◆ Human Brain Mapping 36:378–390 (2015) ◆

A Genome-wide Supported Psychiatric Risk Variant in *NCAN* Influences Brain Function and Cognitive Performance in Healthy Subjects

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Abstract: The A allele of the single nucleotide polymorphism (SNP) rs1064395 in the *NCAN* gene has recently been identified as a susceptibility factor for bipolar disorder and schizophrenia. *NCAN* encodes neurocan, a brain-specific chondroitin sulfate proteoglycan that is thought to influence neuronal adhesion and migration. Several lines of research suggest an impact of *NCAN* on neurocognitive functioning. In the present study, we investigated the effects of rs1064395 genotype on neural processing and cognitive performance in healthy subjects. Brain activity was measured with functional magnetic resonance imaging (fMRI) during an overt semantic verbal fluency task in 110 healthy subjects who were genotyped for the *NCAN* SNP rs1064395. Participants additionally underwent comprehensive neuropsychological testing. Whole brain analyses revealed that *NCAN* risk status, defined as AA or AG genotype, was associated with a lack of task-related deactivation in a large left lateral temporal cluster extending from the middle temporal gyrus to the temporal pole. Regarding neuropsychological measures, risk allele carriers demonstrated poorer immediate and delayed verbal memory performance when compared to subjects with GG genotype. Better verbal memory performance was significantly associated with greater deactivation of the left temporal cluster during the fMRI task in subjects with GG genotype. The current data demonstrate that common genetic variation in *NCAN* influences both neural processing and cognitive performance in healthy subjects. Our study provides new evidence for a specific genetic influence on human brain function. *Hum Brain Mapp 36:378–390, 2015.*

Key words: *NCAN*; rs1064395; fMRI; verbal fluency; verbal memory; left middle temporal gyrus; left temporal pole

INTRODUCTION

Genome-wide significant association has been found between bipolar disorder and the minor allele A of the single nucleotide polymorphism (SNP) rs1064395 in the *NCAN* gene [Cichon et al., 2011]. A subsequent association study revealed that this common variation in *NCAN* also confers risk to schizophrenia, suggesting that *NCAN*

Contract grant sponsor: Deutsche Forschungsgemeinschaft (DFG, German Research Foundation); Contract grant number: KR 3822/2-1

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Received for publication 30 May 2014; Accepted 3 September 2014.

DOI: 10.1002/hbm.22635
Published online 13 September 2014 in Wiley Online Library (wileyonlinelibrary.com).

belongs to a class of susceptibility factors that are shared across affective and psychotic disorders [Mühleisen et al., 2012]. In line with this assumption, the *NCAN* risk allele has been associated with the manic symptom dimension "overactivity" across diagnostic categories [Miró et al., 2012].

NCAN, which is located at chromosomal position 19p13.11, encodes neurocan, a central nervous system (CNS)-specific chondroitin sulfate proteoglycan (CSPG) of the lectican family [Prange et al., 1998]. Neurocan is a significant component of the extracellular matrix (ECM) and is thought to participate in the modulation of neuronal adhesion, neuronal migration, and neurite growth through the interaction with other ECM components and cell surface molecules [Friedlander et al., 1994; Prange et al., 1998; Rauch et al., 2001; Rhodes and Fawcett, 2004; Yamaguchi, 2000; Zimmermann and Dours-Zimmermann, 2008]. Since *NCAN* is highly expressed in the brain especially during (re)modeling stages it may play a significant role in the "fine tuning" of the developing CNS [Rauch et al., 2001].

There is increasing evidence for a role of neurocan in the regulation of synaptic plasticity [Dimatelis et al., 2013; Geissler et al., 2013; Rauch, 2004; Schwarzacher et al., 2006; Zhou et al., 2001]. Neurocan-deficient mice display no obvious aberrations of brain morphology (i.e., the gross anatomical brain development is intact), yet deficits in synaptic plasticity have been observed in these animals [Rauch, 2004; Zhou et al., 2001]. This finding indicates that *NCAN* is responsible for more subtle alterations in the brain that may have an influence on cognitive functioning [Rauch, 2004; Zhou et al., 2001]. In line with this assumption, *NCAN* risk status, defined as AA or AG genotype, has been found to influence cortical folding in the right occipital cortex and the left dorsolateral prefrontal cortex in patients with schizophrenia [Schultz et al., 2014]. Moreover, *NCAN* expression has been localized in the cerebral cortex and the hippocampus of mice and humans, further pointing to an involvement of *NCAN* in higher-order cognition [Cichon et al., 2011].

Neurocognitive impairment is a core feature of bipolar disorder and schizophrenia and can be observed during all stages of the illness [Arts et al., 2008; Bora and Murray, 2014; Hill et al., 2008; Robinson et al., 2006; Szöke et al., 2008]. The most pronounced deficits have been found in the domains of memory, verbal fluency, executive function, and attention [Hill et al., 2008; Robinson et al., 2006; Szöke et al., 2005]. Some cognitive dysfunctions can also be detected in subjects at high genetic risk for developing bipolar disorder or schizophrenia (i.e., in unaffected relatives of patients), indicating an association of the affected cognitive domains with genetic liability to these psychiatric conditions [Arts et al., 2008; Hill et al., 2008; Szöke et al., 2005]. One of the affected domains is verbal fluency, which requires subjects to generate, within a time-limit, as many words as possible that belong to a specific semantic category (semantic verbal fluency) or that begin with a specific letter (phonemic verbal fluency). Particularly, the semantic variant has been suggested as potential endophenotypic marker for schizophrenia and recently also for bipolar disorder [Drysdale et al., 2013; Erol et al., 2012; Hu et al., 2011; Szöke et al., 2008]. Semantic verbal fluency is supposed to rely on multiple cognitive processes, including access to and retrieval from semantic memory, working memory, sustained attention, efficient task initiation, application of a search strategy, and monitoring of responses [e.g., Henry and Crawford, 2004; Reverberi et al., 2014; Ruff et al., 1997].

