NeuroImage 47 (2009) 2016–2022

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Genetic variation in schizophrenia-risk-gene dysbindin 1 modulates brain activation in anterior cingulate cortex and right temporal gyrus during language production in healthy individuals

V. Markov [a,h,*], A. Krug [b], S. Krach [b], C. Whitney [a,h], T. Eggermann [c], K. Zerres [c], T. Stöcker [d,h], N.J. Shah [d,h], M.M. Nöthen [e], J. Treutlein [f], M. Rietschel [f], T. Kircher [g,*]

[a] Department of Psychiatry and Psychotherapy, Medical Faculty, RWTH Aachen University, Pauwelstraße, 30, D-52074 Aachen, Germany
[b] Department of Psychiatry and Psychotherapy, Section of Neuroimaging, Philipps-University, Marburg, Rudolf-Bultmann-Straße 8, D-35039 Marburg, Germany
[c] Institute of Human Genetics, RWTH Aachen University, Pauwelstraße, 30, D-52074 Aachen, Germany
[d] Institute of Neuroscience and Biophysics 3—Medicine, Research Center Jülich, D-52425 Jülich, Germany
[e] Department of Genomics, Life and Brain Center, University of Bonn, Sigmund-Freud-Str. 25, D-53127 Bonn, Germany
[f] Division of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, J 5, D-68159 Mannheim, Germany
[g] Department of Psychiatry und Psychotherapy, Philipps-University Marburg, Rudolf-Bultmann-Straße 8, D-35039 Marburg, Germany
[h] JARA—Translational Brain Medicine

ARTICLE INFO

Article history:
Received 10 November 2008
Revised 6 May 2009
Accepted 21 May 2009
Available online 1 June 2009

Keywords:
DTNBP1
rs1018381
P1578
fMRI
Verbal fluency

ABSTRACT

Genetic variation in dysbindin 1 (*DTNBP1*) gene region tagged by SNP rs1018381 exhibits a linkage with cognitive deficits in patients with schizophrenia and healthy subjects. Language production deficits are core features of schizophrenia with more impairment in semantic than lexical verbal fluency tasks. We investigated the link between brain activation and *DTNBP1* SNP rs1018381 during semantic verbal fluency task in a German healthy population.

46 healthy subjects genotyped for SNP rs1018381 status were divided in heterozygous risk-allele carriers (T/C) and homozygous non-carriers (C/C). Neural correlates of semantic verbal fluency were investigated with functional magnetic resonance imaging (fMRI).

Stronger right hemispherical brain activation in anterior cingulate gyrus (BA 24), superior (BA 22, 38) and middle (BA 21) temporal gyrus was observed in the carriers compared to non-carriers. Brain activations occurred in the absence of task performance differences. No significant correlations were found between personality traits and brain activation differences.

The results point to an influence of genetic variation in *DTNBP1* gene region tagged by SNP rs1018381 on neural correlates of language production. Carriers may exhibit higher processing efforts to reach the same behavioural performance as non-carriers as reflected in activation of schizophrenia-related regions.

Introduction

Schizophrenia is highly heritable (>80%), but the search for chromosomal loci and genes has been slow and arduous because of small single effects and interactions with epigenetic processes and environmental factors (Owen et al., 2002). Nevertheless, molecular studies using positional genetics identified several candidates including catechol-O-methyltransferase (*COMT*), dysbindin 1 (*DTNBP1*), neuregulin 1 (*NRG1*), regulator of G-protein signalling 4 (*RGS4*), *G72*, proline dehydrogenase (*PRODH*), dopamine D2 receptor (*DRD2*), disrupted-in-schizophrenia 1 (*DISC1*) and D-amino acid oxidase (*DAAO*). Recent studies yielded evidence that some of the genes listed above, such as *COMT* (for a review see Harrison and Weinberger, 2005), *DTNBP1* (Burdick et al., 2006; Burdick et al., 2007; Donohoe et al., 2007; Zinkstok et al., 2007; Luciano et al., 2009), *NRG1* (Hall et al., 2006), *DISC1* (Burdick et al., 2005), and *G72* (Goldberg et al., 2006) have an influence on cognition in patients with schizophrenia and healthy subjects.

