DTNBP1 (dysbindin) gene variants modulate prefrontal brain function in schizophrenic patients – support for the glutamate hypothesis of schizophrenia

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Dysbindin (DTNBP1) is a recently characterized protein that seems to be involved in the modulation of glutamatergic neurotransmission in the human brain, thereby influencing prefrontal cortex function and associated cognitive processes. While association, neuroanatomical and cellular studies indicate that DTNBP1 might be one of several susceptibility genes for schizophrenia, the effect of dysbindin on prefrontal brain function at an underlying neurophysiological level has not yet been explored for these patients. The NoGo-anteriorization (NGA) is a topographical event-related potential measure, which has been established as a valid neurophysiological marker of prefrontal brain function. In the present study, we investigated the influence of seven dysbindin gene variants on the NGA in a group of 44 schizophrenic patients. In line with our a priori hypothesis, one DTNBP1 polymorphism previously linked to schizophrenia (rs2619528) was found to be associated with changes in the NGA; however, the direction of this association directly contrasts with our previous findings in a healthy control sample. This differential impact of DTNBP1 gene variation on prefrontal functioning in schizophrenic patients vs. healthy controls is discussed in terms of abnormal glutamatergic baseline levels in patients suffering from schizophrenic illnesses. This is the first report on a role of DTNBP1 gene variation for prefrontal functioning at a basic neurophysiological level in schizophrenic patients. An impact on fundamental processes of cognitive response control may be one mechanism by which DTNBP1 gene variants via glutamatergic transmission contribute to the pathophysiology underlying schizophrenic illnesses.

Keywords: DTNBP1, endophenotype, Go/NoGo, NoGo-anteriorization, prefrontal cortex, schizophrenia

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Dysbindin (DTNBP1) is a recently characterized protein that is part of the dystrophin protein complex in muscle and non-muscle cells and binds to α- and β-dystrobrevins. Variations in the human DTNBP1 gene located on chromosome 6p22.3 have repeatedly been associated with schizophrenia (Bray et al. 2005; Funke et al. 2004; Schwab et al. 2003; Straub et al. 2002; Tochigi et al. 2006; Van den Oord et al. 2003; Williams et al. 2004). However, reported risk alleles and haplotypes differ between studies, and comparison among studies is hampered by the use of different marker sets (Mutsuddi et al. 2006). Currently, DTNBP1 together with NRG1 is the gene most strongly supported as a susceptibility gene for schizophrenia (Kirov et al. 2005; Owen 2005).

Dysbindin is prevalent in axons, particularly with large synaptic terminals such as the mossy fibres in the hippocampus, dorsal cochlear nuclei and cerebellum (Benson et al. 2001). Studies in primary cortical neuronal cultures suggest an influence of DTNBP1 on exocytotic glutamate release and a promotion of neuronal viability through phosphatidylinositol 3 (PI3)-kinase-Akt signalling (Numakawa et al. 2004). In the human brain, DTNBP1 may modulate glutamatergic neurotransmission by multiple postsynaptic and presynaptic mechanisms, partly independent of the dystrophin protein complex and including modulation of other (e.g. gabaergic, dopaminergic) neurotransmitter systems (Harrison & Weinberger 2005; Talbot et al. 2006).

In the dorsolateral prefrontal cortex of patients with schizophrenia, decreased DTNBP1 expression was reported that correlated with DTNBP1 genotype (Weickert et al. 2004). Furthermore, in hippocampus of schizophrenic patients diminished DTNBP1 expression was observed that correlated with increases in vesicular glutamate transporter 1 (Talbot et al. 2004). These changes in DTNBP1 gene expression were considered to contribute to the cognitive deficits observed in schizophrenias.

In line with this view, a dysbindin risk haplotype has been associated with negative symptoms in schizophrenia (DeRosse et al. 2006), and variations in the dysbindin gene were found to impact on cognitive functions mediated by...
the prefrontal cortex (PFC) (Burdick et al. 2006; Donohoe et al. 2007; Takao et al. 2008). Moreover, a recent ‘imaging genetics’ study reported an effect of schizophrenia-related dysbindin variants on electrophysiological measures of PFC function in healthy subjects (Fallgatter et al. 2006). However, to date no study has directly investigated the effect of dysbindin gene variation on PFC function in schizophrenic patients using an imaging genetics approach.