Several functional magnetic resonance imaging (fMRI) studies have reported altered neural responses during verbal fluency paradigms in patients with bipolar disorder and patients with schizophrenia as well as in their unaffected first-degree relatives, indicating that these functional alterations may partly be inherited [Allin et al., 2010; Boksman et al., 2005; Broome et al., 2009; Costafreda et al., 2009, 2011; Curtis et al., 2001, 2007; Fu et al., 2005; Ragland et al., 2008]. However, results have been heterogeneous with activation differences ranging from increased or decreased recruitment of task-relevant brain regions, such as inferior frontal and superior temporal cortical areas [e.g., Boksman et al., 2005; Ragland et al., 2008], to reduced deactivation of areas belonging to the default mode network (DMN) [e.g., Allin et al., 2010], a network of brain regions that are typically more active during rest as compared to various cognitive tasks [Raichle et al., 2001]. These inconsistencies may be explained by methodological differences as well as by differences in clinical or sociodemographic characteristics of study participants.

Altered neural responses during verbal fluency performance, again ranging from differential recruitment of task-relevant brain regions to changes in the suppression of DMN areas, have also frequently been detected in subjects carrying genetic risk variants for bipolar disorder or schizophrenia such as *CACNA1C* or *DAOA/G72* polymorphisms [Kircher et al., 2009; Krug et al., 2009, 2010, 2011; Markov et al., 2009; Mechelli et al., 2008; Papagni et al., 2011; Prata et al., 2008, 2012]. These fMRI findings further point to a partly genetic origin of the functional disturbances in the brain that underlie impaired cognitive abilities in patients [Hill et al., 2008; Savitz et al., 2005].

Given that *NCAN* is supposed to be responsible for changes in the brain that may influence cognitive processing, such as altered synaptic plasticity [Rauch, 2004; Zhou et al., 2001], it is likely that genetic variation in *NCAN* influences performance as well as neural responses during cognitive challenges.

Therefore, the aim of the present study was to explore the effects of common genetic variation in *NCAN* on brain functioning in healthy subjects. First, we investigated the effect of *NCAN* rs1064395 genotype on various cognitive domains known to be impaired in bipolar disorder and schizophrenia, including verbal memory, executive functioning, working memory, processing speed, and attention. We expected decreased performance in risk allele carriers compared to subjects without risk allele, especially on

measures of memory and executive functioning. Second, we explored the effects of *NCAN* rs1064395 genotype on brain activation by means of fMRI. For this purpose, we used a well-tested overt semantic verbal fluency paradigm that has been shown to reliably elicit robust activation in a widespread bilateral language network [Backes et al., 2014, in press; Nagels et al., 2012]. Overt semantic word production, compared to rest, typically activates (pre)frontal and temporal cortical areas as well as the cerebellum [Birn et al., 2010; Heim et al., 2008; Kircher et al., 2011; Nagels et al., 2012]. Moreover, semantic verbal fluency tasks are sensitive for the effects of genetic risk variants for bipolar disorder or schizophrenia on brain function [Backes et al., 2014; Kircher et al., 2009; Krug et al., 2009, 2010, 2011; Markov et al., 2009]. In light of the heterogeneous findings in patients and their unaffected relatives, as reviewed above, we examined whole brain activation and modeled both the baseline and the word generation condition in the group analysis to facilitate the detection of altered task-related deactivations (for details see "Group analyses"). On the one hand, we expected rs1064395 risk status to affect the activation pattern of task-relevant brain areas. Since changes in prefrontal functioning have been frequently found in patients with bipolar disorder and patients with schizophrenia [e.g., Boksman et al., 2005; Curtis et al., 2007; Fu et al., 2005], we hypothesized risk allele carriers to show altered neural responses in the left inferior frontal gyrus, a key prefrontal area for verbal fluency. However, as both hypo- and hyperactivation have been reported, we did not make a prediction regarding the direction of the effect. On the other hand, we hypothesized that risk allele carriers also demonstrate reduced deactivation of brain areas that typically show decreased activation during task execution such as DMN regions, according to recent findings in remitted patients and unaffected first-degree relatives [Allin et al., 2010; Costafreda et al., 2011]. Based upon the evidence of impaired semantic verbal fluency in patients [e.g., Robinson et al., 2006; Szöke et al., 2008], we further expected poorer task performance in risk allele carriers compared to subjects without risk allele.

MATERIALS AND METHODS

Ethics Statement

This study was approved by the ethics committee of the Faculty of Medicine, Philipps-University Marburg, according to the latest version of the Declaration of Helsinki. After a complete description of the procedure, all subjects gave written informed consent to participate in the study.

Subjects

A sample of 110 healthy individuals (aged 18–56 years) of Western- or Middle-European ancestry (mainly German), whose sole native language was German, was investigated. This sample partly overlapped with that of Nagels et al. [2012] and with the control samples of Backes et al. [2014, in press]. Regarding the *NCAN* SNP rs1064395, 3 subjects were homozygous carriers of the A allele, 27 were heterozygous carriers of the A and G allele, and 80 were homozygous carriers of the G allele. Allele frequencies did not deviate from Hardy-Weinberg equilibrium (Fisher's exact test, $P = 0.71$). Due to the small number of individuals with AA genotype, AA and AG carriers were investigated together in an A carrier group. Subjects in the GG genotype group were aged 18–56 years; subjects in the A carrier group were aged 19–55 years. All characteristics of the two genotype groups are listed in Table I.

