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Glutamate receptor delta 1 (*GRID1*) genetic variation and brain structure in schizophrenia

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ABSTRACT

Common genetic variation in the promoter region of the glutamate receptor delta 1 (*GRID1*) gene has recently been shown to confer increased risk for schizophrenia in several independent large samples. We analysed high-resolution magnetic resonance imaging (MRI) data from 62 patients with schizophrenia and 54 healthy controls using voxel-based morphometry (VBM) to assess the effect of single nucleotide polymorphism rs3814614 (located in the *GRID1* promoter region), of which the T allele was identified as a risk factor in a previous association study. There were no effects of genotype or group × genotype interactions on total brain grey matter or white matter, but on regional grey matter. In healthy subjects, we identified a significant effect of rs3814614 genotype in the anterior thalamus (bilaterally), superior prefrontal cortex, and orbitofrontal cortex – in all cases with the homozygous risk genotype TT resulting in higher grey matter density. We did not find this association within the schizophrenia sample, where rs3814614 variation was only associated with grey matter reduction in TT homozygous subjects in medial parietal cortex and increased grey matter in right medial cerebellum. For white matter, we did not find significant genotype effects in healthy controls, and only minor effects within schizophrenia patients in the posterior temporal lobe white matter. Our data indicate that *GRID1* rs3814614 genotype is related to grey matter variation in prefrontal and anterior thalamic brain areas in healthy subjects, but not in patients indicating a potential role of this schizophrenia candidate gene in thalamo-cortical functioning.
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1. Introduction

Glutamatergic neurotransmission has become increasingly important in our understanding of the pathophysiology of schizophrenia. Several putative risk genes identified so far are either related to glutamate turnover, modulation of glutamatergic neurotransmission, or structural proteins important for the development or maintenance of glutamatergic synapses (Allen et al., 2008; Harrison and Owen, 2003; Owen et al., 2005). This improving understanding of the role of glutamate has also given rise to modulation of glutamatergic neurotransmission as a novel pathway to drug discovery, for example using modulators of metabotropic glutamate receptors mGlu2/mGlu3 or NMDA receptor modulators such as D-serine and other compounds, which are still evaluated in ongoing drug trials (Inta et al., 2010; Labrie and Roder, 2010; Patil et al., 2007).

In contrast to several imaging genetics studies that have assessed the impact of catechol-O-methyl-transferase (COMT) as a modulator of dopaminergic neurotransmission on brain structure, function, and cognitive performance (Tan et al., 2007), there are only few studies on genes relevant for the glutamatergic system, especially on prefrontal and frontal-subcortical systems. Such an approach would, however, allow studying the impact of known genetic variation in genes related to glutamatergic neurotransmission (either single

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SNPs or more complex haplotypes) on brain structure (Meyer-Lindenberg and Weinberger, 2006; van Haren et al., 2008).

In a recent study on a large German population, we found evidence for genetic variation within the promoter region of the glutamate receptor delta 1 (*GRID1*) gene as a risk factor for schizophrenia (Treutlein et al., 2009). This gene was initially linked to schizophrenia in a study on an Ashkenazi Jewish population, which investigated several genes of importance to the glutamatergic system, including a locus on chromosome 10q (Fallin et al., 2005). A subsequent case–control study in Han Chinese patients and healthy controls identified four SNPs conferring higher risk for developing schizophrenia (Guo et al., 2007). Based on genome-wide association study (GWAS) data mining and bioinformatic prioritisation, *GRID1* was also suggested to be a candidate gene for schizophrenia (Chen et al., 2011). Furthermore, recurrent deletions in the chromosome 10q22-q23 region including *GRID1*, were shown to be correlated with cognitive and behavioural abnormalities (Balciuniene et al., 2007). Finally, in our recent study on 919 schizophrenia patients and 773 healthy controls recruited from three German University hospitals, evidence was found for three SNPs in the *GRID1* gene/promoter region to be associated with schizophrenia.

In this study, we follow up on our initial *GRID1* finding to examine the impact of genetic variation on brain structure. Based on the three SNPs identified, from which we chose one SNP that had withstood correction for multiple comparisons (Treutlein et al., 2009), we applied voxel-based morphometry (VBM) to high-resolution T1-weighted 3D MRI data of a sub-population of the initial German cohort. Our aim was to test the hypothesis that the *GRID1* SNPs would affect either grey matter or white matter densities, especially in structures reduced in schizophrenia, such as the prefrontal cortex, hippocampus, and thalamus. Identifying such effects would help understanding how this putative risk gene impacts on variation in brain structure, which is one of the best studied intermediate phenotypes for this disorder. Given the limitations induced by multiple testing of larger arrays of SNPs, we specifically selected our SNP markers based on the preceding genetic study of a larger German population. We applied VBM to analyse both grey and white matter, assessing global (total number of grey matter/white matter voxels) as well as regional brain structural variation.