Straub et al. (2002) identified and genotyped 17 SNPs from *DTNBP1* gene, mostly intronic, which showed association to schizophrenia. A number of subsequent studies could replicate the results for several of these SNPs, including the SNP rs1018381 (P 1578 by Straub et al., 2002), in worldwide populations (Schwab et al., 2003; Tang et al., 2003; Van Den Bogaert et al., 2003; van den Oord et al., 2003; Funke et al., 2004; Kirov et al., 2004; Numakawa et al., 2004). Although one meta-analysis pointed to inconsistency of the positively

* Corresponding authors. V. Markov is to be contacted at the Department of Psychiatry and Psychotherapy, RWTH Aachen University, Pauwelsstr. 30, D-52074 Aachen, Germany. Fax: +49 241 8082422. T. Kircher, Department of Psychiatry und Psychotherapy, Philipps-University Marburg, Rudolf-Bultmann-Straße 8, D-35039 Marburg, Germany. Fax: +49 6421 58 68939.
E-mail addresses: vmarkov@ukaachen.de (V. Markov), psychiat@med.uni-marburg.de (T. Kircher).

1053-8119/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2009.05.067

associated genetic variations at *DTNBP1* in the different European samples (Mutsuddi et al., 2006), two meta-analyses supported the more positive than negative association between genetic variations in *DTNBP1* and schizophrenia (Owen et al., 2004; Williams et al., 2005).

In the brain, dysbindin 1 binds to β-dystrobrevin, a member of the dystrophin protein complex (DPC), which has been implicated in synaptic structure and signalling of the glutamatergic neuronal system (Benson et al., 2001). The DPC is located at postsynaptic densities throughout various regions of the brain (Blake et al., 1999), and disruption of its function might result in structural and functional abnormalities in cerebral areas that have been associated with schizophrenia (Straub et al., 2002). Furthermore, presynaptic dysbindin involvement was observed in a study by Numakawa et al. (2004), who suggested that dysbindin 1 regulation of the expression of SNAP25 and synapsin 1 proteins in the presynaptic machinery associated with increased glutamate release. Further, a selective reduction in presynaptic dysbindin 1 expression as well as an inverse correlation of dysbindin 1 levels with VGlut-T-1, the primary vesicular glutamate transporter in the hippocampal formation, has been reported from Talbot et al. (2004). To date, numerous studies have reported decreased dysbindin 1 expression in schizophrenia-related brain regions, such as the hippocampus, dorsolateral prefrontal cortex (DLPFC) and midbrain, amongst other regions (Weickert et al., 2004; Numakawa et al., 2004; Talbot et al., 2004; Coyle, 2006; Weickert et al., 2008). Finally, Harrison and Weinberger (2005) proposed that glutamatergic synaptic alterations and aberrant neural circuitry are considered to be crucial for functional abnormalities in schizophrenia. However, the specific role of genetic variation of dysbindin 1 for malfunctioning glutamatergic neuronal system is still unclear.

Whilst a series of linkage and association studies have implicated the dysbindin 1 SNP rs1018381 with genetic susceptibility to schizophrenia (Straub et al., 2002; Schwab et al., 2003; Tang et al., 2003; Van Den Bogaert et al., 2003; Funke et al., 2004), there is also evidence for no significant results to this marker (Stefanis et al., 2007; Tosato et al., 2007). Although the allele frequencies of SNP rs1018381 differ between HapMap populations (see also Genetic analysis part), associations with the minor T-allele of this SNP were found in a study by Funke et al. (2004) in three independent samples of different ethnic origin (White, Hispanic and African American). In their study the SNP rs1018381 was conceived as a tagging SNP for the risk six-locus haplotype (CTCTAC) that was significantly over-represented in the Caucasian patients when compared with Caucasian controls (Funke et al., 2004). It was the only SNP of this risk-haplotype that showed a significant effect of genotype on cognitive abilities in patients with schizophrenia and healthy volunteers (Burdick et al., 2006). Minor T-allele carriers performed significantly worse on diverse cognitive tests than non-carriers.

Nonetheless, this SNP tags a large number of SNPs, and it is not clear which of these SNPs is responsible for the observed effect (see also Genetic analysis part). Using the web tool Haplotter (http://pritch.bsd.uchicago.edu/data.html) to investigate any marker of *DTNBP1* in HapMap in the CEU population (for a method of *DTNBP1* selection in the HapMap see Voight et al., 2006), it could be shown that the SNP rs1018381 is in perfect LD ($r^2 = 1.0$) with the majority of SNPs (rs11755055, rs9296984, rs2619535, rs2743550, and rs9476860) of top scoring markers in *DTNBP1*. However, in a single SNP query, the integrated haplotype score (iHS) for marker rs1018381 itself (standardized iHS = −1.957) just failed to approach the positive/negative iHS scores of >2/<−2, which were used as a threshold to identify the strongest signals of selection in the study of Voight et al. (2006). Thus, the assumed SNP cannot serve as functional but its role as a significant marker in *DTNBP1* remains crucial.