To rule out current or lifetime psychiatric disorders in participants, the German version of the Structured Clinical Interview for DSM-IV (SCID-I) [Wittchen et al., 1997] was conducted. Subjects with psychiatric history in first-degree relatives were also excluded from the study. Further exclusion criteria were head movement exceeding 3 mm or 3° in any direction, left-handedness, current alcohol/drug abuse or a lifetime history of alcohol/drug dependence, neurological disorders, serious head injury, mental retardation, severe somatic diseases, or any condition that might have an effect on cerebral metabolism or MR safety.

IQ of study participants was estimated with the multiple choice vocabulary test MWT-B ["Mehrfachwahl-Wortschatz-Intelligenz-Test"; Lehrl et al., 1995]. Lateralization quotient was calculated using the Edinburgh Handedness Inventory [Oldfield, 1971]. Subjects also completed the Beck Depression Inventory (BDI) [Hautzinger et al., 1994] and the trait version of the State-Trait Anxiety Inventory (STAI-T) [Laux et al., 1981]. Sex ratio, age, years of education, estimated IQ, lateralization quotient, BDI score, and STAI-T score did not differ between genotype groups as revealed by independent samples *t*-tests (two-tailed) (see Table I).

Genotyping

Genomic DNA was extracted from ethylenediaminetetraacetic acid (EDTA) anticoagulated venous blood according to standard procedures. The *NCAN* SNP rs1064395 was genotyped on a Life Technologies 7900HT Fast Real-Time PCR System using a TaqMan 5′ nuclease assay (TaqMan® SNP Genotyping Assay ID C_8931759_20; Life Technologies). Genotyping accuracy was assessed by running 15% of the sample in duplicates. Reproducibility was 100%.

Neuropsychological Assessment

In addition to the scanning procedure, a neuropsychological assessment was carried out including tests of episodic verbal memory [Verbal Learning and Memory Test, VLMT; Helmstaedter et al., 2001], executive function [Trail Making Test, TMT; Reitan, 1955], visuospatial working memory [Spatial Span, derived from the Wechsler Memory Scale-R; Härting et al., 2000], verbal working memory

TABLE I. Characteristics, neuropsychological data, and verbal fluency data of rs1064395 genotype groups

	AA/AG ($n = 30$)	GG ($n = 80$)	Statistic	P
Sex (male/female)	18/12	49/31	$\chi^2 = 0.01$	0.905
Age	33.73 (10.94)	32.84 (10.75)	$t_{108} = 0.39$	0.699
Years of education	11.7 (1.51)	11.85 (1.45)	$t_{108} = -0.48$	0.634
Estimated IQ	115.27 (14.86)	116.64 (14.77)	$t_{108} = -0.43$	0.666
Lateralization quotient	0.85 (0.22)	0.88 (0.17)	$t_{108} = -0.78$	0.438
BDI	2.23 (2.49)	3.23 (3.53)	$t_{108} = -1.41$	0.161
STAI-T	32.29 (4.96)	33.8 (7.75)	$t_{108} = -0.99$	0.325
Verbal memory				
VLMT immediate recall[a]	53.97 (9.78)	59.34 (7.49)	$F_{1,\ 106} = 9.75$	**0.002**
VLMT delayed recall[b]	11.23 (3.07)	12.88 (2.2)	$F_{1,\ 106} = 9.96$	**0.002**
VLMT recognition[c]	12.7 (2.72)	13.91 (1.89)	$F_{1,\ 106} = 7.19$	0.009
Executive function				
TMT B-A (s)	26.34 (14.84)	28.7 (17.25)	$F_{1,\ 106} = 0.44$	0.51
Working memory				
Spatial span	18.7 (3.47)	17.74 (3.07)	$F_{1,\ 106} = 2.58$	0.111
Letter-number span	16.37 (2.88)	16.08 (2.55)	$F_{1,\ 106} = 0.27$	0.603
Psychomotor speed				
Digit Symbol Test	64.4 (10.86)	62.83 (10.8)	$F_{1,\ 106} = 0.57$	0.452
Attention				
d2 concentration performance	176.5 (39.76)	180.13 (34.68)	$F_{1,\ 106} = 0.15$	0.701
Verbal fluency (fMRI task)				
Correct responses	67.57 (8.68)	69.55 (10.04)	$F_{1,\ 106} = 0.9$	0.346
Error rate (%)	6.27 (4.7)	6.35 (3.74)	$F_{1,\ 106} < 0.01$	0.961

A Bonferroni corrected significance level of $P < 0.005$ was applied for the comparison of neuropsychological measures and fMRI task performance between genotype groups to adjust for multiple testing (10 comparisons). Bold P-values indicate significant differences.
[a]Total number of correctly recalled words of a 15-word list over five immediate free recall trials.
[b]Number of correctly recalled words of the 15-word list in a delayed (approx. 30 min) free recall trial.
[c]Number of correctly recognized words of the 15-word list after the delayed free recall trial.

