions (both $P < 0.001$) relative to frontal (Figure 1), therefore, genetic association analyses utilized novel P300b amplitude data at the Pz electrode.

In analyses of the diagnostic subgroups (Supplementary Figure 1), a main effect of group was also found in the MANOVA comparing CHR, FESZ and their matched controls ($F(2,107) = 7.0$, $P < 0.01$). *Post hoc* analysis revealed no difference in amplitude between CHR and FESZ groups ($P = 0.99$), with both groups significantly lower than controls ($P < 0.01$). The lack of amplitude differences between CHR and FESZ justified inclusion of CHR subjects as cases in all analyses. MANOVA of CSZ and matched controls also revealed a main effect of group ($F(1,31) = 15.5$; $P < 0.001$).

Relationship between novel P300b and attention to auditory stimuli

In order to better understand the neural substrates of novel P300b, we examined its relationship with higher-order cognitive processes. As the auditory novel P300 paradigm is thought to index salience detection, we investigated performance in the Seidman ACPT task that indexes sustained and selective attention to auditory stimuli. We examined three measures, 'vigilance', 'working memory' and 'working memory plus interference'. Performance on all three tasks was significantly impaired in cases relative to controls (Supplementary Table 3). While novel P300b amplitude was not significantly correlated with the vigilance or working memory measures (rho = 0.17, $P = 0.21$ and rho = 0.21, $P = 0.06$, respectively) a significant correlation was found for the 'working memory plus interference' measure (rho = 0.4, $P < 0.001$; Supplementary Figure 2).

To further examine this relationship, 'working memory with interference' performance and current IQ were entered as independent variables in a multiple regression model with novel P300b amplitude as the dependent variable. The model was statistically significant ($F(2,133) = 12.3$, $P = 0.0005$), explaining 14.4% of the variance in novel P300b amplitude. Only 'working memory with interference' significantly contributed to the model (beta = 0.3; $P = 0.001$), suggesting a relationship between novel P300b amplitude and higher-order attentional processes. IQ did not significantly contribute to the model (beta = 0.113; $P = 0.24$), therefore it was not included as a covariate in subsequent genetic association analyses.

Significant association between ZNF804A and novel P300b amplitude

Genetic association analyses of 21 SNPs conferring risk for SZ or impacting putative intermediate phenotypes were performed for each of the three ERP variables, MMN (Fz), oddball P300 amplitude (Cz), and novel P300b amplitude (Pz) (Supplementary Table 4). The only result to attain study-wide significance (that is, $P < 7.9 \times 10^{-4}$) was between the ZNF804A marker, rs1344706, and novel P300b

Table 1. Sociodemographic and clinical information

	Controls (N = 75)	*Cases (N = 68)*	*P-value*	*rs1344706*			
				AA (N = 66)	*AC (N = 54)*	*CC (N = 23)*	*P-value*
Age	26.1 (10.5)	26.4 (12.0)	NS	27.0 (11.8)	25.3 (10.6)	26.2 (11.4)	NS
Male/Female	46/29	49/19	NS	40/26	39/15	16/7	NS
Premorbid IQ	114.6 (15.4)	110.9 (16.3)	NS	111.0 (15.1)	115.6 (15.4)	111.8 (18.7)	NS
Current IQ	117.8 (14.0)	110.3 (15.0)	< 0.01	110.8 (15.5)	118.4 (12.4)	114.4 (16.9)	< 0.05
Education	13.9 (2.7)	12.9 (2.7)	< 0.05	13.5 (2.7)	13.4 (2.8)	13.4 (2.7)	NS
pSES	1.8 (0.9)	2.1 (1.0)	< 0.05	2.2 (1.1)	1.7 (0.77)	1.91 (0.9)	< 0.05
GAF	84.5 (7.7)	49.2 (11.0)	< 0.01	66.0 (20.5)	70.9 (19)	62.7 (20.9)	NS
CPZ equivalents	NA	248.8 (214.7)	NA	343.1 (269.6)	160.2 (118.8)	189.3 (123.6)	NS

Abbreviations: CPZ, chlorpromazine; GAF, global assessment of functioning; NA, not applicable; NS, not significant; pSES, parental socioeconomic status. CPZ equivalents were calculated for subjects on antipsychotic medication ($N = 36$, including 9 CHR, 14 FESZ and 13 CSZ subjects). Values are mean (s.d.). Fisher's Exact Test was used for categorical variables and ANOVA for continuous variables ($P > 0.05$).