2. Methods

2.1. Subjects

We studied 62 patients with DSM-IV schizophrenia (18 female, 44 male; mean age 31.7 years, SD 11.5; age range 18–58 years) and 54 healthy controls (25 female, 29 male; mean age 29.5 years, SD 9.9; age range 18–55 years). Age did not differ significantly between these two groups ($p = 0.291$; two-tailed *t*-test, not assuming equal variances). All study participants had given written informed consent to an imaging and a genetic protocol, which were both approved by the Ethics Committee of the Friedrich-Schiller-University Medical School. The subjects of this sample are a subsample of the of the Jena cohort of the recent German *GRID1* study (Treutlein et al., 2009). All subjects were screened to exclude a history of traumatic brain injury and reading disability, as well as concurrent alcohol or substance dependence. For most patients ($n = 51$) and controls ($n = 44$), we were able to obtain scores on the MWT-B, a German brief test estimate of overall (premorbid) intelligence; this test was chosen to exclude subjects with an IQ below 80, as well as compare premorbid IQ of subjects. Mean scores for patients (106.2; SD 12.7) and for controls (114.7; SD 13.9) differed slightly, but significantly ($p = 0.003$; two-tailed *t*-test assuming unequal variances), consistent with the expectation of slightly reduced overall IQ in schizophrenia patients.

The patients were recruited from in- and out-patient services of the Jena University Hospital's Department of Psychiatry. They were diagnosed according to DSM-IV criteria using a semi-structured interview. Dosages of antipsychotic medication were available for all but three patients, and chlorpromazine (CPZ) equivalent doses were calculated with CPZ equivalents for second-generation antipsychotics taken from a recent survey of fixed-dose placebo-controlled clinical studies (Woods, 2003), yielding a mean CPZ dose of 496.36 mg (SD 478.47) with seven patients being off antipsychotic medication at the time of recruitment and scanning. Psychopathological ratings, obtained from trained clinical raters, were available for 50 patients and showed a mean PANSS total score 68.8 (SD 27.2), mean positive symptom score 29.7 (SD 11), mean negative symptom score 48.5 (SD 23), and mean general symptom score of 35.2 (SD 14.6).

Healthy controls were volunteers recruited through newspaper advertisements and word of mouth. They were all screened using a semi-structured interview to exclude any concurrent or history of schizophrenia or other axis I psychiatric disorders, known axis II disorder, or neurological or major medical conditions. None of the controls took psychotropic medication.

2.2. Genotyping and SNP selection

DNA was extracted from blood samples and analysed as described previously (Treutlein et al., 2009). In that previous study, a GoldenGate assay (Illumina, San Diego, USA) was used to analyse a total of initially 22 SNPs located across a transcriptional regulatory region of the *GRID1* gene on chromosome 10q. All subjects were of German ancestry, and only one patient and one control had a parent of presumably non-German Caucasian origin.

The selection of single nucleotide polymorphism (SNP) for these analyses was based on the findings from the German *GRID1* study. Since the two previous association studies on *GRID1* (in Ashkenazi Jewish and Han Chinese populations, resp.) analysed different SNPs, we first focussed our SNP selection to the three SNPs that were significant in the initial German study, i.e. rs3814614, rs10749535, and rs11201985. Of these three SNPs, rs3814614 was selected as the SNP with the highest odds ratio (1.177) of the three markers, and which also survived a two-step correction for multiple comparisons in a sub-sample of the total cohort (Treutlein et al., 2009). In addition, the other two SNPs (rs10749535 and rs11201985) were found to be completely correlated with each other in both patient and control samples, thus yielding no additional information.

Genotype distribution of the controls and patients did not deviate from Hardy–Weinberg Equilibrium (Controls: Exact Test, $p = 0.4$; Patients: Exact Test, $p = 1.0$; http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). The call rate for rs3814614 in this sub-sample of the Treutlein et al. study was 98.38% of the initial Jena sample.

The distribution of genotypes for *GRID1* rs3814614 in the two groups and the gender ratios for each subgroup are given in Table 1. In order to test for differences in distributions of IQ (derived from MWT-B measures), we performed a univariate ANOVA with diagnosis and genotype as between-subject factors and age as

Table 1
Genotype distributions of *GRID1* rs3814614 in schizophrenia patients (Sz) and healthy controls (HC) with gender ratio (f: female; m: male).

GRID1 rs3814614	Genotype	HC	Sz
	C C	16 (10f, 6m)	12 (1f, 11m)
	C T	30 (10f, 20m)	31 (9f, 22m)
	T T	8 (5f, 3m)	19 (8f, 11m)

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covariate: this model did not show a significant overall effect ($F(7,87) = 2.016$; $p = 0.062$), nor significant effects for age ($F(1,87) = 0.26$; $p = 0.611$), genotype ($F(2,87) = 0.666$; $p = 0.516$) or diagnosis × genotype interaction ($F(2,87) = 0.422$; $p = 0.657$), but only a significant effect for diagnostic group ($F(1,87) = 6.885$; $p = 0.01$), thus indicating that IQ was affected by diagnostic group, but not the other variables in this sample, and importantly not differently across the subgroups created by genotyping.

2.3. Imaging data acquisition and analysis

MRI data were obtained on a 1.5 Tesla scanner (Magnetom Vision plus, Siemens, Erlangen, Germany) using a T1-weighted 3D FLASH sequence (TR 15 ms, TE 5 ms, flip angle 30°, field-of-view 256 mm, 192 slices, voxel dimensions ($1 \times 1 \times 1$) mm^3). Images were visually inspected for artefacts and sample homogeneity was ascertained by determining the standard deviation of segmented grey matter and white matter images of the entire sample as well as for the controls and patients separately.