[Letter-Number Span; Gold et al., 1997], psychomotor speed [Digit Symbol Test, derived from the Hamburg-Wechsler Intelligence Test-R; Tewes, 1991], and attention [d2 Test of Attention; Brickenkamp, 2002].

fMRI Task and Stimuli

The semantic verbal fluency paradigm applied here has already been used successfully in previous investigations in healthy subjects and patients with major depressive disorder [Backes et al., 2014, in press; Nagels et al., 2012]. In this paradigm, a block design was employed with 10 blocks for each of two alternating conditions: word generation and baseline. At the beginning of each word generation block, an instruction slide with a German noun was shown for 3 s, followed by a fixation cross. From then on, subjects had to overtly name, within 12 s, as many members of the category the noun represented (e.g., say "dog," "cat," "eagle" [...] after the word "animal" had been shown). The appearance of the word "silence" (presented for 3 s) indicated the beginning of the baseline condition, in which the hash mark "#" was presented for 12 s. During this resting phase, participants were required to be silent. Subsequently, a new category name indicated the next word generation block. The following 10 categories were applied in fixed order: animals, sports, clothes, professions, fruit, vehicles, furniture, flowers, hobbies, and spices. It has been demonstrated that the use of such a block design with relatively short task (overt speech) and rest (no speech) periods of about 10 s is a useful strategy for reducing task-related motion artifacts while optimally detecting blood oxygen level dependent (BOLD) signal changes [Birn et al., 2004, 2010].

In a previous study with healthy subjects, analyses with a finite impulse response (FIR) model indicated that the timeframe of 12 s for word generation is optimal with regard to the time course of the BOLD signal in the present paradigm [Nagels et al., 2012]. Moreover, based on the number of produced words for the 10 word generation blocks, a high internal consistency (Cronbach's $\alpha = 0.82$) has been identified for this task [Nagels et al., 2012].

Stimuli were presented in white color on a black background with Presentation software package (version 14.1.09.21.09, Neurobehavioral Systems Inc.). Subjects' responses were recorded using a scanner-compatible microphone and Audacity software (version 1.2.6., Softonic International S. L.). To familiarize participants with the task and the rules for word generation (see "Verbal Fluency Data Evaluation"), a test session with two exemplary

categories was conducted prior to the scanning procedure. These categories were not part of the fMRI investigation.

Verbal Fluency Data Acquisition

The overt speech production in the scanner was recorded with a 40 dB noise-reducing microphone system (FOMRI-II, Optoacoustics Ltd.) allowing for online speech synchronization. A dual adaptive filter system [for technical details see, e.g., Stephens et al., 2010] subtracted the reference input (MRI noise) from the source input (speech signal) and filtered the speech production instantly while the overt output was recorded. The optic microphone was mounted on the head coil and wired to the sound filter box. The output port was directly wired to the audio in-line plug of the notebook sound card. All audio files were saved and afterwards transcribed into text files.

Verbal Fluency Data Evaluation

The transcripts were analyzed regarding the number of produced words (including errors) and checked for incorrect responses. The following answers were counted as errors: non-members of the given category, repetitions or grammatical variations of words produced during the same block, and words having the same stem as the preceding one. The resulting measures were (a) correct responses (i.e., the total number of correctly generated words) and (b) the error rate (i.e., the total number of incorrect responses relative to the total number of generated words).

fMRI Data Acquisition

Imaging was performed on a 3 Tesla Tim Trio MR scanner (Siemens Medical Systems) at the Department of Psychiatry and Psychotherapy, Philipps-University Marburg. Functional data were acquired with a T2*-weighted echo planar imaging (EPI) sequence sensitive to BOLD contrast (64×64 matrix, 224 mm $\times$ 224 mm field of view, 40 slices, 3.5 mm slice thickness, repetition time = 2.5 s, echo time = 30 ms, flip angle = 90°). Slices covered the whole brain and were positioned transaxially parallel to the anterior-posterior commissural line (AC-PC). The initial 3 of the 130 collected functional images were excluded from further analysis to remove the influence of T1 stabilization effects. To minimize head movements, subjects' heads were fixated with foam pads.

Neuropsychological and Verbal Fluency Data Analysis

The effects of genotype on neuropsychological measures and fMRI task performance (correct responses and error rate) were assessed via one-way ANOVAs with age and sex as covariates of no interest. A Bonferroni corrected significance level of $P < 0.005$ was applied to adjust for multiple testing (10 comparisons). Statistical analysis was carried out with SPSS (version 15.0, SPSS Inc.).

fMRI Data Analysis

Preprocessing

Functional imaging data were analyzed using SPM8 (v4290) standard routines and templates (Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm) running on MATLAB 7.7.0.471 (R2008b) (The MathWorks, Inc.). After slice-time correction (to the 20th slice), functional images were realigned (motion-corrected) and normalized to standard MNI (Montreal Neurological Institute) space (resulting voxel size: 2 mm $\times$ 2 mm $\times$ 2 mm). To increase the signal-to-noise ratio and to compensate for inter-subject anatomical variation, functional scans were spatially smoothed with an 8 mm full-width-at-half-maximum (FWHM) Gaussian kernel. As overt articulation in the scanner could lead to increased head movement, head movement parameters were carefully checked for each participant, and subjects with head movement exceeding 3 mm or 3° in any direction were excluded. Four subjects exceeded this limit and were not included in the study. Thus, head movement parameters of all participants in our final sample ($n = 110$) were in an acceptable range, similar to previous reports [e.g., Kircher et al., 2009; Krug et al., 2011; Markov et al., 2009].

Single subject analyses

On the first level, we used a block design to model single-subject BOLD responses during the 10 word generation and 10 baseline blocks. The instruction periods were included as separate condition of no interest. To account for differences in the individual number of words produced, the word generation condition was modulated with the number of words generated by each subject within each block using the parametric modulation function of SPM8.

Additionally, the six movement parameters of the realignment procedure (motion correction, see "Preprocessing") were entered as regressors of no interest to account for any residual head movement-related effect. To remove low-frequency drifts from time series, a high-pass filter with a cut-off period of 128 s was applied.

