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Genetic association study of the P300 endophenotype in schizophrenia

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ABSTRACT

Objective: Although reduced amplitude of the P300 event-related potential is a well-documented intermediate phenotype of schizophrenia, little is known about its genetic underpinnings in patients with schizophrenia. This study aims to examine associations between P300 and a range of candidate genetic variants, selected from either candidate gene studies or genome-wide association studies, in a large sample of patients with schizophrenia.
Methods: P300 amplitude at the midline parietal electrode and 193 single nucleotide polymorphisms (SNPs) in 67 genes were assessed in 336 patients with schizophrenia. The association between each SNP and P300 amplitude, controlled for illness duration and gender, was evaluated. Associations at $p<.01$ were considered of potential relevance, while Bonferroni correction was applied to determine formal statistical significance (Bonferroni-corrected threshold of significance $p=.0003$).
Results: Of the 193 selected SNPs, 4 SNPs showed potentially relevant association with P300 amplitude at a significance level of $p<.01$. One of these SNPs, rs1045642 in ABCB1, was most convincingly associated with P300 amplitude, reaching formal (Bonferroni-corrected) significance, while there was evidence for possible association with rs1572899 in DISC-1, rs6265 in BDNF and rs1625579 in MIR137.
Conclusion: Genetic variation in ABCB1 may be associated with P300 amplitude in patients with schizophrenia. This result may encourage further efforts to elucidate the genetic underpinnings of P300 generation.

1. Introduction

The auditory P300 is an event-related potential (ERP) that is typically elicited by an auditory target stimulus serving as the signal for the participant to execute a predefined task, such as pushing a button or counting. It is named after its typical peak 300 ms after the target stimulus. The P300 is believed to reflect a summation of simultaneous brain processes, including directed attention and contextual updating of working memory (Turetsky et al., 2007; van der Stelt and Belger, 2007). It is described by its amplitude and latency. Two subcomponents can be distinguished: the P3a subcomponent with a predominantly frontal distribution, which reflects the unexpectedness of the stimulus and the P3b subcomponent with a predominantly parietal distribution, reflecting cognitive processing of task-relevant or contextually salient stimuli (Turetsky et al., 2007).

Reduced amplitude of the auditory P300, especially the P3b subcomponent, has been consistently reported in patients with schizophrenia (Turetsky et al., 2007; van der Stelt and Belger, 2007). Although reduced P300 amplitude is not specific to schizophrenia, the observed deficits are distinguishable in several aspects from the P300-deficits in Alzheimer disease (marked latency prolongation), alcoholism (more visual than auditory abnormalities) and depression (state-dependent abnormalities), suggesting different underlying neural mechanisms (Souza et al., 1995; Salisbury et al., 1999; Turetsky et al., 2007). However, since P300 amplitudes at centro-parietal sites in patients with bipolar disorder manifesting psychotic symptoms were not distinguishable from those of patients with schizophrenia, it was suggested that decreased P300 amplitude at these sites may mark functional psychosis in general (Bestelmeyer et al., 2009).

The heritability of the P300 was established by several twin studies (O'Connor et al., 1994; Bestelmeyer et al., 2009; Hall et al., 2009). Moreover, part of the genetic contribution to the P300 waveform is shared with the genetic contribution to schizophrenia (Hall et al., 2007) and family members of patients with schizophrenia also show significantly reduced P300 amplitudes compared to the general population, although to a lesser degree than their ill relatives (Bramon et al., 2005).

Reduced P300 amplitude was found in first-episode patients, recent-onset, chronic patients and even people at ultra-high risk for psychosis (van Beijsterveldt et al., 2001; Umbricht et al., 2006; Turetsky et al., 2007; van der Stelt and Belger, 2007; van Tricht et al., 2011). Given these findings, a decrease in the amplitude of the P300 is commonly accepted as an intermediate phenotype for schizophrenia (Turetsky et al., 2007; van der Stelt and Belger, 2007).

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Although a body of evidence supports reduced P300 amplitude as an intermediate phenotype for schizophrenia, there is limited knowledge of the actual genetic underpinnings of P300 generation in general, and P300 disruption in schizophrenia specifically (Turetsky et al., 2007; van der Stelt and Belger, 2007). Blackwood et al. (2001) reported a reduction of P300 amplitude in a large Scottish family multiply affected with schizophrenia. In this family, a balanced translocation of chromosome 1 and 11, disrupting the DISC1 gene, was strongly associated with both the diagnosis of schizophrenia and reduced P300 amplitude (Blackwood et al., 2001). In addition, reduced P300 amplitude was also observed in unaffected carriers of the translocation (Blackwood et al., 2001). Further studies used a candidate gene approach to examine the association with P300 amplitude in healthy controls (Tsai et al., 2003a; Tsai et al., 2003b; Mulert et al., 2006; Vogel et al., 2006; Gallinat et al., 2007; Schofield et al., 2009; Shaikh et al., 2011), patients with a diagnosis of depression (Chen et al., 2002) or addiction (Johnson et al., 1997; Hill et al., 1998; Berman et al., 2006; Antolin et al., 2009), as well as patients with schizophrenia (Gallinat et al., 2003; Bramon et al., 2006; Golimbet et al., 2006; Bramon et al., 2008; Wang et al., 2009; Shaikh et al., 2011). These studies commonly focused on a single gene, often even limited to a single nucleotide polymorphism (SNP). This is potentially problematic since there are increasing concerns about the candidate gene approach, as the high prevalence of false-positive findings in genetic research may not always be adequately taken into account, especially in the context of undisclosed multiple statistical comparisons (Sullivan, 2007). On the other hand, sample sizes are often too small to allow for the use of genome-wide chips, suggesting that a candidate gene approach with a larger number of SNPs and adequate control for multiple testing may be the most viable option for the research of candidate endophenotypes (Greenwood et al., 2011). Therefore, the current study examined a range of candidate SNPs, selected from either candidate gene studies or genome-wide association studies, in a sample of 336 patients with a schizophrenia spectrum disorder.