Out of the different electroencephalographic (EEG) and event-related potential (ERP) measures, the NoGo-antiorieriation (NGA) as a neurophysiological marker of PFC function and cognitive response control seems particularly suited for the application in imaging genetics studies because of its simple application and high interindividual stability (Fallgatter et al. 1997) as well as excellent short- and long-term test-retest reliability (Fallgatter et al. 2001, 2002a). The NGA represents the topographical difference between Go- and NoGo-ERPs, reflects medial prefrontal cortex activity (Barry & Rushby 2006; Fallgatter et al. 2002b) and is altered in schizophrenic patients (Fallgatter & Müller 2001).

Based on these findings, we hypothesized a modulation of the NGA in schizophrenic patients by the same variations in DTNBP1 previously investigated in healthy controls (Fallgatter et al. 2006). To test this hypothesis, we examined the association of seven single nucleotide polymorphisms (SNPs) in the DTNBP1 gene with the NGA in a Caucasian sample of schizophrenia patients. In the previous control sample, the A-allele of DTNBP1 rs2619528 was found to be associated with enhanced medial prefrontal functioning in terms of significantly higher values of the NGA. Rs2619528 was therefore the primary target SNP for the present investigation.

Materials and methods

Sample

The study was approved by the Ethics Committee and by the research conference of the Department of Psychiatry, Psychosomatics, and Psychotherapy of the University of Wuerzburg (Germany). Forty-four in-patients (22 females, 22 males; 39 right-handed, 5 left-handed; mean age: 36.3±9.8 years) from the Department of Psychiatry, Psychosomatics, and Psychotherapy of the University of Wuerzburg, who were treated under the diagnosis of a schizophrenic illness according to Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria, were included. All of them gave their written informed consent after extensive description of the study procedures. Mean duration of illness was 132.6 months (±49.4), with a mean of 5.0±6.4 previous in-patient treatments. The antispsychotic treatment at the time-point of investigation consisted of 483.4±376.1 mg in chlorpromazine equivalents (calculated according to the specifications of Laux and colleagues on clinical-empirical equivalent doses of typical and atypical antipsychotic drugs; Laux et al. 2000). Eighteen patients received typical (perazine (n=13), perazine + flupentixol (n=2), flupentixol (n=1), haloperidol (n=1), chlorpromazine + perphenazine (n=1)) and 18 patients atypical antipsychotics (risperidone (n=8), clozapine (n=5), olanzapine (n=3), amisulpride (n=1), ziprasidone (n=1)); 8 patients received no antipsychotic medication at the time of testing. Psychometric assessments with two scales used to measure symptom severity in schizophrenic patients (Brief Psychiatric Rating Scale/BPRS; Positive and Negative Syndrome Scale/PANSS) resulted in a BPRS score of 37.2±10.6, a PANSS total score of 58.6±15.7, a PANSS positive score of 13.8±4.7 points (7-item subscale assessing the severity of positive symptoms), a PANSS negative score of 14.4±5.5 (7-item subscale assessing the severity of negative symptoms) and a PANSS global score of 30.4±8.4 points (16-item subscale assessing the severity of global symptomatology). No significant depressive or anxiolytic symptomatology was present at the time-point of investigation, as indicated by a Hamilton Depression Rating Scale (HDRS) score of 8.9±5.5 points. Compared with the previously published sample of healthy controls (Fallgatter et al. 2006), the current group of schizophrenic patients did not significantly differ regarding the handedness (Fisher’s exact test: P = 0.57) or gender distribution (χ² = 0.16, P = 0.69); however, with a mean age of 26.5±7.2 years, the previous control sample was significantly younger than the current sample of patients (t₁₉₅ = 5.50, P < 0.001).

NGA measurement with the continuous performance test

Patients sat on a comfortable chair in front of a computer screen in a dimly lit and electrically shielded room to perform the Continuous Performance Test (CPT) during a 21-channel EEG recording. The instruction was to press a response button with the index finger of the right hand as fast as possible, whenever a letter ‘O’ appearing on the computer screen was directly followed by a letter ‘X’. Accuracy and speed were emphasized equally during the instruction. A short training session was performed, in order to ensure a correct understanding of the task. During the CPT task with a total length of about 13 min, 400 letters were presented sequentially for 200 milliseconds each in a pseudo-randomized order with an interstimulus interval of 1650 milliseconds. The whole task consisted of 400 letters, with 114 primer conditions (O), 57 Go (O–X) and NoGo (O, any other letter) conditions and 172 distractors (other letters, or letter X without a preceding O).