amplitude (beta = 4.38, $P = 1.03 \times 10^{-4}$; Table 2), explaining 7.2% of the total variance. The 'A' allele for this A/C SNP, which has previously been associated with increased risk for SZ and psychosis, was associated with higher novel P300b amplitude (Figure 2). Since diagnostic group could be a potentially confounding variable, analyses including group (CHR, FESZ, CSZ and controls) as a covariate were also performed, but this had a negligible effect on the results (beta = 4.21, $P = 1.38 \times 10^{-4}$). Therefore, subsequent *post hoc* analyses did not include diagnostic group as a covariate. In addition, MANCOVA of P300b amplitude with genotype as a between factor and pSES and current IQ as covariates revealed a main effect of genotype ($F(2,138) = 3.64$, $P = 0.029$) and no interaction effect of the covariates, indicating that these variables are not confounding the ZNF804A association. Amplitude was significantly lower in CC genotype than AA ($P < 0.05$), while amplitude of AA and AC did not differ ($P = 0.99$).

Association analyses of only cases or only controls revealed that the ZNF804A association was strongly driven by the control subjects (beta = 6.35, $P = 9.08 \times 10^{-5}$), with little contribution from the cases when CHR, FESZ and CSZ diagnostic groups were analyzed separately or together (Table 2). Association results were similar between males and females. Restricting analyses to participants of Caucasian ancestry ($N = 87$), the largest ancestry group represented in this study, yielded a relatively similar effect size (beta = 3.34; $P = 0.012$) as in the entire study population (beta = 4.38).

Table 2. Association analyses for ZNF804A SNP rs1344706 and novel P300b amplitude

Group	N	*Beta*	P-*value*
All subjects	143	4.38	1.03×10^{-4}
Combined cases	68	1.97	0.21
CHR	27	2.75	0.33
FESZ	25	0.88	0.74
CSZ	16	3.21	0.61
Controls	75	6.35	9.08×10^{-5}

Abbreviations: CHR, clinical high risk; CSZ, chronic schizophrenia; FESZ, first episode of schizophrenia.

We also examined whether the association detected between ZNF804A and novel P300b amplitude at the Pz electrode was supported by analyses of other electrodes. Associations were detected with novel P300b amplitude at other parietal, central and frontal electrodes ($4.2 \times 10^{-4} < P < 0.064$; Figure 3), although the

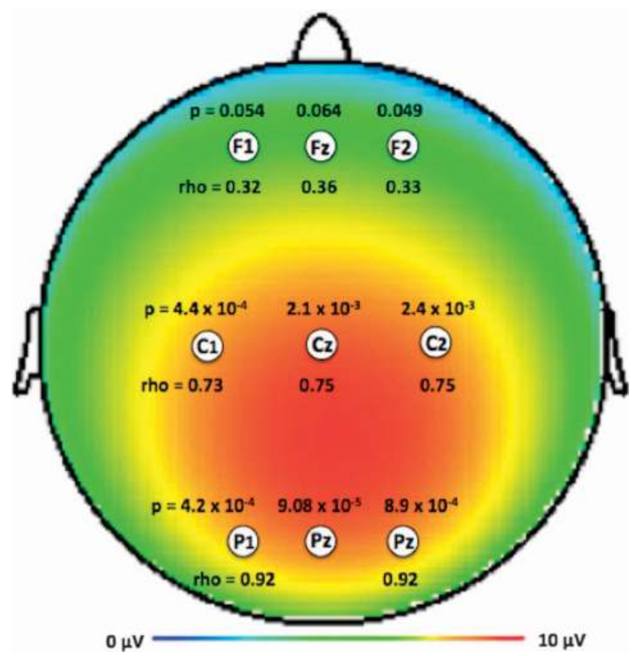

Figure 3. Distribution of ZNF804A rs1344706 association with novel P300b amplitude in controls. Rs1344706 association results with novel P300b amplitude at latency between 315 – 390 msec are shown for frontal, central and parietal electrodes (uncorrected *P*-values above electrode labels). Correlation between novel P300b amplitude at the Pz electrode and amplitudes at other electrodes (Spearman's rho values below electrode labels) decreases stepwise from parietal to central to frontal sites as the strength of rs1344706 association with novel P300b amplitude decreases (that is, less significant *P*-values). All Spearman's rho correlations significant at $P < 0.01$.

