

NCAN Cross-Disorder Risk Variant Is Associated With Limbic Gray Matter Deficits in Healthy Subjects and Major Depression

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Genome-wide association studies have reported an association between NCAN rs1064395 genotype and bipolar disorder. This association was later extended to schizophrenia and major depression. However, the neurobiological underpinnings of these associations are poorly understood. NCAN is implicated in neuronal plasticity and expressed in subcortical brain areas, such as the amygdala and hippocampus, which are critically involved in dysfunctional emotion processing and regulation across diagnostic boundaries. We hypothesized that the NCAN risk variant is associated with reduced gray matter volumes in these areas. Gray matter structure was assessed by voxel-based morphometry on structural MRI data in two independent German samples (healthy subjects, $n = 512$; depressed inpatients, $n = 171$). All participants were genotyped for NCAN rs1064395. Hippocampal and amygdala region-of-interest analyses were performed within each sample. In addition, whole-brain data from the combined sample were analyzed. Risk (A)-allele carriers showed reduced amygdala and hippocampal gray matter volumes in both cohorts with a remarkable spatial overlap. In the combined sample, genotype effects observed for the amygdala and hippocampus survived correction for entire brain volume. Further effects were also observed in the left orbitofrontal cortex and the cerebellum/fusiform gyrus. We conclude that NCAN genotype is associated with limbic gray matter alterations in healthy and depressed subjects in brain areas implicated in emotion perception and regulation. The present data suggest that NCAN forms susceptibility to neurostructural deficits in the amygdala, hippocampus, and prefrontal areas independent of disease, which might lead to disorder onset in the presence of other genetic or environmental risk factors.

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INTRODUCTION

Bipolar disorder (BD), major depressive disorder (MDD), and schizophrenia (SCZ) are highly heritable neuropsychiatric disorders. Genome-wide association studies have implicated several genes in these disorders, and have revealed a high degree of overlap in terms of common genetic factors (Lee *et al*, 2013). Although each of the identified genome-wide significant variants leads to only a marginal increase in individual disease risk, they may provide insights into the etiology and molecular pathways involved in these disorders. In recent years, imaging genetics has emerged as a powerful tool for exploring the involvement of previously identified genetic variants in the etiology of psychiatric disorders. This

approach characterizes the influence of genetic variants on brain structure and function that are the consequence of normal and abnormal biological pathways (Dannlowski *et al*, 2010, 2011, 2014; Erk *et al*, 2010; Stein *et al*, 2012).

One of the most recently identified genome-wide significant candidates for BD is the single-nucleotide polymorphism (SNP) rs1064395 in *Neurocan* (NCAN) (Cichon *et al*, 2011). Subsequent candidate studies also found associations of this SNP with SCZ ($p = 2.28 \times 10^{-3}$, Mühleisen *et al*, 2012) as well as with MDD in the overall PGC MDD sample ($p = 0.013$, www.broadinstitute.org/mpg/ricopili) as well as in the German sample ($p = 0.009$) suggesting that variation in NCAN is a susceptibility factor across psychiatric diagnostic categories.

NCAN is a brain-specific chondroitin sulfate proteoglycan (CSPG), which is mainly expressed during early brain development (<http://www.informatics.jax.org/assay/MGI:4826610>, Schultz *et al*, 2014). It is widely expressed throughout the mammalian brain, including subcortical structures, such as the hippocampus and the amygdala (Cichon *et al*, 2011). NCAN is a component of the extracellular matrix, where it is involved in cell adhesion,

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cell migration, and neurite growth (Rauch *et al*, 2001). In gene expression studies of Brodman areas 8/9 and 46/10, as well as cerebellum, NCAN is differentially regulated in MDD patients compared with controls in Brodman area 46/10 (<https://www.stanleygenomics.org/>).