For analysis of MRI data, we used the VBM5 package, a freely available toolbox (http://dbm.neuro.uni-jena.de/vbm/vbm5-for-spm5/) implemented in SPM5 software (Statistical Parametric Mapping, Institute of Neurology, London, UK; http://www.fil.ion.ucl.ac.uk). This VBM approach derives from optimised VBM approaches (Ashburner and Friston, 2000), but considers a unified segmentation approach (Ashburner and Friston, 2005). Each individual image is spatially normalised into standard MNI space (Montreal Neurological Institute) and segmented into different tissue classes of grey matter (GM), white matter (WM), and CSF (including other tissue). Normalisation includes both linear components, which correct for position and overall brain differences (incl. brain size), as well as non-linear transformations, which take into account individual regional differences in brain anatomy. We also applied modulation, a step used to correct for volume changes introduced by the non-linear transformation component, which was applied to both GM and WM segmented images. For segmentation, we applied a Hidden Markov Random Field model using prior information regarding the spatial distribution of tissue types (Cuadra et al., 2005), and also using a spatial constraint by specifying a minimum number of voxels that must be connected within a tissue type. This approach improves signal-to-noise ratio and minimises changes of misclassification of voxels to a particular tissue type. To further improve segmentation, a clean-up procedure is implemented using conditional dilations and erosions to remove unconnected voxels especially on the grey/white-matter borders as well as CSF borders. We also applied a threshold during VBM analysis to remove voxels with a low probability of GM (or WM, resp.), which was set to 0.2 (i.e. voxels with less than 0.2 probability of being classified as GM, or WM, resp.). This threshold is rather conservative (normally chosen to be between 0.1 and 0.2), but reduces the chances of false positive results, esp. at the tissue borders. Finally, images were smoothed with a Gaussian kernel (FWHM of 12 mm for GM, and 15 mm for WM images). Significant clusters were evaluated for anatomical localisation; in addition to visual inspection, we also applied the Anatomical Automatic Labelling (AAL) toolbox initially implemented by Tzourio-Mazoyer and colleagues on the basis of anatomical parcellation of a single subject high-resolution T1-weighted data set (Tzourio-Mazoyer et al., 2002).

2.4. Statistical analyses

First, we assessed overall (global) effects of group and genotype, as well as group × genotype interaction for the total brain GM and WM, resp. Since VBM5 segments the brain scans into GM, WM and CSF/other tissues, it also gives the total number of voxels for each of the tissue type, which then can be multiplied with the voxel volume (in our case 1 mm^3) to obtain an accurate estimate of total brain GM, and WM, resp. (CSF was excluded for these analyses, since this often includes other types of tissue like bone, meninges etc.). We used SPSS (Version 16.0 for Mac, SPSS Inc., Chicago, USA) to calculate ANOVAs for GM and WM separately, defining group (schizophrenia; controls) and *GRID1* rs3814614 genotype (CC; CT; TT genotypes) as between-group factors. We tested for main effects of factors group and genotype, as well as the interaction between these two, and assumed unequal variances due to the distribution in the subgroups.

Secondly, we assessed regional brain structural effects of group, genotype, and the interaction of both, based on the VBM5 analyses and the general linear model implemented in SPM. For all main statistical analyses of VBM data, we used a uniform threshold of $p < 0.001$ (uncorrected). The applied general linear model included group (schizophrenia; healthy control) and *GRID1* rs3814614 genotype (CC; CT; TT), as well as the nuisance variables age and gender; hence, the influence of the latter two variables was controlled for on the whole-sample level, allowing to compensate for both potential confounding effects as well as differing gender distributions in subgroups.

For the VBM analyses, we first tested effects of group on GM to evaluate the basic pattern of schizophrenia-related structural changes. We then tested the genotype effects within the healthy control sample only, and then within the schizophrenia patient sample only (separately for GM and WM). Finally, we computed a group × genotype interaction (also, separately for GM and WM).

3. Results

For SNP rs3814614, consistent with the previously reported higher frequency of the T allele in the previous association study indicating this being a risk allele in our German population (Treutlein et al., 2009), T allele and TT genotype were more frequent in the patient sample. Chi square test for allelic association test was significant between patients and controls (allele distribution: $p = 0.047$, df = 1; genotype distribution: $p = 0.103$, df = 2).

3.1. Effects of group and genotype on global GM and WM

There was no group × genotype interaction for either total GM or WM. The mean total brain GM and WM for the two groups, as well as the subgroups divided by *GRID1* rs3814614 genotype are given in Table 2. ANOVA did not show a significant effect of diagnostic group on either total brain GM or WM (although there was a trend-level increase of total brain WM in patients). Genotype had no significant effect on total brain GM or WM within the healthy controls, and likewise no effect within the schizophrenia patients.

Table 2
Total grey matter (GM) and white matter (WM) volumes (in mm^3, derived from VBM voxel counts) in schizophrenia patients (Sz) and healthy controls (HC), and subgroups stratified by *GRID1* rs3814614 genotype: mean values (SD in brackets).