EEG recording

The EEG was recorded from 21 scalp electrodes, which were properly positioned with electrode cream according to the International 10-20 system (Fp1, Fp2, F3, F4, F7, F8, T3, T4, C3, C4, T5, T6, P3, P4, O1, O2, Fpz, Fz, Cz, Pz and Oz). For the registration of eye movements, three additional electrodes were placed at the outer canthi of both eyes and below the right eye. Linked mastoids were used as the recording reference. All electrode impedances were constantly kept below 5 kΩ. A 32-channel DC amplifier (Brain Star System) and the Neuroscan data acquisition software were used for EEG recording. The A/D rate was 256 Hz; the hardware filter was set to a bandpass of 0.1–70 Hz.

Data analysis

A computerized artifact rejection was applied which excluded all segments with amplitudes exceeding 50 μV in any of the EEG or ERP channels within the first 700 milliseconds after stimulus onset. The remaining artifact-free EEG segments for all correct responses at the behavioural level (button press in the Go condition, no button press in the NoGo condition) were then averaged to one Go and one NoGo-ERP for each patient. A mean of 46.0±9.7 and 49.0±6.8 epochs were available for the Go and NoGo conditions, respectively. For the NoGo-ERP, the time-point of the most positive peak at electrode position Cz within a P300 time window (290–480 ms) was used to calculate the two-dimensional topography by means of the centroid method (Lehmann 1987), whereas the respective peak at electrode position Pz was used for the Go condition. A coordinate system with the integers 1–5 at the respective electrode rows in the anterior-posterior direction (‘1’ = level of electrode position Fpz; ‘5’ = level of electrode position Oz) was used to quantify the centroids of the Go- and NoGo-ERPs. For a more detailed description of the centroid method, please confer the work of Lehmann (1987) and our previous publications (e.g. Fallgatter et al. 1997). Finally, the NGA, defined as the distance between the individual Go and NoGo centroid within a P300 time window (290–480 ms), was used to calculate a Go- and NoGo centroid above Cz (third row >= 3) would result in an arbitrary NGA value of 1 (4–3 ≤ 1). The grand...
average curves of the two patient groups (see below) at the two relevant electrode positions (Cz, Pz) are displayed in Fig. 1, along with the topographical maps of the P300 distribution for the Go and NoGo conditions, respectively.

Genotyping
DNA was extracted from whole blood samples using standard protocols. Seven SNPs (Table 1) that cover the DTNBP1 gene and its 5′ and 3′ flanking region were selected based on the initial study (Straub et al. 2002), and further reports showing association with schizophrenia as listed in the SZGene database (http://www.szgene.org; Allen et al. 2008). Samples were genotyped by Custom TaqMan SNP genotyping 5′ exonuclease allelic discrimination assays (Applied Biosystems, Darmstadt, Germany). Preparation of the polymerase chain reaction (PCR) reaction mixtures was performed by Genesis Workstation RSP 150 (Tecan, Crailsheim, Germany). For PCR amplification and allelic discrimination, the ABI Prism 7900 HT Sequence Detection System and SDS software version 2.1 (Applied Biosystems) were used. Genotypes were assigned automatically for allelic discrimination data with SDS auto caller as well as manually for individual absolute quantification data, both resulting in concordance rates of 100%. Quality values were >98.8% (genotyping error rates: <1.2%) for all SNPs with the exception of SNP rs1000117, which therefore was further genotyped via direct sequencing.

All polymorphisms were in Hardy–Weinberg equilibrium (P > 0.05; assessed by online resource www.kursus.kvl.dk/shares/vetgen/Popgen/genetik/applets/kitest.htm; for genotype frequencies, see Table 1).