Group analyses

On the second level, parameter estimates of the word generation and the baseline condition were entered into a 2×2 ANOVA using a full factorial design with genotype (A versus GG) as between-subjects factor and condition (word generation versus baseline) as within-subjects factor. Age and sex were included as covariates of no interest. To reveal differential effects of genotype on whole brain activation during word generation versus baseline, genotype

× condition interaction effects were examined. This procedure, which has been proven to be useful for group analyses in previous studies [e.g., Backes et al., 2014, in press; Dietsche et al., 2014] not only takes into account potential differences between groups in baseline activity, but also allows for a more precise analysis of activation patterns compared to subtractive designs [cf. Fernández-Corcuera et al., 2013; Gusnard and Raichle, 2001; Pomarol-Clotet et al., 2008]. It provides the opportunity to evaluate whether greater activation in one genotype group (e.g., A) reflects a greater task-related increase of activation compared to the other group (e.g., GG) or whether it is due to a weaker task-related decrease in activation in this group (here: A). This is important since it is increasingly recognized that reduced deactivation plays a significant role in bipolar disorder and schizophrenia and might even represent an enduring, trait-like variable in these disorders [Fernández-Corcuera et al., 2013; Pomarol-Clotet et al., 2008].

In addition to the interaction effects, the effects of condition and genotype were examined. For all analyses, *t*-contrasts were used to reveal the directionality of the effects. We report findings that are significant at $P < 0.05$ on the cluster-level after family-wise error (FWE) correction for multiple comparisons as implemented in SPM8. For the anatomical localization, the functional activations were assigned to probabilistic cytoarchitectonic areas with the SPM Anatomy Toolbox (version 1.6) [Eickhoff et al., 2005]. Brain activations were plotted on the anatomical MRIcroGL (version 12/12/2012, http://www.mccauslandcenter.sc.edu/mricrogl/) template.

RESULTS

Neuropsychological and Verbal Fluency Data

Neuropsychological results and fMRI task performance are summarized in Table I. Genotype had a significant effect on verbal memory performance as assessed with the VLMT. Compared to subjects with GG genotype, A allele carriers showed significantly decreased immediate and delayed free recall performance as well as a trend for decreased recognition performance (see Table I). There were no further significant effects of genotype on neuropsychological measures. Regarding semantic verbal fluency performance in the scanner, there were no significant effects of genotype. Thus, genotype groups did not differ with respect to the number of words produced (correct responses) or the relative number of errors made (error rate).

fMRI Data

Effects of condition

Word generation compared to baseline activated a commonly observed widespread semantic verbal fluency network [Backes et al., 2014, in press; Nagels et al., 2012] including areas in the cerebellum, the superior temporal gyrus, the precentral gyrus, the postcentral gyrus, the supplementary motor area, the insular cortex, and the inferior frontal gyrus. Maxima in this network, which encompassed 36,495 voxels, were found in the right precentral gyrus ($x = 48$, $y = -10$, $z = 36$; $t = 15.7$), the right cerebellum ($x = 16$, $y = -62$, $z = -20$; $t = 15.33$), and the left cerebellum ($x = -18$, $y = -62$, $z = -20$; $t = 14.97$).

Baseline compared to word generation also activated a widespread neural network including mainly areas in the inferior parietal lobule, the precuneus, the posterior cingulate cortex, the superior medial gyrus, the middle and inferior temporal gyri, the angular gyrus, the supramarginal gyrus, and the inferior occipital gyrus. Maxima in this network, which encompassed 58,757 voxels, were found in the right middle cingulate cortex ($x = 8$, $y = -52$, $z = 34$; $t = 18.39$) and the left inferior parietal lobule ($x = -52$, $y = -62$, $z = 42$; $t = 17.91$).

Effects of genotype

No suprathreshold cluster was found.

Genotype × condition interaction effects

The interaction analysis yielded one cluster of 815 voxels with two maxima in the left middle temporal gyrus (MTG) ($x = -60$, $y = -32$, $z = -14$; $t = 4.41$ and $x = -56$, $y = -10$, $z = -22$; $t = 4.27$) and a third maximum in the left temporal pole (TP) ($x = -36$, $y = 6$, $z = -20$; $t = 4.08$). In this cluster, A allele carriers demonstrated greater activation during word generation versus baseline compared to subjects with GG genotype (see Fig. 1). The inclusion of task performance, lateralization quotient, and estimated IQ as additional covariates did not alter these results.

To further explore brain activation in the MTG/TP, fMRI data were extracted as the first eigenvariate of the entire cluster. *Post hoc* paired *t*-tests (two-tailed) with a Bonferroni corrected significance level of $P < 0.025$ (two tests) revealed that in subjects with GG genotype, activity in the left MTG/TP decreased significantly from baseline to word generation ($t_{79} = 7.55$, $P < 0.0001$), while in A allele carriers, activity did not change significantly ($t_{29} = -0.04$, $P = 0.966$).