The subcortical distribution of NCAN expression shows remarkable parallels to regions implicated in neuroanatomical models of major psychoses on the basis of numerous studies reporting functional and structural aberrations in the amygdala and hippocampus, which have been linked to dysfunctions of emotion processing and regulation (Phillips and Swartz, 2014; Strakowski *et al*, 2012). Particularly, gray matter volume abnormalities in the amygdala and hippocampus appear to be a common feature of affective disorders and SCZ alike (Brown *et al*, 2011; Redlich *et al*, 2014; Stratmann *et al*, 2014). Therefore, it should be a plausible hypothesis that NCAN genotype is associated with subcortical gray matter structure.

In an early imaging genetics study, Schultz *et al* (2014) demonstrated that NCAN rs1064395 genotype influenced cortical folding in the occipital and prefrontal cortex. The authors hypothesized that this may establish disease susceptibility during neurodevelopment. However, the investigation was restricted to cortical structures only, and involved a relatively small sample. To our knowledge, no study to date has investigated NCAN genotype effects on either subcortical structures or gray matter volumes across the entire brain. The aim of the present study therefore was to investigate an association between NCAN rs1064395 and regional gray matter volume in a large sample of healthy subjects ($N=512$), and in a second, clinical sample of patients with MDD ($N=171$). Brain-wide local gray matter volume was measured using voxel-based morphometry (VBM). In each sample, hippocampal and amygdala region-of-interest (ROI) analyses were performed. In addition, analyses of whole-brain data were conducted for each sample separately and the combined sample.

As the minor allele A of rs1064395 has been shown to confer a risk effect that increases disease susceptibility, whereas the G-allele is likely to confer a protective effect (Cichon *et al*, 2011; Mühleisen *et al*, 2012), we hypothesized that carriers of the NCAN genotypes AA and AG would exhibit reduced gray matter volumes, particularly in the amygdala and hippocampus, as well as in other structures with an involvement in emotion perception and regulation, such as prefrontal areas.

MATERIALS AND METHODS

Sample Characteristics

Two independent samples from separate studies have been collected at the University of Münster, at the same MRI scanner with identical sequences. Both samples were of Central European ancestry with an age range of 18–65 years. None of the subjects from either study have been included in any previous report regarding NCAN genotype.

Sample 1 comprised $N=520$ healthy subjects. All subjects were thoroughly investigated by experienced psychologists and were free from any lifetime history of psychiatric disorders according to DSM-IV criteria, assessed by means of the SCID interview. The data of eight subjects had to be

excluded from the analyses due to the presence of anatomical abnormalities (abnormally enlarged ventricles) or artifacts discovered in the structural MRI Images checked by visual inspection and identification as extreme outliers in the check data quality function of the VBM8-toolbox (>3 SD from mean homogeneity). The remaining scans of $N=512$ subjects (mean age 33.3 ± 11.6 ; $N=223$ male, $N=289$ female) were clear of such problems.

Sample 2 (clinical sample) comprised $N=176$ hospitalized inpatients with MDD and a current depressive episode, as diagnosed with the SCID interview, all under current antidepressant treatment. Comorbid diagnoses of substance-related disorders or psychotic disorders were exclusion criteria. Five data sets were removed after the quality check described above, leaving $N=171$ patients (mean age 38.6 ± 11.7 ; $N=66$ male, $N=105$ female) in sample 2.

Common exclusion criteria for both samples were any neurological abnormalities including stroke, epilepsy, multiple sclerosis, Parkinson's disease or dementia, head trauma or unconsciousness, and the usual MRI contraindications (metal implants, cardiac pacers, and so on). The study was approved by the Ethics Committee of the University of Münster. After complete description of the study to the participants, written informed consent was obtained.

Verbal intelligence was estimated by the Mehrfachwahl-Wortschatz-Intelligenztest (multiple-choice vocabulary intelligence test; Lehrl, 1995). Depression level and trait anxiety were assessed by means of the BDI (Beck and Steer, 1987) and STAI-trait (Laux *et al*, 1981) questionnaire.

