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Association of GRM3 polymorphism with white matter integrity in schizophrenia

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ABSTRACT

Background: While the functional disconnectivity hypothesis of schizophrenia has received considerable attention, fewer studies have investigated the contribution of genotype to structural connectivity between brain regions either in schizophrenia patients or in healthy controls. In this study, we obtained diffusion tensor imaging (DTI) data and genome-wide single nucleotide polymorphism (SNP) data from 74 cases and 87 age- and gender-matched controls.
Methods: We used independent component analysis (ICA) to analyze fractional anisotropy (FA) values and correlated FA values with 121 SNPs in genes associated with myelination and/or schizophrenia risk.
Results: Using ICA, we identified 6 maximally independent components in which the majority of the voxels corresponded to known white matter (WM) tracts. Among these WM-enriched components, two had FA values that were significantly decreased in patients. In addition, we examined the relationship between FA values and genotype and found that a SNP located in the intronic region of the metabotropic glutamate receptor 3 gene, *GRM3*, shows a significant correlation with FA values in a component containing tracts from the cortico–cerebellar–thalamic–cortical circuit of patients but not controls.
Conclusions: Our findings strengthen the evidence for an association between *GRM3* genotype and schizophrenia and suggest a role for glutamate neurotransmission in the establishment and maintenance of myelinated fibers.

1. Introduction

The functional disconnectivity hypothesis of schizophrenia states that symptoms of schizophrenia could be explained by a loss of connectivity between dispersed brain regions. Many imaging and genetic studies support this hypothesis and point to altered oligodendrocyte function and myelin microstructure in schizophrenia. Diffusion tensor imaging (DTI) studies (reviewed in Bora et al., 2011; Fitzsimmons et al., 2013) have demonstrated decreased FA and thus decreased integrity of several WM tracts in patients with schizophrenia across many reports including ours (Caprihan et al., 2011; White et al., 2011, 2013). These results are supported by many post-mortem studies showing that several myelin-associated genes are expressed at lower levels in the brains of schizophrenia patients as well as genetic studies that show an association between schizophrenia and several myelin- and oligodendrocyte-associated genes (reviewed in Feng, 2008; Takahashi et al., 2011).

While post-mortem studies have suggested a link between myelin-related gene expression and schizophrenia, few studies have integrated DTI data and genotype. Among these, Voineskos et al. (2013) recently reported that SNPs in myelin-specific genes such as *MAG* and *CNP* predict FA values in many WM tracts in both patients and controls. Also, another recent study demonstrated an association of a SNP in *CNTNAP2* and FA values in the uncinate fasciculus of controls and patients (Clemm von Hohenberg et al., 2013). These reports were focused on a few myelin genes and SNPs and the contributions of genes participating in other signaling pathways that affect white matter development and function, such as those involving glutamate and dopamine have not been evaluated to date.

In this study, we compared the genotypes at 121 schizophrenia- and myelin-related SNP loci to fractional anisotropy images from 74 schizophrenia subjects and 87 controls. We hypothesized that individual SNPs in genes associated with white matter integrity or schizophrenia would correlate with FA values in our patient sample. We used independent component analysis (ICA) to process DTI data into maximally ICs to investigate the association between structural connectivity and genotype. Our results uncovered a novel relationship between the glutamate receptor 3 *GRM3* gene and WM integrity in the patients.

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2. Methods

2.1. Participants

The subjects for this study were participants in the multisite Mind Clinical Imaging Consortium (MCIC), which is comprised of investigators at four research sites: the University of New Mexico (UNM), the University of Minnesota (MINN), Massachusetts General Hospital (MGH), and the University of Iowa (IA) (Gollub et al., 2013). The cross section of individuals from the MCIC study with both genetic and DTI data consisted of 74 patients with schizophrenia and 87 controls matched for age and sex. White/non-white status was included as a covariate for all linear regression analyses that compared cases and controls (Table 1). All participants provided written informed consent, and the Institutional Review Board at each site approved this project. Participants in the control group were excluded if they had any physical or neurological disorder; a history of any Axis I psychiatric disorder including substance abuse; or a first degree relative diagnosed with schizophrenia or bipolar disorder. All participants in the patient group had received a diagnosis of schizophrenia, or schizoaffective disorder. This diagnosis was confirmed upon their entry into the study using the Structured Clinical Interview for DSM-IV-TR Disorders (Williams et al., 1992) or the Comprehensive Assessment of Symptoms and History (Andreasen et al., 1992). Patients were excluded if they had ever been diagnosed with any other psychiatric disease or with epilepsy, had a history of head injury, had a history of substance abuse or dependence within the past month, or had an intelligence quotient equal to or less than 70. The severity of positive and negative symptoms for the patient group was assessed using the Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen, 1984) and the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1983).