A series of current studies supported the influence of this and other SNPs of this risk-haplotype on cognitive abilities in healthy individuals. Luciano et al. (2009) showed significant associations between the SNP rs1018381 and verbal ability, the SNPs rs2619528 and rs1011313 and executive function, and the SNP rs2619522 and memory, speed and executive function in two cohorts. Furthermore, the SNPs rs2619522 and rs760761 were associated with lower sustained attention in healthy volunteers (Stefanis et al., 2007). Finally, only the SNP rs1018381 implemented in the risk six-locus haplotype (CTCTAC) reported by Burdick et al. (2006) and Funke et al. (2004) seemed to be characteristic for cognitive deficits in patients with schizophrenia and healthy individuals.

Deficient language production is one of the core symptoms in patients with schizophrenia. Results from meta-analyses (DeLisi, 2001; Sitskoorn et al., 2004; Szöke et al., 2004; Snitz et al., 2006) have provided evidence that this deficiency has a strong genetic component. Verbal fluency is a widely employed measure to assess alteration in language production in schizophrenia patients (for a review see Bokat and Goldberg, 2003). Two meta-analyses have shown that patients with schizophrenia were more impaired on semantic (the word generation is constrained by a specified category, e.g. “tools”) relative to lexical (the participants are asked to generate as many words as possible beginning with a specified letter, e.g. “S”) fluency tasks, which has been taken as evidence for a selective semantic deficit (Bokat and Goldberg, 2003; Henry and Crawford, 2005). Further, Chen et al. (2000) have found that semantic verbal fluency abnormality can serve as a familial trait marker for schizophrenia.

In healthy populations, cerebral correlates of semantic verbal fluency comprise a left-lateralized fronto-temporal network (for a review on word production see Indefrey and Levelt, 2004). Due to the demand on organizing and controlling the semantic search, validation and retrieval processes, the most consistent activations have been found in the lateral and medial prefrontal cortex including the inferior frontal gyrus and anterior cingulate cortex, complimented by lateral and polar temporal areas, the thalamus or hippocampal region (Markowitsch, 1995; Cabeza and Nyberg, 2000; Crespo-Facorro et al., 2004; Demonet et al., 2005; Basho et al., 2007; Kircher et al., 2008; Ragland et al., 2008; Whitney et al., 2009). Functional imaging studies of verbal fluency in patients with schizophrenia report aberrant activation patterns in prefrontal (Yurgelun-Todd et al., 1996; Boksman et al., 2005; Schaufelberger et al., 2005), anterior cingulate (Fletcher et al., 1996) and temporal structures (Yurgelun-Todd et al., 1996; Kuperberg et al., 2007). Such neural abnormalities have been interpreted within the fronto-temporal dysconnectivity hypothesis (Friston and Frith, 1995). Further, when patients with schizophrenia are compared with healthy controls during semantic verbal fluency tasks, stronger activation has been found in the left prefrontal and motor cortex, right anterior cingulate gyrus, bilateral insula, right putamen, bilateral middle and STG, left inferior and superior parietal lobule, left lingual gyrus, left cuneus and precuneus, and cerebellum (Ragland et al., 2008).

The aim of the current study was to investigate the influence of the SNP rs1018381 of the *DTNBP1* gene on neural correlates of verbal fluency processes in healthy volunteers. Consistent with the findings in the literature (Markowitsch, 1995; Cabeza and Nyberg, 2000; Crespo-Facorro et al., 2004; Demonet et al., 2005; Basho et al., 2007; Kircher et al., 2008; Ragland et al., 2008; Whitney et al., 2009), differential activation for semantic but not for lexical verbal fluency in the lateral and medial prefrontal cortex, right anterior cingulate gyrus, bilateral insula, bilateral middle and STG, left inferior and superior parietal lobule, and cerebellum was expected. Stronger brain activations in some or all of these cerebral regions in risk-allele carriers compared with non-carriers and correlations between them and verbal fluency task performance were hypothesized.

Materials and methods

Participants

A sample of 521 subjects (268 men, 253 women) was recruited from students of the RWTH Aachen University. Advertisements were