Group/sub-group	Tissue	HC	Sz
Total group	GM	763.1 (69.7)	753.4 (81.2)
CC	GM	761.4 (15.8)	785.7 (16.6)
CT	GM	756.8 (11.5)	744.6 (14.8)
TT	GM	790.1 (36.6)	747.4 (20.7)
Total group	WM	489.2 (63.5)	504.6 (67)
CC	WM	470.9 (12.8)	536.4 (23.4)
CT	WM	503.1 (11.9)	509.3 (11)
TT	WM	473.4 (26)	476.8 (13.4)

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We also excluded the potentially confounding effects of IQ and medication using Spearman correlation, which showed no significant effects for IQ in all subjects with MWT-B scores ($n = 95$) for either grey matter ($r = 0.013$; $p = 0.904$) or white matter ($r = -0.066$; $p = 0.525$), nor within the patient sample in either grey matter ($r = 0.024$; $p = 0.868$) or white matter ($r = -0.101$; $p = 0.479$). Also, we did not find a significant effect of medication (CPZ equivalents were available for $n = 59$ of 62 patients) on either total grey matter ($r = 0.007$; $p = 0.960$) or white matter ($r = 0.098$; $p = 0.461$).

3.2. VBM results of regional brain structural effects of group

Comparison of GM maps between healthy controls and schizophrenia patients showed a pattern of decreased grey matter density in patients in several cortical and subcortical areas, including the dorsolateral, ventrolateral, and orbitofrontal prefrontal cortices, including the frontopolar cortex, and furthermore insular cortex (bilaterally), right middle and inferior temporal cortex, thalamus (mostly anterior nuclei), and anterior medial temporal lobe (incl. amygdala), as well as cerebellum. An overview of results is shown in the figure of the supplementary material (Supplementary Fig. 1).

For WM, we did not observe any significant differences in white matter density between schizophrenia patients and healthy controls.

3.3. VBM results of regional brain structural effects related to genotype

Within the healthy controls, we observed significant effects of *GRID1* rs3814614 genotype on regional grey matter ($p < 0.001$, uncorrected) with significant clusters in the anterior thalamus (bilaterally), left lateral prefrontal cortex (middle frontal gyrus, close to superior frontal junction), as well as smaller clusters in the right dorsolateral prefrontal cortex and left orbitofrontal cortex (gyrus rectus), and the left precentral gyrus. A maximum intensity projection of the findings is shown in Fig. 1. As can be seen from parameter estimates (grey matter density plots of maximum intensity voxels of each cluster), the observed effect did in all case arise from the TT homozygous subjects, who had higher grey matter density compared to the CC homozygous and CT heterozygous subjects.

For the white matter analysis among the healthy control subjects, we did not find any significant differences related to genotype.

Within the schizophrenia patients, we observed significant effects of *GRID1* rs3814614 genotype on regional grey matter ($p < 0.001$, uncorrected) in the right medial cerebellum and an area in the medial parietal cortex between the central and precuneal region. Results are shown in Fig. 2. While for the cerebellar cluster there appeared to be a linear relation with TT homozygous subjects showing highest grey matter density (CC homozygous subjects being lowest and CT heterozygous subjects being intermediate), this was the opposite for the parietal cluster, where CC homozygous subjects had highest grey matter concentrations.

For the white matter analysis of genotype rs3814614 effects within schizophrenia patients (shown in Fig. 3), we found significant clusters in two smaller posterior temporal lobe white matter areas, but not in frontal lobe white matter.

Testing the interaction of group × genotype for the VBM findings ($p < 0.001$, uncorrected), we did not observe significant interaction effects for grey matter analysis and only for a small (<20 voxel) cluster for white matter analysis. However, taking into account that interaction effects would be expected to be much smaller than a main genotype effect in either diagnostic group alone, we performed an exploratory analysis at a lower threshold of $p < 0.01$. For grey matter, this basically confirmed the pattern of diverging findings observed in the separate cohort analysis, i.e. showing significant interaction effects in the prefrontal and thalamic areas significant in the healthy control analysis, as well as the cerebellum cluster seen in the schizophrenia group analysis. Likewise, this pattern was observed for the white matter analysis, where, however, we saw additional effects (only at $p < 0.01$) at multiple white matter clusters in the frontal lobe (underneath the left superior and middle frontal gyri and right gyrus rectus) and the posterior temporal lobe white matter.

An overview of the findings of effects of genotype within each group is given in Table 3 listing significant clusters with anatomical label, location of maximum intensity voxel, and number of voxels in that cluster.

4. Discussion

In this study, we provide an analysis of the effects of single SNP marker (rs3814614) in a recently identified candidate gene coding for a delta glutamate receptor on brain structure in healthy controls and patients with schizophrenia. Our results suggest that variation within this marker of a schizophrenia-associated gene has effects on prefrontal and anterior thalamic regions, with a dissociation of effects between healthy and schizophrenia-affected populations. The T allele of *GRID1* rs3814614, which has previously been shown to be overrepresented in schizophrenia patients (Treutlein et al., 2009), gives rise to higher grey matter density in the above areas in healthy controls, but not schizophrenia patients, who show such effects only in a medial cerebellar region. In addition, our group comparison of patients vs. controls demonstrates that our sample is representative of the well-documented changes in schizophrenia.