2. Materials and methods

2.1. Subjects

The sample of this study was recruited between October 1999 and November 2006. Psychiatric diagnoses according to DSM-IV criteria were established by experienced psychiatrists affiliated with the University Centre at Louvain, Belgium, and responsible for the patient's treatment. In the University Centre, P300 analysis forms part of a comprehensive neurological and neurophysiological assessment conducted in inpatients, after clinical stabilization. Conform to international guidelines (De Hert et al., 2009), patients receive an elaborate physical health screening including assessment of fasting glucose, lipids and other parameters as described previously (van Winkel et al., 2006; De Hert et al., 2010). On this occasion, they were asked for permission to store a blood sample for genetic analyses and for the anonymous analysis of clinical data recorded during their treatment. The study was approved by the standing ethics committee.

2.2. P300-recording

P300 data were recorded using a Neurofax Portable Electroencephalograph EEG-7414 (Nihon Kohden Corporation, Tokyo). During the recording patients were seated in a slightly reclined chair and were asked to fix their gaze at a mark approximately 1 meter in front of them. Evoked responses were recorded with 3 mid-line electrodes (Fz, Cz and Pz), positioned according to the international 10/20 system and online referenced to left and right ear-electrodes (A1 and A2). Electro-oculogram (EOG) was recorded in order to reject P300-epochs distorted by eye-movement artefacts. All electrodes were attached with a skin-electrode impedance of less than 5 kOhm.

120 sinus tones of 800 Hz (standard) and 30 sinusoidal tones of 1470 Hz (deviant), both with a duration of 40 ms and an intensity of 70 dB sound pressure level, were presented binaurally through earphones. Each inter-stimulus interval (ISI) was 1 s. Standard and deviant tones were mixed randomly. Patients were asked to push a button as quickly as possible when hearing a deviant tone. Data were collected with a sampling rate of 1024 Hz and with a high cut-off at 70 Hz. The event-related potentials (ERP) elicited by correctly processed standard (without push on button) and deviant tones (push on button) were averaged separately for each subject, using the EEG epochs from 100 ms pre-stimulus to 600 ms post-stimulus. The obtained curves (Fz, Cz, Pz and EOG) were displayed on a LCD-screen and for each electrode the N100 and P300 peaks after the deviant tone were manually indicated. The most negative deflection between 50 ms and 150 ms post-stimulus was considered as the N100, the most positive deflection between 250 ms and 400 ms as the P300. The obtained curves and the indicated peak values were printed and the paper report was stored in the patient's file. For 336 patients both DNA and P300 data were available. Because a reduction of P300 amplitude over the midline parietal electrode Pz was described as a very robust finding in patients with schizophrenia (Turetsky et al., 2007; van der Stelt and Belger, 2007), P300 amplitude at Pz was *a priori* used for all analyses. During the retrieval of the P300-data, the printed curves were visually inspected. The amplitude of the averaged EOG-curves exceeded the amplitude of the P300-waves in 145 patients. Although EOG-artefacts are not expected to affect measurements at Pz, analysis of the entire sample of 336 patients was complemented with a sensitivity analysis in the sample of 191 patients for whom the averaged EOG curves did not exceed the P300 amplitude.

2.3. Genetic variation

A previous study of our group, which examined molecular-genetic interactions with cannabis, selected a total of 179 SNPs, 152 of which passed quality control and were subsequently analyzed (van Winkel and GROUP Investigators, 2011). Gene selection in this study was based on previous evidence of association with schizophrenia, involvement in dopamine or endocannabinoid signaling or an involvement in the regulation of environmental influences including epigenetic mechanisms. Since this selection included the most studied candidate genes for schizophrenia prior to the genome-wide studies, this set was used as the starting point for our SNP selection. This set was updated with 24 SNPs either showing association with schizophrenia at grade 'A' or 'B' level in the SzGene database (Allen et al., 2008) (update 26 February 2010) or identified by genome-wide association studies (situated in PGBD1, NRGN, NOTCH4, PDE4B, TCF4, TPH1, HTR2A, RELN, MDGA1, CCKAR, DRD4, APOE, GWAS 11p14.1, PLXNA2, GABRB2, SRR, ANK3, CACNA1C, ZNF804A, MHC, MIR137). Finally, a set of 10 SNPs was selected for intended pharmacogenetic studies (in ABCB1, ADH1C, AS3MT, CYP17A1, CYP1A2, CYP2D6, FOXA2, GSTP1, SOD2). It was decided to also analyze these SNPs in the context of this study, since for one of these SNPs, rs1045642 in ABCB1, an association with P300-amplitude was found in a sample of healthy controls using a data-driven analysis with 384 SNPs in 222 genes, which survived stringent correction for multiple testing (Liu et al., 2009).

The sample used in this article is completely independent of the GROUP sample that was used to examine the molecular-genetic interactions with cannabis (van Winkel and GROUP Investigators, 2011). The sample analyzed here is part of a larger sample (UPC-CUL sample) previously described in the context of metabolic syndrome (van Winkel et al., 2010), which now has been genotyped for the same markers as the GROUP sample with the intention of replication of the molecular-genetic cannabis findings. From the UPC-CUL sample, only the patients for whom a P300 measurement was available ($n = 336$) were included in this current study.

The selected SNPs were determined by Sequenom (Hamburg, Germany) using the MassARRAY iPLEX platform at the facilities of