Figure 1: Go/NoGo grand average waveforms. Grand average curves for the CPT Go (thin) and NoGo condition (bold line) in patients with an A/G (n = 15; top) vs. G/G (n = 29; bottom) genotype of rs2619528 of DTNBP1. The maps display the brain electrical field at the time-point of the respective P300 peak for the Go (the upper one of each pair of maps) and NoGo (lower maps) condition as well as respective difference potentials (NoGo-Go).
**Statistical analysis**

Based on the results of our previous study in healthy controls (Fallgatter et al. 2006), we primarily tested the single a priori hypothesis, that an association of the NGA and dysbindin variants would occur for rs2619528 with a significance level of \( P = 0.05 \) (uncorrected \( \alpha \) level as only one test was applied). Additional exploratory analyses were performed on the dependent variable NGA for all seven assessed SNPs (compare Fig. 2 and Supporting information Appendix S1). Differences in NGA between genotype and haplotype groups were assessed by two-tailed \( t \)-tests for independent samples, parametric or non-parametric analyses of variance, as appropriate because of the distribution of residuals and the number of categories of the independent variable. Further analyses on behavioural performance measures as dependent variables [reaction times (RTs), commission errors, omission errors] and on Go/NoGo centroids were explored, if an individual SNP or haplotype showed nominal association with the NGA. In addition, exploratory analyses of P300 amplitudes and latencies were conducted in this case.

Linkage disequilibrium (LD) was analysed by Haploview version 3.32 (http://www.broad.mit.edu/mpg/haploview). Haplotypes from six SNPs (not including rs1000117 due to low pairwise \( D' \) measures) were reconstructed on an individual level by PHASE, version 2.11 based on a probability of reconstructed pairs of haplotypes for each individual >0.9. Three individuals had to be excluded because of lower probabilities of the reconstructed haplotypes. Haplotypes with a frequency >10% in the sample were analysed as present vs. not present in an exploratory manner.

**Results**

The CPT behavioural data were as follows: mean RT in Go trials 585.2 ± 163.3 milliseconds, number of omission errors (i.e. button presses after an NoGo trial) 1.82 ± 2.40 and number of omission errors (i.e. missed button presses after a Go trial) 6.71 ± 8.74. The NGA as our primary measure of prefrontal brain function had a mean value of 0.64 ± 0.46 (range from −0.83 to 1.50) electrode distances for the whole group of 44 schizophrenic patients.

According to our a priori hypothesis, the mean NGA differed significantly between patients carrying one A-allele (A/G) of rs2619528 and patients homozygous for the G-allele (G/G) (\( \chi^2 = 2.23, \ P < 0.05 \); Table 2, see also Supporting information Appendix S2). However, the direction of this difference in the present sample of schizophrenic patients (A/G vs. G/G: 0.43 ± 0.55 vs. 0.74 ± 0.38) was opposite to the one reported previously in healthy controls [A/A (n = 1) + A/G (n = 17): 1.00 ± 0.44 vs. G/G (n = 30): 0.58 ± 0.52, \( P < 0.007 \); Fallgatter et al. 2006], whereas genotype frequencies did not differ between the two samples (\( \chi^2 = 0.12, \ P = 0.73 \)).

On a behavioural level, patients of the rs2619528 A/G subgroup did not differ significantly from patients of the G/G group with respect to omission errors (A/G: 7.53 ± 10.93; G/G: 6.28 ± 7.55; Mann–Whitney \( U = 208.5, Z = -0.22, P = 0.82 \), commission errors (A/G: 2.40 ± 3.16; G/G: 1.52 ± 1.90; Mann–Whitney \( U = 197.5, Z = -0.52, P = 0.61 \)) or RTs (A/G: 614 ± 73 milliseconds; G/G: 570 ± 150.2 milliseconds; \( t_{42} = 0.84, P = 0.40 \)). Behavioural data showed no significant association with age (RT: \( r = 0.20, P = 0.20 \); omission errors: Rho = 0.06, \( P = 0.72 \); commission