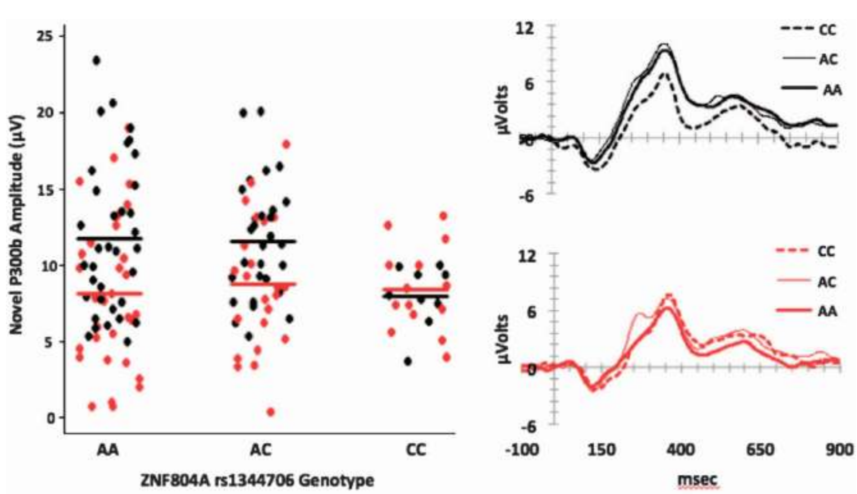

Figure 2. ZNF804A rs1344706 association with novel P300b amplitude. Left: scatterplot of novel P300b amplitude at the Pz electrode by rs1344706 genotype in controls (black dots) and cases (red dots). Horizontal lines depict average amplitude in controls or cases. Right: novelty oddball task grand average waveforms at the Pz electrode by genotype in controls (top panel; black lines) and in cases (bottom panel; red lines).

results were less significant than for the Pz electrode. The strength of the ZNF804A association with novel P300b amplitude decreased from parietal sites (P1, Pz, P2) to central sites (C1, Cz, C2) to frontal sites (F1, Fz, F2), paralleling the stepwise decrease in the correlations between novel P300b amplitude at the Pz electrode and amplitudes at central and frontal electrode sites (Figure 3). These results suggest that the ZNF804A association may be more specific for mechanisms indexed by novel P300b amplitude at parietal sites.

DISCUSSION

ZNF804A was among the first genes associated with risk of SZ by GWAS with statistical evidence surpassing genome-wide significance.[4,7] The 'A' allele of SNP rs1344706 in ZNF804A was associated with increased risk of psychosis, with an odds ratio of 1.11 in a combined analysis of SZ and bipolar disorder.[4] Subsequent studies focused on ZNF804A strongly supported association with SZ.[5–11] ZNF804A is expressed in the brain, and the risk allele of rs1344706 has been associated with decreased mRNA expression during fetal brain development.[80] Knockdown of ZNF804A in neural progenitor cells changes expression of genes pertaining to neuronal outgrowth and migration, synapse formation, and cell adhesion, all of which have been implicated in the pathophysiology of SZ.[81] Animal and cell culture studies have shown that ZNF804A is associated with expression of COMT, an enzyme that degrades catecholamines, and the DRD2 dopamine receptor.[82] These data provide a possible link between novel P300, ZNF804A and dopaminergic signaling, as there is evidence that the novel P300 ERP component is mediated by catecholaminergic activity.[83] *In silico* studies have also suggested possible involvement of ZNF804A in regulation of oligodendrocyte proliferation and differentiation.[84] This might impact neural connectivity, and in fact ZNF804A has been associated with connectivity between brain regions that are important in processing of auditory stimuli.[18,27–30,85]