Previous studies on the links between genetic variation relevant to glutamatergic neurotransmission have focused on genes encoding for metabotropic glutamate receptors/subunits, such as GRM3 (Tan et al., 2007) or G72, which is assumed to influence NMDA regulated neurotransmission (Goldberg et al., 2006). The GRID family of receptors differs in many aspects from the other glutamate receptors, esp. through their modulation of glutamatergic function (Schmid and Hollmann, 2008). Originally identified in mice (Yamazaki et al., 1992), the delta 1 as well as the delta2 subunits have been classified as part of the ionotropic glutamate receptor family mainly based on sequence homology. In rodents, *GRID1* is expressed in the cortex and hippocampus, and

Table 3
Overview of significant clusters ($p < 0.001$, uncorr.) of grey matter (GM) and white matter (WM) analyses of genotype effects in healthy controls (HC) and schizophrenia patients (Sz); co-ordinates refer to local maxima; only clusters larger than 40 voxels are included.

Anatomical region	Number of voxels	Co-ordinates
Genotype effects in HC (grey matter)		
Left frontal middle gyrus	1545	−35; 9; 54
Anterior thalamus (bilateral)	590	−5; −6; 10
Right frontal middle gyrus	80	35; 40; 8
Right postcentral gyrus	112	−51;-13; 25
Medial orbitofrontal cortex/gyrus rectus	47	−8; 47; −15
Genotype effects in Sz (grey matter)		
Right cerebellum	219	16; −53; −42
Right paracentral lobule	755	6; −35; 56
Genotype effects in Sz (white matter)		
Right inferior temporal lobe white matter	678	52; −28; −23
Left inferior/lateral temporal lobe white matter	590	−52; −50; −11

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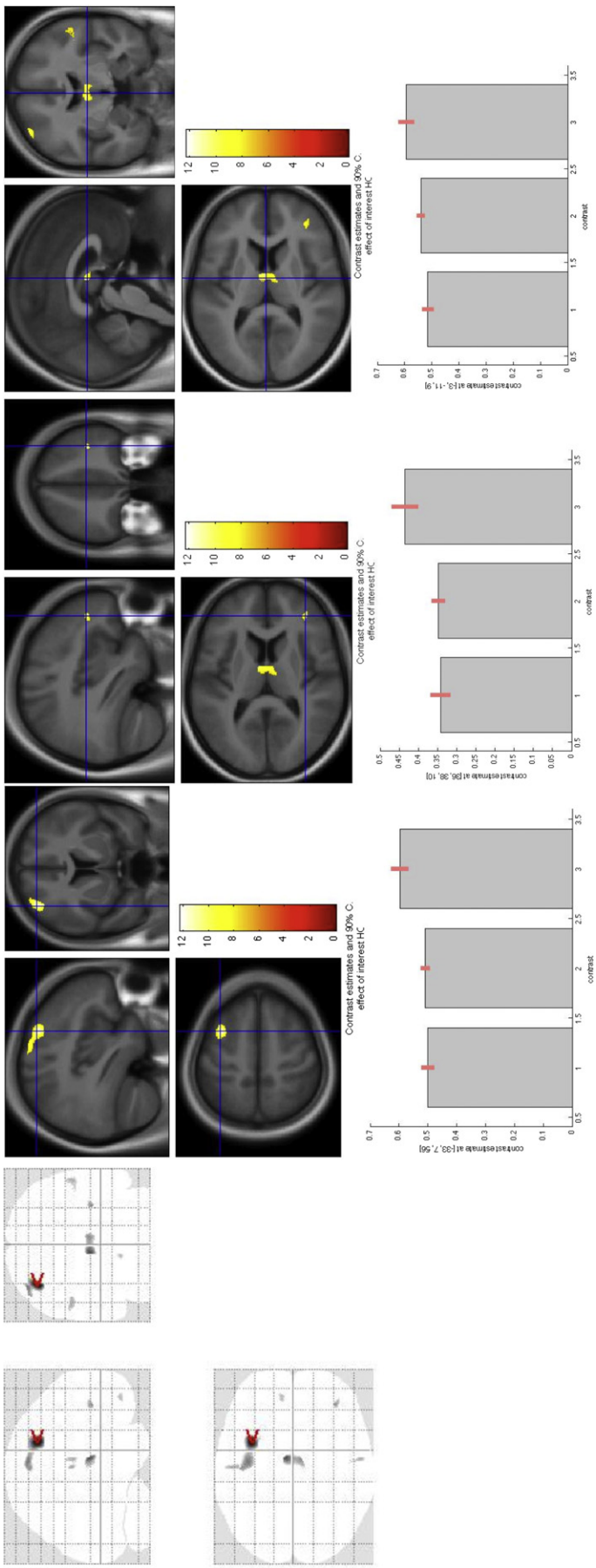

Fig. 1. Voxel-based morphometry (VBM) analysis of effects of *GRID1* rs3814614 genotype on regional grey matter in 54 healthy controls ($p < 0.001$, uncorrected): Maximum intensity projection (MIP) and selected axial sections of significant clusters with corresponding plot of parameter estimates (grey matter density) for maximum intensity voxel.