We additionally examined potential associations between left MTG/TP activation during task execution and those neuropsychological measures for which a significant effect of genotype had emerged (i.e., immediate and delayed verbal memory), separately for both genotype groups via partial correlation analyses (two-tailed) with age and sex as covariates of no interest. A Bonferroni corrected significance level of $P < 0.013$ was applied to adjust for multiple testing (four analyses). In subjects with GG genotype, a significant negative association between left MTG/TP activation during word generation and delayed free recall performance was found ($r = -0.28$, $P = 0.012$). Thus, subjects with greater deactivation in the MTG/TP demonstrated better long-term verbal memory

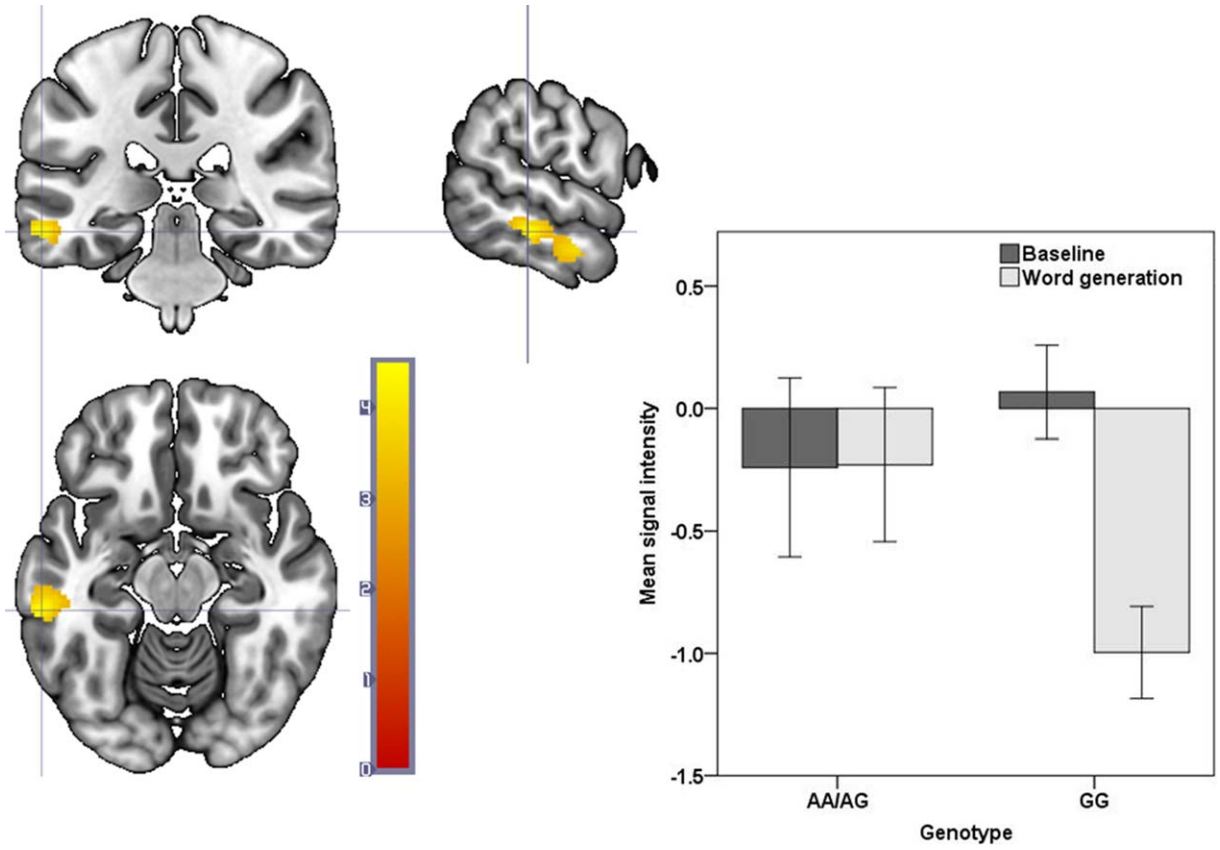

Figure 1.

Results of the *NCAN* rs1064395 genotype × condition interaction analysis ($P < 0.05$, cluster-level FWE corrected). Compared to subjects with GG genotype, subjects with A allele showed a lack of task-related deactivation in a cluster extending from the left middle temporal gyrus to the left temporal pole ($x = -60$, $y = -32$, $z = -14$). The colorbar displays the corresponding z-values. Bar graphs display the mean signal intensity during baseline and word generation for the whole cluster. Error bars indicate 95% confidence intervals. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

performance (see Fig. 2). There was also a trend for a negative correlation between MTG/TP activation and immediate free recall performance in subjects with GG genotype ($r = -0.23$, $P = 0.046$). In A allele carriers, no significant associations were found.

DISCUSSION

This is, to our knowledge, the first fMRI study demonstrating effects of the recently identified risk variant in the *NCAN* gene on human brain function. Our data provide first evidence that rs1064395 risk status impacts on both neural processing and cognitive performance even in healthy individuals without any psychiatric disease, which accords with previous research suggesting effects of *NCAN* on neurocognitive functioning [e.g., Schultz et al., 2014; Zhou et al., 2001]. When compared to subjects with GG genotype, risk allele carriers demonstrated poorer verbal memory performance as assessed with the VLMT as well as a lack of task-related deactivation during semantic verbal fluency in the left lateral temporal cortex, namely in the left MTG and TP. These findings are in line with evidence that *NCAN* is expressed in the cerebral cortex and the hippocampus [Cichon et al., 2011] as well as with research indicating a role of neurocan in the regulation of synaptic plasticity [Dimatelis et al., 2013; Geissler et al., 2013; Rauch, 2004; Schwarzacher et al., 2006; Zhou et al., 2001].

The observed functional alteration in the left MTG/TP might represent a mechanism by which rs1064395 influences susceptibility to affective and psychotic disorders in healthy subjects. Functional as well as structural alterations of this brain area, particularly cortical thinning and decreased gray matter volume, have been observed in both patients with bipolar disorder and patients with schizophrenia as well as in unaffected first-degree relatives of patients [Chen et al., 2007; Elvsåshagen et al., 2013; Hu et al., 2013; Kupferschmidt and Zakzanis, 2011; MacDonald et al., 2009; Sprooten et al., 2013].