**Table 1:** Polymorphisms of the DTNBP1 (dysbindin) gene

<table>
<thead>
<tr>
<th>dbSNP ID*</th>
<th>Allele</th>
<th>SNP location</th>
<th>SNP position†</th>
<th>Genotype frequencies</th>
<th>Minor allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1474588</td>
<td>G/C</td>
<td>3’ flanking region</td>
<td>15 569 688</td>
<td>G/G = 18, G/C = 21, C/C = 5</td>
<td>0.35</td>
</tr>
<tr>
<td>rs2619539</td>
<td>C/G</td>
<td>Intron 5</td>
<td>15 728 834</td>
<td>C/C = 19, G/C = 15, G/G = 10</td>
<td>0.40</td>
</tr>
<tr>
<td>rs2112207</td>
<td>A/G</td>
<td>Intron 4</td>
<td>15 736 091</td>
<td>A/A = 33, A/G = 5, G/G = 0</td>
<td>0.06</td>
</tr>
<tr>
<td>rs1011313</td>
<td>G/A</td>
<td>Intron 3</td>
<td>15 741 411</td>
<td>G/G = 30, A/G = 11, A/A = 3</td>
<td>0.19</td>
</tr>
<tr>
<td>rs2619528</td>
<td>G/A</td>
<td>Intron 3</td>
<td>15 757 808</td>
<td>G/G = 29, A/G = 15, A/A = 0</td>
<td>0.17</td>
</tr>
<tr>
<td>rs885773</td>
<td>A/G</td>
<td>5’ flanking region</td>
<td>15 777 465</td>
<td>A/G = 4, G/G = 40</td>
<td>0.05</td>
</tr>
<tr>
<td>rs1000117</td>
<td>T/G</td>
<td>5’ flanking region</td>
<td>15 981 968</td>
<td>T/T = 18, G/T = 23, G/G = 3</td>
<td>0.33</td>
</tr>
</tbody>
</table>

†UCSC Genome Browser, Human May 2004 Assembly (http://genome.ucsc.edu/cgi-bin/hgGateway).
Table 2: Comparing DTNBP1 genotypes with respect to the electrophysiological parameter of prefrontal brain activation (NGA)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>n</th>
<th>NGA mean</th>
<th>NGA SD</th>
<th>F (df)/χ² (df)*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1474588</td>
<td>C/C</td>
<td>5</td>
<td>0.70</td>
<td>0.4</td>
<td>F = 0.37 (2)</td>
<td>0.693</td>
</tr>
<tr>
<td></td>
<td>C/G</td>
<td>21</td>
<td>0.57</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>18</td>
<td>0.69</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2619539</td>
<td>C/C</td>
<td>19</td>
<td>0.64</td>
<td>0.5</td>
<td>χ² = 2.75 (2)</td>
<td>0.252</td>
</tr>
<tr>
<td></td>
<td>C/G</td>
<td>15</td>
<td>0.56</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>10</td>
<td>0.74</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3213207</td>
<td>A/A</td>
<td>39</td>
<td>0.69</td>
<td>0.4</td>
<td>χ² = 5.01 (1)</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>5</td>
<td>0.19</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1011313</td>
<td>A/A</td>
<td>3</td>
<td>1.1</td>
<td>0.1</td>
<td>χ² = 3.83 (2)</td>
<td>0.147</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>11</td>
<td>0.58</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>30</td>
<td>0.61</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2619528</td>
<td>A/G</td>
<td>15</td>
<td>0.43</td>
<td>0.5</td>
<td>F = 4.98 (1)</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>29</td>
<td>0.74</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs885773</td>
<td>A/G</td>
<td>4</td>
<td>0.54</td>
<td>0.7</td>
<td>χ² = 0.007 (1)</td>
<td>0.935</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>40</td>
<td>0.65</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1000117</td>
<td>G/G</td>
<td>3</td>
<td>0.64</td>
<td>0.2</td>
<td>χ² = 3.75 (2)</td>
<td>0.154</td>
</tr>
<tr>
<td></td>
<td>G/T</td>
<td>23</td>
<td>0.51</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>18</td>
<td>0.80</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

df, degree of freedom; SD, standard deviation.
*Kruskal–Wallis χ².

Discussion

This study is the first to report an association of a frequent variant of the DTNBP1 gene (AG/GG of rs2619528) with an electrophysiological measure of prefrontal brain function (NGA) in patients with schizophrenia, thereby complementing our previously reported findings in a sample of healthy participants (Fallgatter et al. 2006).
The observation that dysbindin gene variants modulate prefrontal cortex function is consistent with the fact that dysbindin is associated with glutamatergic neurotransmission. Glutamate is broadly distributed throughout the central nervous system including the (pre-) frontal cortex (e.g. Weickert et al. 2004), which implies that a general impact of this neurotransmitter and associated proteins on (pre-) frontal cortex function is highly probable. Previous findings confirm this assumption, showing an impact of the glutamatergic neurotransmitter system on the PFC and associated neuropsychological (executive) functions (Aultman & Moghaddam 2001; Nicolle & Baxter 2003). Of particular interest for the present study is the apparent impact of N-methyl-D-aspartate (NMDA) receptor neurotransmission in the medial PFC of the rat on measures of response inhibition (Murphy et al. 2005). A protein such as dysbindin influencing the glutamate neurotransmitter system is thus to be expected to affect PFC functions as well. This has also been shown previously, both in healthy control samples (impact of DTNBP1 genotype on general cognitive ability; Burdick et al. 2006) and in patients with schizophrenia (patients with the risk haplotype showed an impaired spatial working memory performance as compared with patients who were non-risk carriers; Donohoe et al. 2007). The observed impact of dysbindin gene variants on PFC neurophysiology in healthy controls and patients with schizophrenia is in line with these findings. It is also in line with functional magnetic resonance imaging (fMRI) studies showing a significant impact of DTNBP1 gene variation on frontal cortex activation during working memory (Markov et al. 2010) and episodic memory tasks (Thimm et al. 2010) in healthy control samples.