The present investigation detected significant association between ZNF804A and amplitude of the P300b electrophysiological component elicited by a novelty oddball task. The novel P300 component represents a brain mechanism dedicated to re-orienting attention to salient and novel events in the environment.[24,25] Novel P300 is of particular interest since SZ is characterized by core cognitive deficits, notably, general difficulty in concentrating attention on relevant stimuli while ignoring unimportant stimuli,[86] including during the CHR period.[87,88] Indeed, the cases in our study exhibited lower P300b amplitude compared to controls, which is supported by prior reports of high-risk, recent onset, and chronic SZ subjects.[19,20,89,90] Interestingly, we found that the risk-associated allele of rs1344706 was related to increased novel P300b amplitude, in contrast to the lower amplitude observed in SZ cases. This result implies that ZNF804A has pleiotropic effects on ERPs and SZ, in that the allele associated with increased risk of SZ is also related to higher P300b amplitude, opposite to that observed in SZ. Previous studies have also suggested that genes implicated in SZ etiology may exhibit pleiotropic effects on intermediate phenotypes including ERPs, gray matter volume, cognitive performance and sensorimotor gating.[11,44,69,91] While this study is limited by a small sample size and mixed population that may increase the likelihood of false positive results, our findings are supported by those of O'Donoghue *et al.*[92] (this issue), who found a remarkably similar relationship between the rs13444706 risk allele and increased P300 amplitude elicited by an auditory oddball task. Although O'Donoghue *et al.* did not utilize the same novel oddball task, both oddball P300 and novel P300 are elicited in response to rare, deviant and salient stimuli.[26,93,94] Therefore, their results strongly support our findings and provide independent evidence for pleiotropic effects of ZNF804A on SZ and regulation of brain processes mediating ERPs.

Our finding of association with novel P300b amplitude in controls but not in cases, which was also observed by O'Donoghue *et al.*,[92] could be due to several factors. One potential scenario is that the brain pathology present in individuals at high risk or diagnosed with SZ may disrupt the ZNF804A effect on P300 amplitude and thereby mask the association with elevated P300 amplitude in cases. It is also possible that other genetic, epigenetic and environmental risk factors that are highly loaded in cases but not controls influence ERPs such that the ZNF804A effect is obscured. Alternatively, the small case- and control-only samples had low statistical power, so failure to detect an association in some analyses is not surprising. Additional studies are needed to confirm our findings, elucidate the function of ZNF804A in SZ and brain function, and clarify its interaction with other risk factors.

Investigation of the functional significance of ZNF804A association with SZ has focused on brain structure and connectivity between neural circuits during performance of cognitive or emotional tasks, domains particularly affected in SZ.[95,96] The risk allele of rs1344706 is hypothesized to modulate connectivity between the dorsolateral prefrontal cortex and hippocampus, specifically during working memory tasks, in both healthy controls[43,85] and SZ subjects.[97] These studies are pertinent to our results, as they indicate that ZNF804A may modulate specific neural circuitry that has been implicated in the dynamics of novel P300.[18,27–30] As novel P300 indexes a complex dynamic process in which detection of the novel event occurs via the anterior cingulate,[26] among other regions, and habituation to the novel event via recruitment of working memory through the dorsolateral prefrontal cortex and hippocampus,[18,27–29] our genetic association results with novel P300b are in line with published studies demonstrating ZNF804A association with modulation of connectivity between these brain regions.[85,97,98] Thus, the present investigation supports the notion that ZNF804A regulates specific and dynamic brain processes, possibly at the interface between the salience and executive networks.[32,99]

On reviewing the extant literature, some studies suggest that SZ subjects carrying the rs1344706 risk allele may have milder impairments than non-carriers. For example, SZ risk allele carriers have larger regional gray matter volume and relatively intact cognitive performance,[11] indicating that ZNF804A may delineate a subtype of SZ with preserved brain function in some domains. However, other studies have found greater impairment in SZ subjects carrying the risk allele, including heightened psychotic symptoms.[100] In healthy controls, risk allele carriers appear to be more impaired, with impaired attention,[91] deleterious effects on gray matter volume and cortical thickness and neural activity reported in default network areas[45,91] and the Theory of Mind network.[101] However, the latter studies only evaluated controls, therefore it is unknown whether these deleterious effects would also be observed in SZ subjects. It should also be kept in mind that different approaches have been used across studies (for example, grouping subjects of different genotypes) and that correction for multiple testing has not consistently been performed, which could result in inconsistent or spurious results. Future studies of both SZ and healthy subjects across multiple domains will be important in elucidating the role of ZNF804A in brain function and risk of SZ.

In conclusion, our finding of significant association between ZNF804A and novel P300b amplitude concurs with prior literature suggesting a specific role of this gene in modulating brain circuits that are neurobiological substrates of this ERP component.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)