Compared with sample 1, participants in sample 2 (i) were significantly older (38.6 ± 11.7 vs 33.3 ± 11.6 years, $t(681) = 5.16$, $p < 0.001$); (ii) reported fewer years of education (14.2 ± 2.2 vs 14.8 ± 2.1 years, $t(681) = 3.25$, $p = 0.001$); and (iii) displayed lower verbal IQ scores (111.4 ± 13.4 vs 116.0 ± 12.5 , $t(594) = 3.84$, $p < 0.001$). As expected, compared with sample 1, participants in sample 2 showed higher scores on both the BDI (23.3 ± 11.1 vs 2.4 ± 3.1 , $t(662) = 37.9$, $p < 0.001$), and the STAI (58.8 ± 9.3 vs 32.2 ± 6.9 , $t(615) = 37.0$, $p < 0.001$). The two samples did not differ in terms of gender, $\chi^2(1) = 1.29$, $p = 0.26$, or NCAN genotype distribution, $\chi^2(2) = 1.33$, $p = 0.51$.

DNA Extraction and Genotyping

Genomic DNA was prepared from whole blood using standard procedures. Genotyping was performed using a TaqMan 7900HT Fast Real-Time PCR System (Life Technologies), and the SNP Genotyping Assay ID C_8931759_20 for the NCAN polymorphism rs1064395. Accuracy was assessed by duplicating 15% of the sample. Reproducibility was 100%.

Structural MRI Acquisition and Morphometry

A detailed description of both the MRI methodology and statistical approach is provided elsewhere (Baune *et al*, 2012a, b; Dannlowski *et al*, 2012, 2014; Opel *et al*, 2014; Stacey *et al*, 2014). Briefly, T1-weighted high-resolution anatomical images were acquired using a 3 T-MRI scanner (Gyrosan Intera 3 T, Philips Medical Systems, Best, NL), and a 3D fast gradient echo sequence ('Turbo Field Echo'). TR = 7.4 ms, TE = 3.4 ms, FA = 9°, 2 signal averages,

inversion prepulse every 814.5 ms, acquired over a field of view of 256 mm (FH) × 204 mm (AP) × 160 mm (RL, nominal slice selection direction) with 1-mm resolution in all directions, frequency encoding in FH direction, phase encoding in AP and RL direction, reconstructed to cubic voxels of 0.5 × 0.5 × 0.5 mm.

The VBM8-toolbox (<http://dbm.neuro.uni-jena.de/vbm>) was used for pre-processing all structural images using default parameters. Images were bias-corrected, tissue classified, and normalized to MNI space using linear (12-parameter affine) and nonlinear transformations, within a unified model including high-dimensional DARTEL normalization. Gray matter segments were modulated by the nonlinear components only in order to preserve actual gray matter values locally (modulated GM volumes). Use of these procedures precludes the need for further correction for total brain volume.

As mentioned above, homogeneity of gray matter images was checked using the covariance structure of each image with all other images, as implemented in the check data quality function. The modulated gray matter images were smoothed with a Gaussian kernel of 8 mm FWHM.

Group statistics were calculated using SPM8 in each sample separately. To investigate the effect of rs1064395, *t*-tests were conducted using genotype group (A-carriers vs GG homozygotes) as a between-subjects factor. Age and gender were added to the models as nuisance regressors. The scanner gradient system was upgraded in the course of recruitment ('Master' Gradient System to 'Quasar Dual' Gradient System). Although the MRI sequence used before and after the gradient system upgrade was identical, the scanner upgrade was modeled as a regressor of no interest.

In a first step, a ROI approach was applied, restricting the analyses to the bilateral amygdala and hippocampus. An anatomical mask was generated using the Anatomy Toolbox (http://www.fz-juelich.de/SharedDocs/Downloads/INM/INM-1/DE/Toolbox/Toolbox_20.html). The mask encompassed all parts of the amygdala and the hippocampus, as defined on the basis of probabilistic cytoarchitectonic maps (Amunts et al, 2005). To control for multiple statistical testing within the ROI, a cluster-level false-positive detection rate was maintained at $p < 0.05$ using a voxel-level threshold of $p < 0.01$ and a cluster extent (k) empirically determined by Monte Carlo simulations ($n = 5000$ iterations), as reported previously (Baune et al, 2012a, b; Dannlowski et al, 2012). This was performed using the AlphaSim procedure, which accounts for spatial smoothness (estimated from the residuals), as implemented in the REST toolbox (<http://restfmri.net/forum/index.php>). The empirically determined cluster threshold for the bilateral hippocampus/amygdala mask was $k = 160$ voxels.