However, although the same DTNBP1 SNP was found to impact on prefrontal cortex function in patients with schizophrenia and our previous sample of healthy controls (Fallgatter et al. 2006), we did not truly replicate the previous findings, because the direction of this association was exactly the opposite in both groups. In the present sample of patients, the A-allele was associated with lower NGA values (indexing worse prefrontal brain function), whereas the same allele was associated with higher values of the NGA (i.e. better prefrontal function) in healthy controls. But although the opposite impact of DTNBP1 genotypes on the NGA in healthy controls vs. schizophrenic patients was unexpected, it is not entirely implausible.

The glutamatergic neurotransmitter system has frequently been suggested to be involved in the pathophysiology of schizophrenias (Goff & Coyle 2001; Kim et al. 1980; Moghadam & Krystal 2003; Nanitos et al. 2005). On the assumption of a deficient glutamatergic neurotransmission in patients with schizophrenia (Laruelle et al. 2005), a differential impact of genes coding for proteins that are involved in the glutamate system (e.g. dysbindin) in patients vs. healthy controls is not at all surprising. If one further assumes that – analogous to the dopamine system (Seamans & Yang 2004) – a medium level of glutamate is required for an optimal function of the prefrontal cortex, this ‘inverted u-shaped function’ could very well explain the current results. The G-allele of rs2619528 may optimize prefrontal brain function in schizophrenic patients (i.e. higher NGA values) by increasing glutamatergic neurotransmission, but may at the same time lead to a dysfunctional hyperglutaminergic state (i.e. lower NGA values) in the brain of healthy controls, who have a generally higher level of prefrontal glutamate as compared with schizophrenic patients (see Fig. 3; for the influence of DTNBP1 on glutamate release/exocytosis and vesicle biogenesis in neurons, see Chen et al. 2008). Such an inverted ‘u-shaped relation’ between glutamatergic tone and cognitive/PFC functioning is actually highly plausible, because – on the one hand – reduced glutamatergic activity has been associated with impaired processes of learning and memory (Magnusson 1998; Magnusson et al. 2007; Parwani et al. 2005; Winters & Bussey 2005), while – on the other hand – excessive glutamate concentrations are known to act neurotoxic (for review, see McEntee & Crook 1993; see also Riedel et al. 2003). Differences in the baseline level of the neurotransmitter would therefore underlie the differential effect of genes involved in the glutamate system in patients vs. controls. Similar considerations have previously been made for the dopaminergic system, where catechol-O-methyl transferase (COMT; a major enzyme involved in dopamine metabolism) was found to differentially influence PFC function in healthy controls and patients with schizophrenias (Ehlis et al. 2007b;
Figure 3: Hypothesized relationship between prefrontal level of glutamate and frontal lobe function. Hypothetical model on the relationship between prefrontal glutamate concentration and prefrontal brain function as indexed by the NGA. Mean NGA values were marked for the four study groups (schizophrenic patients/Sz and healthy controls with AG vs. GG genotype of rs2619528 of the DTNBP1 gene). Patients with schizophrenia are characterized by particularly low baseline glutamate concentrations (glutamate hypothesis). Owing to the assumed inverted u-shaped relation, dysbindin genotypes would be expected to affect patients and controls differently with respect to their prefrontal cortex function.

see also Tunbridge et al. 2006). It has to be acknowledged that such a model, although attractive, is highly speculative at this time and needs to be corroborated by further research.