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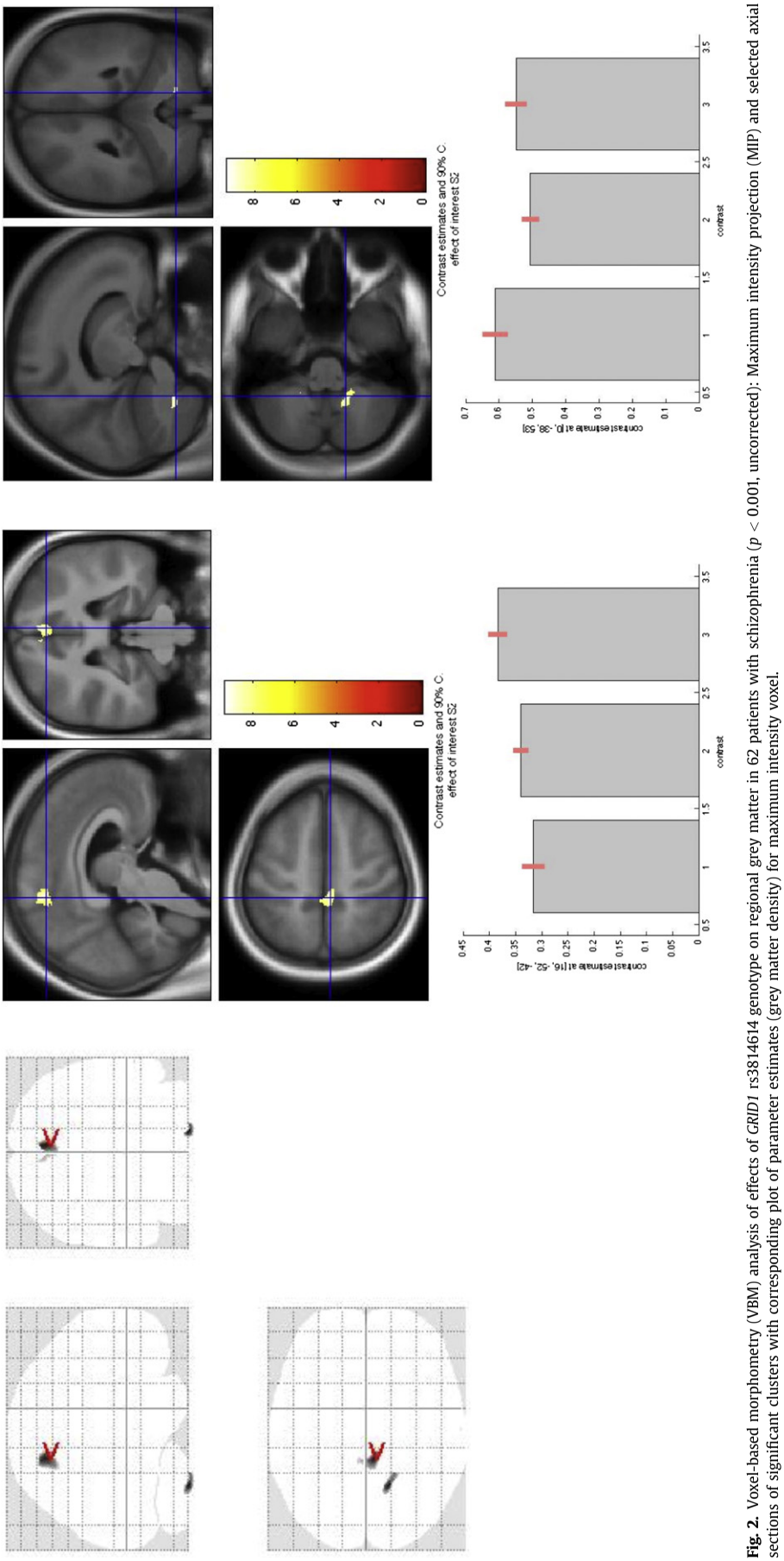

Fig. 2. Voxel-based morphometry (VBM) analysis of effects of *GRID1* rs3814614 genotype on regional grey matter in 62 patients with schizophrenia ($p < 0.001$, uncorrected): Maximum intensity projection (MIP) and selected axial sections of significant clusters with corresponding plot of parameter estimates (grey matter density) for maximum intensity voxel.