In a second step, exploratory whole-brain analyses were conducted in order to detect brain areas for which no *a priori* hypothesis existed, but which were potentially affected by NCAN genotype. The statistical threshold for whole brain analyses was $p < 0.001$, $k = 10$. The overlap of genotype effects in the two samples was assessed at the same threshold by calculating all voxels surviving $p < 0.01$ in both sample 1 and sample 2, thereby yielding a combined threshold of $p < 0.001$ (cluster threshold $k = 10$ was used as well).

To explore whether genotype effects were influenced by age or gender, two supplementary models were conducted

in the healthy sample, modeling the interaction term of genotype × age and genotype × sex. These analyses were restricted to all areas showing significant genotype effects in this sample (as listed in Table 2).

Finally, genotype effects were additionally calculated for the combined sample ($N = 683$). The previously used nuisance regressors were included (age, gender, and gradient upgrade). In the combined sample, sample (healthy vs MDD) was modeled as an additional regressor, also including the interaction term of sample and genotype group. From this model, also main effects of sample (healthy vs MDD) were calculated.

RESULTS

Genotyping Results

Sample 1 comprised $N = 123$ A-allele carriers (12 AA homozygotes, 111 AG heterozygotes) vs $N = 389$ GG homozygotes. Given the small number of AA homozygotes, these subjects were grouped together with the AG-carriers for the purposes of the present analyses. Genotype frequencies did not deviate from Hardy-Weinberg equilibrium (HWE), (exact test: $p = 0.24$). Sample 2 comprised $N = 124$ GG homozygotes vs $N = 47$ A-carriers ($N = 3$ AA, $N = 44$ AG). Again, allele frequencies did not deviate from HWE (exact test: $p = 1$). Table 1 shows the sociodemographic and questionnaire data of the two samples according to rs1064395 genotype.

Table 1 Sample Characteristics According to NCAN rs1064395 Status

Sample 1 ($N = 512$ healthy subjects)			
	AA/AG ($N = 123$)	GG ($N = 389$)	<i>p</i> -value ^a
Age	33.9 ± 12.8	33.1 ± 11.3	0.50
Sex (m/f)	48/75	175/214	0.25
Verbal IQ ^b	115.6 ± 13.0	116.2 ± 12.4	0.68
Years of education	14.5 ± 2.0	14.9 ± 2.1	0.09
STAI-trait ^c	32.1 ± 6.5	32.3 ± 7.0	0.90
BDI ^d	2.7 ± 3.8	2.3 ± 2.9	0.20
Sample 2 ($N = 171$ MDD patients)			
	AG/AA ($N = 47$)	GG ($N = 124$)	<i>p</i> -value
Age	38.6 ± 12.5	38.7 ± 11.5	0.96
Sex (m/f)	15/32	51/73	0.27
Verbal IQ ^b	110.5 ± 13.3	111.7 ± 13.5	0.62
Years of education	14.1 ± 2.3	14.2 ± 2.2	0.68
STAI-trait ^c	60.7 ± 7.5	58.0 ± 9.8	0.12
BDI ^d	24.3 ± 10.2	22.9 ± 11.4	0.48

^aAccording to *t*-tests or χ^2 -tests. ^bAssessed with the German language Mehrfachwahl-Wortschatz-Test (multiple-choice vocabulary test (Lehrl, 1995)). Data were available for $N = 449$ (sample 1) and $N = 147$ (sample 2) subjects.

^cState-Trait Anxiety Inventory. Data were available for $N = 476$ (sample 1) and $N = 141$ (sample 2) subjects. ^dBeck Depression Inventory. Data were available for $N = 501$ (sample 1) and $N = 163$ (sample 2) subjects.