Another factor that might lead to differences in baseline glutamate levels – and might therefore affect the impact of DTNBP1 – is the participants’ age. Both glutamate content and glutamate receptor expression and binding have been found to show a strong age-related decline (see e.g. Chang et al. 2009; Court et al. 1993; Dawson et al. 1989; Ohtani et al. 2004; Piggott et al. 1992). Therefore, a protein such as DTNBP1 directly affecting the synaptic release of glutamate (see Chen et al. 2008) might very well differentially impact cortical function in different age groups. As the previous sample of healthy participants was on average about 10 years younger than the current group of patients (see Materials and methods), it cannot be excluded that this age difference at least partly accounts for the opposing influence of DTNBP1 genotype in the two samples.

A closer look at the electrophysiological results showed not only one component of the Go-NoGo-related activation pattern to be compromised in the rs2619528 AG subgroup of the present sample of schizophrenic patients but also that both response execution and inhibition-related potentials contributed to the difference between the two genotype groups with respect to the compounded NGA measure. This is in contrast to the previous findings in healthy controls, where it was mainly the topography of the Go centroid that led to the reported finding. So, while in healthy controls the process of response execution was apparently different between the two genotypes, in schizophrenic patients dysbindin groups differed somewhat in processes of execution as well as inhibition of a primed motor response. Both of these – in itself statistically non-significant – differences added to the global difference in the measure of the NGA; in other words, the relationship between both processes on a neurophysiological level was altered, which is the crucial concept underlying the NGA.

On a functional level, the intronic SNP rs2619528 might be of possible relevance due to its position closely to a copy number variation site (CNV_1172734; http://genome.ucsc.edu/). Recently, Xu et al. (2009) showed that individually rare inherited copy number variants are more frequent in cases with familial or sporadic schizophrenia compared with unaffected controls, and affect almost exclusively genic regions. Furthermore, rs2619528 is located within a transcription factor binding site as analysed by MatInspector (http://www.genomatix.de/products/). This site might be important for the transcription factor ‘downstream immunoglobulin control element’ (VS\DICE.01), which is critical for B-cell activity and specificity. A direct link between alterations in the immune system and schizophrenia is suggested, as the percentage of CD19(+) B-lymphocytes was increased in patients during the acute state of psychosis compared with healthy volunteers (Maino et al. 2007).

Concerning the study’s limitations, it should be remarked that despite apparent differences in brain function between rs2619528 groups, we did not find any corresponding differences on a behavioural level (error rates, RTs). The fact that underlying differences in neuronal activation are not reflected in the patients’ performance might well be explained by the easiness of the applied version of the CPT, which entailed relatively long interstimulus intervals and presentation times. This particularly demanding task was specifically designed to produce clear-cut electrophysiological results, but is rather unsuited for the detection of performance differences between different patient samples and control subjects (e.g. schizophrenias; Fallgatter & Müller 2001; Fallgatter et al. 2003). It is therefore plausible to expect differences in brain function without accompanying behavioural effects when comparing different genetic subgroups of one clinical sample with this particular task.

Another point of criticism may certainly be the rather small sample size, e.g. compared to sample sizes employed in classical genetic association studies based on categorical phenotypes such as a psychiatric diagnosis. However, it has to be noted that compared to association studies with genetically complex behavioural traits, robust gene-brain activity correlations allow the investigation of substantially smaller sample sizes in study designs utilizing dimensional intermediate phenotype approaches such as the present one (Ehlis et al. 2007a; Fallgatter et al. 2004). Nevertheless, replication in independent and preferably larger samples, possibly also with other functional paradigms of prefrontal brain function, is warranted.

In summary, this is the first report of an impact of DTNBP1 gene variants on electrophysiological measures of prefrontal brain function in a sample of schizophrenic patients. While
glutamate neurotransmission and, more specifically, variations in the human DTNBP1 gene have been associated with schizophrenic illnesses before, speculations that an increased risk for schizophrenia by dysbindin risk haplotypes may be mediated by their influence on prefrontal cortex function (Donohoe et al. 2007) have – until now – never been confirmed on a basic neurophysiological level. The present findings support such a supposition, providing direct evidence for an impact of DTNBP1 genotypes on PFC function in patients suffering from schizophrenia. This impact on fundamental processes of cognitive response control might be one mechanism by which DTNBP1 gene variants contribute to the pathophysiology underlying schizophrenic illnesses.

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Supporting Information

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

**Appendix S1** Pairwise LD in DTNBP1 polymorphisms.

**Appendix S2** Distribution of NGA values across all participants.

**Appendix S3** Results of the haplotype analysis.

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