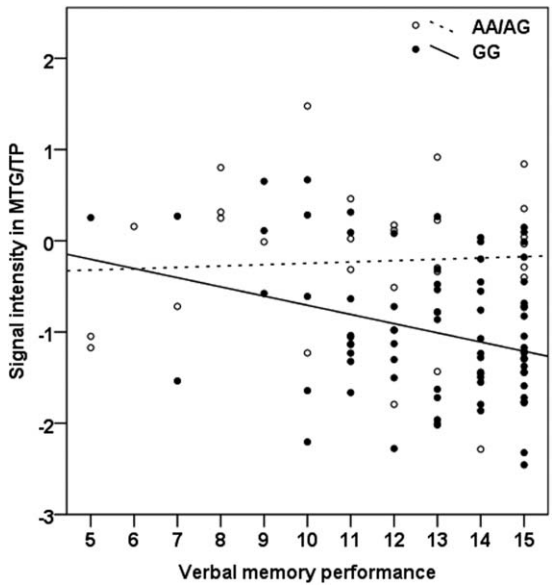

Figure 2.

Scatter plot depicting the association between verbal memory performance (number of correctly recalled words of a 15-word list in a delayed free recall trial) and activation in the left MTG/TP (middle temporal gyrus/temporal pole) cluster during word generation for both *NCAN* rs1064395 genotype groups. In subjects with GG genotype, a Bonferroni corrected partial correlation analysis revealed a significant negative association between verbal memory performance and left MTG/TP activation ($r = -0.28$, $P = 0.012$).

However, the precise mechanisms through which *NCAN* influences neural processing and cognitive functioning remain to be clarified. In light of the temporal expression pattern of *NCAN* and the functions of neurocan known to date [for review see Avram et al., 2014], it is possible that the observed effects of rs1064395 on brain functioning result from changes in brain development. *NCAN* is highly expressed in the mammalian brain during early development, particularly in late embryonic and early postnatal phases [Cichon et al., 2011; Milev et al., 1998]. As an important component of the ECM, neurocan is thought to be involved in neuronal adhesion and migration as well as in neurite growth [Rhodes and Fawcett, 2004; Yamaguchi, 2000; Zimmermann and Dours-Zimmermann, 2008]. Alterations in the unique molecular composition of the ECM due to a genetic upregulation or downregulation of neurocan, for instance, might interfere with these key cellular events during brain development, leading to structural and functional changes within specific brain areas [cf. Berretta, 2012]. In a previous imaging genetics study, *NCAN* risk status has already been shown to influence cortical folding, a sensitive marker for neurodevelopmental alterations, in patients with schizophrenia [Schultz et al., 2014]. Since the effects were found in occipital and prefrontal areas, the authors suggest a role of *NCAN* not only on higher cognitive functions (top-down processing) but also on early visual processing (bottom-up processing).

Neuropsychological and Verbal Fluency Findings

One aim of the present study was to explore the effects of rs1064395 on semantic verbal fluency and various other cognitive domains. Although pronounced effects of rs1064395 genotype were found on neural responses, semantic verbal fluency performance did not differ between genotype groups. Since brain imaging measures are supposed to be more proximal to the biological pathways that mediate genetic risk than overt behavioral responses, this finding might reflect greater penetrance of genetic risk variants at the level of brain function as compared to the level of cognitive measures [Meyer-Lindenberg and Weinberger, 2006; Rose and Donohoe, 2013]. However, the absence of genotype effects on semantic verbal fluency performance may also be attributed to the relatively small time window for word generation in the fMRI paradigm (12 s) compared to the classical neuropsychological test with a time limit of 60 s.

Regarding the other neuropsychological domains tested, a significant effect of rs1064395 genotype was observed solely on verbal memory, with *NCAN* risk status being related to poorer immediate and delayed free recall performance. This finding is in line with the observation that neurocan-deficient mice display reduced maintenance of late-phase hippocampal long-term potentiation (LTP) when compared to wild-type mice, suggesting an association of neurocan with learning and memory [Rauch, 2004; Zhou et al., 2001]. Moreover, neuropsychological reports indicate that verbal memory is one of the most impaired cognitive domains in bipolar disorder and schizophrenia [Arts et al., 2008; Hill et al., 2008; Robinson et al., 2006]. Decreased verbal memory performance can also be found in unaffected relatives of patients [Arts et al., 2008; Hill et al., 2008; Whyte et al., 2005], which points to a genetic basis of such deficits and underpins the sensitivity of verbal memory measures for genetic influences.

In contrast to the VLMT, which requires the recall of specific words from a recently studied list (episodic verbal memory), semantic verbal fluency relies on the retrieval of words that belong to "overlearned" semantic categories such as *animals* (semantic verbal memory). This might be the reason why genotype effects were observed for episodic verbal memory, but not for semantic verbal fluency.

fMRI Findings

A major aim of the present study was to explore the effects of *NCAN* rs1064395 genotype on brain activation patterns during word generation versus baseline. First, we predicted genotype effects on key cortical regions of the semantic verbal fluency network, especially on the left inferior frontal gyrus. However, our results do not provide

any evidence for genotype-related activation differences in this brain region or other prefrontal areas. Thus, the present data do not support the assumption that *NCAN* risk status might contribute to the alterations of prefrontal brain functioning during verbal fluency that have been observed in patients with bipolar disorder or schizophrenia and their unaffected relatives [e.g., Allin et al., 2010; Boksman et al., 2005; Costafreda et al., 2009; Curtis et al., 2007; Fu et al., 2005].