Region-of-Interest Analysis of the Bilateral Amygdala and Hippocampus

In healthy subjects (sample 1), a significant rs1064395 genotype effect on bilateral amygdala, and hippocampus gray matter volumes was observed. Carriers of one or two risk (A) alleles had significantly decreased gray matter volumes compared with subjects with GG status (left: $x = -26, y = 0, z = -23, t(507) = 3.05, k = 323, p = 0.0012$; right: $x = 18, y = -12, z = -9, t(507) = 3.31, k = 436, p = 0.0005$). There were no significant differences between AA homozygotes and AG-carriers.

In depressed patients (sample 2), very similar effects in the same direction were observed (left: $x = -16, y = 0, z = -20, t(166) = 2.87, k = 182, p = 0.0023$; right: $x = 30, y = -13, z = -20, t(166) = 3.43, k = 305, p = 0.0003$).

Whole-Brain Data

In healthy subjects, several areas were found showing lower gray matter volume in A-carriers compared with GG homozygotes, including the amygdala and hippocampus, cerebellum, prefrontal, particularly orbitofrontal areas, and in the temporal and occipital cortex (see Table 2). There was no area showing larger gray matter volumes in A-carriers compared with GG homozygotes.

In the depressed sample, clusters showing effects in the same direction were found in the hippocampus, superior frontal gyrus and cerebellum (Table 2).

Table 2 Results of Whole-Brain Analyses of NCAN rs1064395 Genotype Effects on Local Gray Matter Volume in the Healthy Sample ($N = 512$) and the Patient Sample ($N = 171$)

Anatomical region	Side	Cluster size	x	y	z	Z-score
<i>Sample 1 (healthy sample)</i>						
Cerebellum, FFG	L	2131	-27	-57	-29	4.19
ITG, MTG, TP	R	154	52	2	-39	3.88
Orbital parts of MidFG, IFG, SFG; SFG, MidFG	L	427	-52	51	12	3.84
IPG, postcentral gyrus	R	25	44	-37	54	3.69
MTG	R	162	69	-30	9	3.64
HC, PHG, amygdala	R	36	15	-3	-18	3.29
STG	L	18	-52	-42	18	3.27
SFG, MidFG	R	10	22	60	24	3.25
Calcarine gyrus	L	25	-2	-94	-9	3.24
ITG, IOG	L	11	-56	-66	12	3.20
ITG, FFG	R	10	38	3	-38	3.16
<i>Sample 2 (patient sample)</i>						
HC	R	45	16	-9	-9	3.66
SFG	R	11	15	51	19	3.32
Cerebellum	L	23	-8	-39	-11	3.26

Abbreviations: FFG, fusiform gyrus; HC, hippocampus; IFG, inferior frontal gyrus; IOG, inferior occipital gyrus; ITG, inferior temporal gyrus; MidFG, middle frontal gyrus; MTG, middle temporal gyrus; PHG, parahippocampal gyrus; SFG, superior frontal gyrus; STG, superior temporal gyrus; TP, temporal pole.

Coordinates are given in MNI space.

The clusters within the two samples showed spatial overlap ($k = 334$ voxels surviving $p < 0.01$ in both groups, see Figure 1). The two largest overlapping clusters were located in the bilateral amygdala/hippocampus head (left: $x = -21, y = -1, z = -21, k = 92$; right: $x = 15, y = -3, z = -18, k = 189$). Checking this overlapping area regarding the affected hippocampal and amygdala substructures (Amunts et al, 2005) by means of the Anatomy-Toolbox yielded a major involvement of the basolateral amygdala (right: 25.3%, left: 84.3% of the overlapping area) and superficial amygdala (right: 62.7%, left: 13.2%), with additional minor involvements of the entorhinal cortex, subiculum, and the hippocampus-amygdala transition area. Other overlapping areas were found in the insula/temporal pole ($x = -39, y = 9, z = -15, k = 19$) and cerebellum ($x = -6, y = -42, z = -12, k = 17$).

There were no genotype \times age or genotype \times gender interactions within the areas showing main effects of genotype.

In our whole-brain approach within the entire sample ($N = 683$), we detected five clusters influenced by genotype surviving a whole-brain correction. These were located in the bilateral amygdala/hippocampus/parahippocampal gyrus, lateral orbitofrontal cortex, and cerebellum/fusiform gyrus (see Supplementary Table 1 and Figure 1).