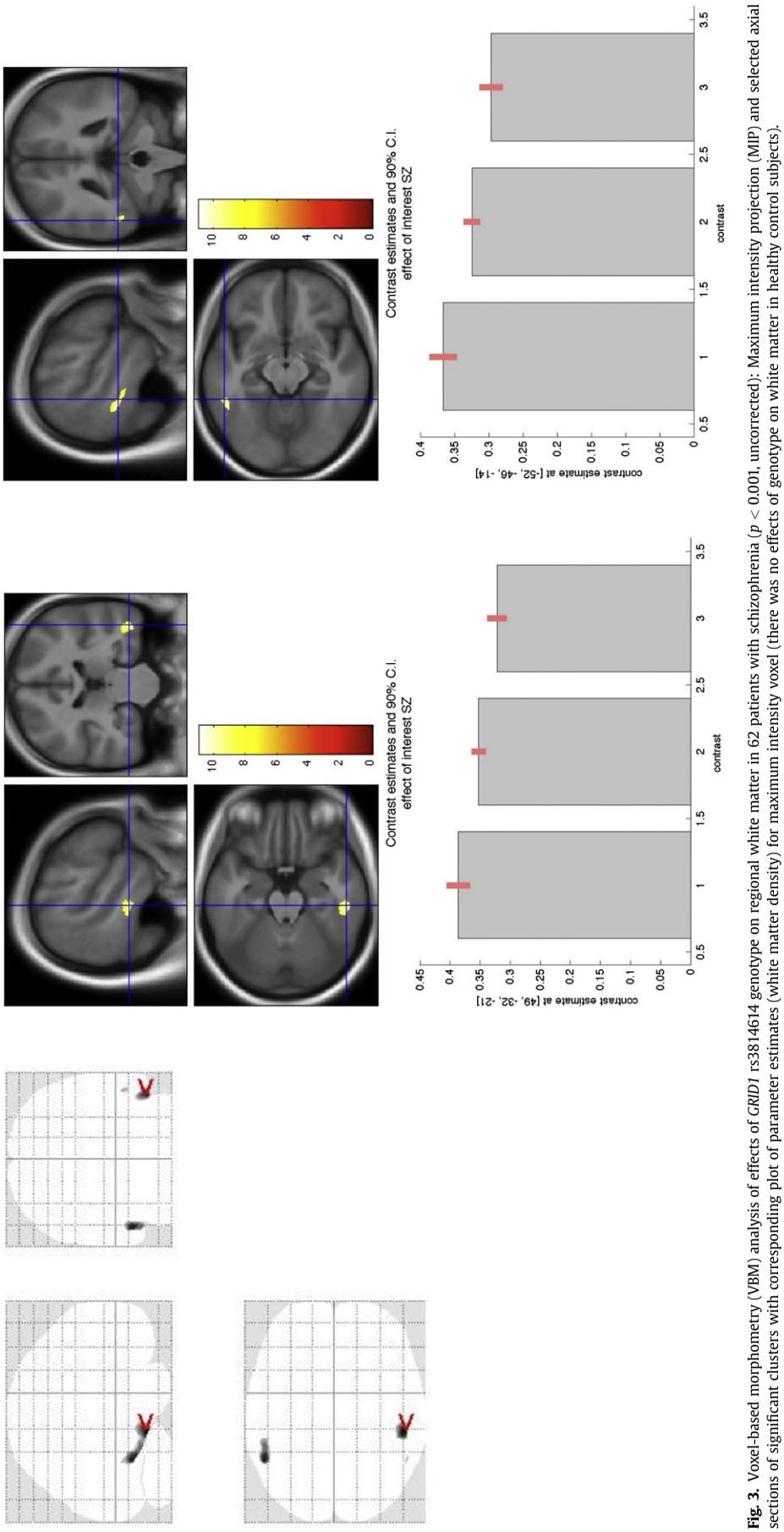

Fig. 3. Voxel-based morphometry (VBM) analysis of effects of *GRID1* rs3814614 genotype on regional white matter in 62 patients with schizophrenia ($p < 0.001$, uncorrected): Maximum intensity projection (MIP) and selected axial sections of significant clusters with corresponding plot of parameter estimates (white matter density) for maximum intensity voxel (there was no effects of genotype on white matter in healthy control subjects).

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transiently during development in the caudate/putamen, while GluR delta2 is abundant in the cerebellum (Naur et al., 2007; Schmid and Hollmann, 2008). In humans, it is expressed in several brain regions, including hippocampus, amygdala, and thalamus, but hardly any other (non-brain) tissues (Nagase et al., 1999). Unlike other glutamate receptor subunits, however, *GRID1* and *2* apparently do not form ion channels, but rather act through gating of and interaction with other subunits (Schmid and Hollmann, 2008). Both delta receptors, *GRID1* and *GRID2*, were shown to induce presynaptic differentiation (Kuroyanagi et al., 2009). Of particular interest is a most recent study, which showed that glutamate delta 1 receptor knock out mice show not only hyperactive and aggressive behaviour, but also deficits in social interaction; these behaviours were associated with lower GluA1 and GluA2 subunit expressions in the prefrontal cortex and higher GluA1 expression in the amygdala of knock out mice (Yadav et al., 2012).

Our findings in healthy subjects suggest that variation of SNP rs3814614 in the gene encoding for *GRID1* contributes to brain structural variation, especially in areas of a frontothalamic system. The presence of effects in both the thalamus and areas in the prefrontal cortex (in healthy subjects) indicates that thalamo-cortical projections might be a circuitry affected by this gene, or that gene products influence might influence structural integrity of these circuits. The nuclei of the anterior thalamus project, among others, to several prefrontal areas in primates, including rostral premotor areas (McFarland and Haber, 2002), and receive direct hippocampal projections (Aggleton et al., 2010; Xiao et al., 2009), thus providing an important link for several cognitive functions. Abnormalities of the anterior thalamus, as seen in our group comparison of patients vs. controls, have also been reported in post mortem studies (Byne et al., 2009), and specifically affecting mostly parvalbumin-labelled thalamo-cortical projections neurons rather than GABAergic interneurons or related indicators (Danos et al., 1998; Thompson et al., 2009). In addition, a number of imaging studies have shown alterations not only in the thalamus (e.g. Agarwal et al., 2008; Corradi-Dell'Acqua et al., 2012; Smith et al., 2011), but also in tracts linking the thalamus with the prefrontal cortex (Kim et al., 2008), thus providing a potential link to cellular findings on aberrant thalamo-cortical functioning (Pinault, 2011).