Second, we hypothesized that risk allele carriers also show reduced deactivation of brain areas that typically show lower activation during task execution compared to baseline such as DMN regions, according to recent findings in remitted patients and unaffected first-degree relatives [Allin et al., 2010; Costafreda et al., 2011]. Consistent with this assumption, our fMRI data indicate that *NCAN* risk status is associated with a lack of task-induced deactivation in a large left lateral temporal cluster extending from the left MTG to the left TP, a brain area that was part of the network activated during baseline as compared to word generation. This finding is in line with evidence suggesting that reduced task-induced deactivation might represent an enduring, trait-like variable in bipolar disorder and schizophrenia [e.g., Fernández-Corcuera et al., 2013; Pomarol-Clotet et al., 2008].

Moreover, since lateral temporal cortex extending into the TP has been described as one of the anatomical components of the DMN [Andrews-Hanna, 2012; Buckner et al., 2008], activation differences between genotype groups might be interpreted in terms of altered DMN functioning. There are increasing reports of common DMN dysfunctions, particularly compromised DMN suppression, in schizophrenia and affective disorders [Anticevic et al., 2012; Broyd et al., 2009; Buckner et al., 2008; Whitfield-Gabrieli and Ford, 2012]. Since greater suppression of the DMN is associated with better performance on various cognitive tasks, compromised suppression might contribute to the cognitive impairments observed in these disorders [Anticevic et al., 2012; Whitfield-Gabrieli and Ford, 2012]. In the present study, homozygous carriers of the G allele, which is likely to confer a protective effect [Cichon et al., 2011; Mühleisen et al., 2012], also showed both greater task-related deactivation and better cognitive performance, albeit in a task conducted outside the scanner, when compared to risk allele carriers. First evidence for a genetic basis of altered DMN patterns stems from functional DMN changes in unaffected first-degree relatives of patients [Meda et al., 2012; Whitfield-Gabrieli et al., 2009]. In addition, previous fMRI studies on verbal fluency have reported decreased deactivation of DMN-related areas not only in patients with bipolar disorder or schizophrenia and their unaffected first-degree relatives but also in subjects carrying a genetic risk variant for these psychiatric conditions [Allin et al., 2010; Costafreda et al., 2011; Papagni et al., 2011]. The hypothesis that *NCAN* genotype may be associated with functional alterations of the DMN could be tested in future studies that directly assess this brain network, for example by additionally applying a resting state approach.

Alternative explanations for the present rs1064395 genotype-related activation differences also have to be considered. Lateral temporal cortex has been reported as part of the DMN, yet there are several other brain regions that more consistently show task-related deactivations such as medial prefrontal cortex, posterior cingulate cortex, and inferior parietal lobules [Andrews-Hanna, 2012; Buckner et al., 2008; Whitfield-Gabrieli and Ford, 2012]. Since the pattern of task-induced deactivations may vary depending on the nature of the task and its specific demands [Andrews-Hanna, 2012], it is possible that the differential activation pattern in the left MTG/TP reflects genotype-related differences in the strategies used to perform the semantic verbal fluency task.

An additional finding of the present study is that greater deactivation of left MTG/TP during word generation significantly correlated with better performance on an external verbal memory task in subjects with GG genotype. Thus, it may be speculated that the observed functional difference in the left MTG/TP might not be specific for semantic verbal fluency processing, but instead indicate a more general *NCAN* risk status-related alteration of neural responses during cognitive challenges that involve verbal memory. Moreover, this finding suggests that the ability to reduce activity in specific brain areas during cognitive processing might be an advantageous feature of brain functioning in GG genotype subjects that could be absent or reduced in risk allele carriers, who showed both lower cognitive performance and a lack of deactivation.

Limitations

This investigation was limited by the relatively small size of the risk allele carrier group, which included 30 subjects with either AA or AG genotype, yet the number of risk allele carriers was sufficient for the detection of significant genotype effects. Moreover, it is important to replicate the observed genotype effects in independent samples. Future studies might consider genotyping a larger number of subjects and subsequently selecting subsets of subjects to achieve a better balance between risk allele carriers and those individuals without risk allele.

Another limitation relates to the interpretation of the present results in light of the findings obtained in patients with bipolar disorder and patients with schizophrenia. The observed pattern of decreased task-related deactivation and lower verbal memory performance in *NCAN* risk allele carriers overlaps with findings described in patients, yet activation differences in prefrontal brain areas were not detected in the present study. In this context, it has to be kept in mind that study participants and their first-degree relatives had no history of any psychiatric disorder. Although risk allele carriers showed lower verbal memory performance compared to subjects with GG genotype, this

finding cannot be interpreted as impairment since performance was in the normal range in both genotype groups.

CONCLUSION

In the present study, a neuropsychological test battery and a well-tested semantic verbal fluency paradigm that reliably elicits robust activation in a bilateral language network were used to explore the effects of the *NCAN* SNP rs1064395, a susceptibility factor for bipolar disorder and schizophrenia, on cognitive performance and brain function in healthy subjects. The results demonstrate that rs1064395 genotype affects verbal memory performance as well as neural responses during semantic verbal fluency. Our data further provide evidence for a link between these measures, which might indicate that we detected a more general rs1064395 risk-associated alteration of brain function. These findings can serve as a basis for future work to further examine the effects of *NCAN* genotype on neural processing in specifically tailored experiments. Based on the present results, future studies should, for example, investigate the effects of rs1064395 on the neural correlates of verbal memory.

Taken together, we present new evidence for a specific genetic impact on neural processing and cognitive functioning in healthy subjects. The precise mechanisms through which rs1064395 might exert its influence on human brain function remain to be elucidated.

ACKNOWLEDGMENT

The DFG (grant sponsor) had no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication. The authors declare that they have no conflict of interest.

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