Matching several previous studies, effects of diagnosis (healthy subjects vs controls) were found in the hippocampus, amygdala, insula, thalamus, and orbitofrontal cortex, among others (see Supplementary Table 2). However, there was no area in the entire brain showing a significant genotype \times sample interaction, indicating that genotype effects were comparable in healthy subjects and in depressed patients.

DISCUSSION

The present study investigated the impact of a single-nucleotide variant in NCAN, which has previously associated with BD, SCZ, and MDD, on gray matter volume. The investigations were performed in a large sample of healthy controls, which is a first-time finding in the literature, and were repeated in a second sample of hospitalized depressed patients. The two samples showed comparable associations of genotype and brain structure with an overlap of genotype effects mapping predominantly to the bilateral amygdala and hippocampal subfields. In the combined sample, the amygdala/hippocampus clusters survived correction for entire brain volume, as did areas in the left orbitofrontal cortex and cerebellum/fusiform gyrus. As these findings were obtained in healthy subjects and a clinical sample alike, they might indicate a disease-independent genetic effect on corticolimbic brain circuitries.

Previous neuroimaging studies have highlighted the role of the amygdala and the hippocampal formation in the etiology of BD, SCZ, and MDD. For example, several functional neuroimaging studies have shown that amygdala dysfunction is a key feature of BD (Almeida and Phillips, 2012; Blond et al, 2012; Fournier et al, 2013; Grotegerd et al, 2013, 2014; Lim et al, 2013), SCZ (Pankow et al, 2013; Rauch et al, 2010), and MDD (Dannlowski et al, 2007; Groenewold et al, 2012; Siegle et al, 2007; Stuhmann et al, 2011, 2013; Suslow et al, 2010). Also structural imaging studies have demonstrated decreased amygdala volumes in BD (Blumberg et al, 2003; Foland-Ross et al, 2013; Redlich et al, 2014) and gray matter

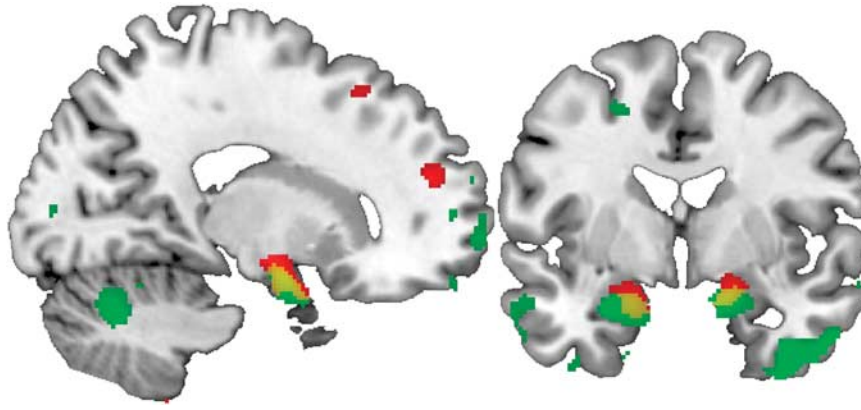


Figure 1 Coronal ($y = -2$) and sagittal ($x = 17$) views of the amygdala and the hippocampus region depicting the effect of *NCAN* rs1064395 genotype on gray matter volumes in the two samples, as corrected for gender and age and rendered on a standard brain template in MNI space. Green, results from sample 1 (healthy subjects) showing $N = 389$ GG homozygotes $> N = 123$ A-carriers with a threshold of $p < 0.01$. Red, results from sample 2 (MDD) showing $N = 124$ GG $> N = 47$ A-carriers at the same statistical threshold. Yellow, overlap between the clusters of each sample with a combined threshold of $p < 0.001$.

volume reductions have been described in the hippocampus of BD patients (Hajek *et al*, 2012a, b; Redlich *et al*, 2014), although the respective literature is less consistent than that for the amygdala (Blumberg *et al*, 2003; Hartberg *et al*, 2011).