GRID1 expression has been demonstrated in human thalamic post mortem tissue (Nagase et al., 1999), and a recent study using real-time RT-PCR to measure *GRID1* messenger ribonucleic acid levels in the dorsolateral prefrontal cortex found reduced expression in schizophrenia (Zhu et al., 2009), but to our knowledge there are no systematic post mortem studies on differential expression across different cortical/subcortical brain regions. Hence, while the present literature suggests expression differences to be present in adult brain, we cannot infer on the timing of a putative effect of aberrant *GRID1* expression or function in the thalamus or prefrontal cortex.

Our finding of a disease effect on anterior thalamus voxels contrasts with a genotype effect only in healthy controls but not patients, suggesting a differential effect related to either the expression of the schizophrenia disease phenotype or genetic liability. This could be related to several factors. Genetic variation in the *GRID1* gene might affect other receptors and molecular pathways involved in schizophrenia pathology. Zhu and colleagues recently identified a micro-RNA in the *GRID1* intron 2, which targets several schizophrenia susceptibility genes, suggesting that variation in the *GRID1* gene might not be limited to this gene product only (Zhu et al., 2009). An effect on other gene products might explain the lack of a significant direct effect of *GRID1* (rs3814614) on brain structure in schizophrenia in our sample. Given the interaction of several pathways and epistatic effects between schizophrenia risk genes, the effect on brain structure could thus differ between diagnostic groups. Hence, even though the T risk allele might be overrepresented in schizophrenia, it might not be sufficient on its own to interact in the emerging pathology leading to disease manifestation. Also we need to consider that even if the risk gene affects brain structure, its effects might be obscured by those of other genes or factors – either related or unrelated to this particular gene or its products. Therefore, the effect of other genes, developmental pathology, or effects related to the onset of schizophrenia (as well as confounding factors) acting on thalamic morphology might in fact have contributed to the lack of significant impact of this *GRID1* genotype in the patient population. Also, it is unclear whether the effect of *GRID1* SNP rs3814614 in healthy subjects might be related, in part, to compensation, i.e. grey matter increase related to counteract a developmental effect introduced by subtle glutamatergic dysfunction associated with this gene marker.

While we have accounted for or excluded several putative confounds, there are a few limitations to be considered. First, we need to consider sample size, which is a limiting factor when dividing a reasonably large sample into subgroups. However, all our subgroups stratified by genotype were sufficiently large, and the overall sample size is in the regions suggested by a recent survey of imaging genetics studies (van Haren et al., 2008) and modelling of false positive effects (Meyer-Lindenberg et al., 2008). Second, our association of *GRID1* with prefrontal and thalamic grey matter should be substantiated with expression analyses in post mortem tissues of these areas, which are not yet available. Third, we need to consider a limitation inherent to the VBM-based analysis of white matter derived from T1-weighted images. While white matter VBM has been used in several studies, identifying morphometric abnormalities in schizophrenia (e.g.(Hulshoff Pol et al., 2004)), it does not depict qualitative microstructural changes such as those identified with diffusion tensor imaging (DTI). Therefore, complementary DTI analyses might be useful for further elucidation of effects on white matter. Finally, we need to consider medication and IQ as potentially confounding variables. While patients and controls did significantly differ in an estimate of IQ, our more detailed ANOVA analyses have shown that there was an effect only for the factor diagnosis, but no significant effects of age or genotype, and no significant diagnosis by genotype interaction for the IQ data, which indicates that IQ was not affected by the other factors and not distributed differently across the genotype subgroups. Controlling for medication, however, is more complex since CPZ equivalents might not be a useful single confounding variable. Although antipsychotics have repeatedly been shown to affect brain structure (even though effects are often small to moderate), the regional distribution differs markedly across single drugs, and we therefore cannot completely rule out effects of medication within the patient sample.

In conclusion, we have demonstrated that schizophrenia-associated genetic variation in *GRID1* affects grey matter of the anterior thalamus and left lateral prefrontal cortex in healthy controls, but not schizophrenia patients, which might indicate an association of the schizophrenia candidate gene *GRID1* with frontothalamic functioning.

Conflict of interest

The authors declare that they have no relevant conflicts of interest that might influence the study design, data acquisition, interpretation, or other parts of this work.

Contributors

I.N., R.G.M.S., M.M.N., S.C., and H.S. designed the study.

I.N., C.C.S., C.S., St.S., J.R.R., H.S., and R.G.M.S. contributed to patient recruitment and scanning.

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I.N., C.C.S., J.T., T.W.M., T.D., S.C., M.R., M.M.N., R.G.M.S, and H.S. contributed to the collection, processing, and analysis of DNA samples.

R.M., S.S., C.G., and I.N. contributed to the imaging data analysis.

I.N. wrote the first draft of the manuscript and all authors approved the final version.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jpsychires.2012.08.026.

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