Further research has also demonstrated structural alterations in the amygdala, hippocampus, and prefrontal areas in MDD and SCZ. Gray matter deficits of the hippocampus, for example, are one of the most frequently described imaging finding in MDD (MacQueen and Frodl, 2010; Stratmann *et al*, 2014), which was also found in the present study, and gray matter volume reductions in the amygdala and hippocampus have also been confirmed in meta-analyses of SCZ data (Shepherd *et al*, 2012). As the amygdala and the hippocampus have a key role in the generation and regulation of affective states, gray matter structural deficits might correspond to the genetic association findings for *NCAN*, which cross the diagnostic boundaries for the major psychoses (Miró *et al*, 2012).

NCAN is a CSPG. CSPGs are crucial for ECM organization, and participate in cell adhesion and migration. They are thus crucial for neuroplasticity, neuronal growth, and axon guidance during development, as well as for regenerative processes in neurons following central nervous system injury (Avram *et al*, 2014). The synergistic effects of cell proliferation and differentiation are the basis for the development of gray matter structure (Zilles *et al*, 2013), and the lack of CSPGs results in improved axonal regeneration and recovery (Galtrey and Fawcett, 2007). A plausible hypothesis therefore is that rs1064395 A-allele status leads to an upregulation of *NCAN*, which results in turn in growth inhibition. One possible explanation for the observed effects of *NCAN* variation on grey matter structure in the amygdala and hippocampus could lie in the interplay of the protein with binding partners, which are only present in the regions in question. However, to date, only few binding partners of *NCAN* have been described, among them PTPRS (Shen *et al*, 2009) and *NCAM1*, *L1CAM*, and *contactin2* (Rauch *et al*, 2001) as well as other proteoglycans (www.string-db.org). None of these proteins are exclusively expressed in the amygdala and/or hippocampus.

However, the precise molecular mechanisms through which *NCAN* is involved in the etiology of psychiatric disorders, and impacts on gray matter volume, still await elucidation. This process will require molecular genetic studies, including, for example, the search for tissue-specific binding partners by screening amygdala- and hippocampus-specific cDNA libraries using an adapted yeast two-hybrid system.

The effects of *NCAN* genotype appear to be disease independent as they have been found in healthy subjects and a patient sample alike and interaction effects of genotype and disease status were missing. It seems that these disease-independent effects form susceptibility to structural brain deficits that may be in turn associated with mental illness. Other genetic or environmental risk factors in patients (or protective factors in healthy subjects) probably need to be present to exhibit psychopathology (or prevent disease onset).

The results agree well with a recent study (Raum *et al*, 2015), showing that *NCAN* rs1064395 A-carriers have significant deficits in memory tasks associated with hippocampal function compared with GG homozygotes, and show differences in brain activation in temporal areas, which were also affected by genotype in our healthy sample. Conversely, different results were obtained in a study by Schultz *et al*, who reported *NCAN* genotype effects on cortical folding only in schizophrenia patients but not in healthy controls. However, sample sizes were weak ($N = 65$ healthy subjects), and the morphometric methods and the patient sample differed much compared with the present study.

The present study had limitations. First, the current lack of data concerning the functional properties of the *NCAN* SNP rs1064395 renders our statements regarding potential molecular mechanisms speculative. Furthermore, behavioral and clinical correlates of reduced amygdala, hippocampal, and PFC gray matter volume remain to be investigated in more detail. The inclusion of a second healthy sample would have represented a truly independent second sample compared with a patient sample. As patients and controls were not matched for age and other sociodemographic data, the effects of sample on gray matter volumes must be taken

with care. Finally, we cannot exclude the possibility that the observed effect was driven by another genetic variant that is in linkage disequilibrium with rs1064395.

In summary, the present study is the first to demonstrate an influence of a cross-disorder risk variant in NCAN on subcortical brain structure in healthy and depressed subjects. In our imaging genetics approach, the risk allele showed strong impact on gray matter volume in brain areas critically involved in emotion processing, which also show functional and structural abnormalities in BD, MDD, and SCZ. The present data suggest that the increased disease susceptibility in risk allele carriers might be mediated by neurostructural deficits in the amygdala, hippocampus, and prefrontal areas, leading to dysfunctional emotion processing and regulation.

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