2.2. DTI acquisition and preprocessing

DTI data was acquired at each of four sites: IA, MGH, MINN and UNM. The patient/control count for each site is 15/38 for IA, 25/18 for MGH, 20/16 for MINN, and 14/15 for UNM. For imaging parameters and details at each site please see White et al. (2011, 2013). Data were preprocessed in FSL and FA images were calculated (Caprihan et al., 2011). FNIRT, a non-linear registration algorithm, was used to normalize FA images of each subject to an FA template in the Montreal Neurological Institute (MNI) which was downsampled to $2 \times 2 \times 2$ mm^3 images and then smoothed with a 8 mm full width half maximum Gaussian kernel.

2.3. Independent component analysis

ICA analysis is a blind source separation technique that is widely used in imaging studies, especially in fMRI. Briefly, ICA attempts to identify maximally independent components from the imaging data. ICA was performed in MATLAB using the Group ICA fMRI Toolbox (GIFT) software (http://icatb.sourceforge.net) to extract 20 ICs from a subject-by-voxel FA matrix (Erhardt et al., 2011) combining DTI data from both patients and control subjects. We estimated 20 components based on previous studies using a similar approach (Caprihan et al., 2011).

2.4. WM characterization of independent components

The Z-score maps of each component were overlaid on the Ch2better template with a threshold of $z > 2$ (Fig. 1). Next using SPM8 we matched the suprathreshold voxels in each component to 20 different major white matter tracts contained in the John Hopkins DTI atlas (Mori et al., 2005). We then computed the percentage of suprathreshold voxels in each component that mapped to these tracts as well as the percentage of each tract that mapped to our components. Twelve of the twenty components were found to map less than 60% to all major WM tracts combined and were excluded from our analysis. Of the remaining eight components, two were found upon visualization to contain voxels almost entirely along the edge of the brain or in the ventricles and were also excluded, leaving a total of six components that mapped more than 60% of WM tracts and were included in our analysis (components A–F, Fig. 1).

2.5. Genetic analysis

All subjects provided whole blood sample and DNA was extracted using the PAXgene Blood DNA Kit (Qiagen) and genotyped using Illumina's Human Omni Quad 1M Beadchips (Illumina, San Diego). GenomeStudio was used to make the final genotype calls. A series of quality control procedures following the recommendation by Anderson et al. (2010) was performed in PLINK (Purcell et al., 2007), including less than 5% per-sample missingness, less than 5% per-locus missingness, samples' gender match, heterozygosity within 3 standard deviation, relatedness <0.18 (no 2nd degree or closer relatives), Hardy–Weinberg equilibrium $<1 \times 10^{-6}$, and minor allele frequency (MAF) >0.05 (Chen et al., 2012). Although the vast majority of subjects self-reported racial status, we validated this information by computing the population structure from the genotyping data. Specifically, we applied PLINK multidimensional scaling analysis to our samples and used the HapMap3 as the reference. This analysis not only indicated that genotyping-based race largely agreed with the self-reported data but also enabled the classification of 8 subjects without self-reported race (Table 1). Then, a list of 288 myelin and/or schizophrenia related SNPs was compiled for further analysis using SZGene database (http://www.szgene.org), Jungerius et al. (2008) and various other literature sources (Supplementary Table 1). Out of these 288, 140 were available from the chip, although four failed quality control (genotyping rate < 95%), nine failed Hardy–Weinberg equilibrium and five others were excluded because they had a minor allele frequency of less than 5% in our sample. This left a final list of 121 SNPs corresponding to 81 different myelin and/or schizophrenia-related genes (Supplementary Table 1). Allele frequency differences between cases and controls were determined using a χ^2 analysis. We found that none of the SNP had any significant difference in allele distribution between control subjects and

Table 1
Demographic information of subjects included in this study. All participants were members of the Mind Clinical Imaging Consortium (MCIC) (Gollub et al., 2013).

	Schizophrenia (n = 74)	Controls (n = 87)	P value (test)
Age			
Mean (SD)	34.8 (11.1)	32.7 (11.2)	0.41 (two sample Kolmogorov–Smirnov test)
Range	18–60	18–57	
Sex (M/F)	53/21	52/35	0.16 (χ^2 test)
Race/ethnicity			
White	53	81	4.2×10^{-4} (Fisher's exact test)
Black	16	3	
Asian	4	3	
Native American	1	0	

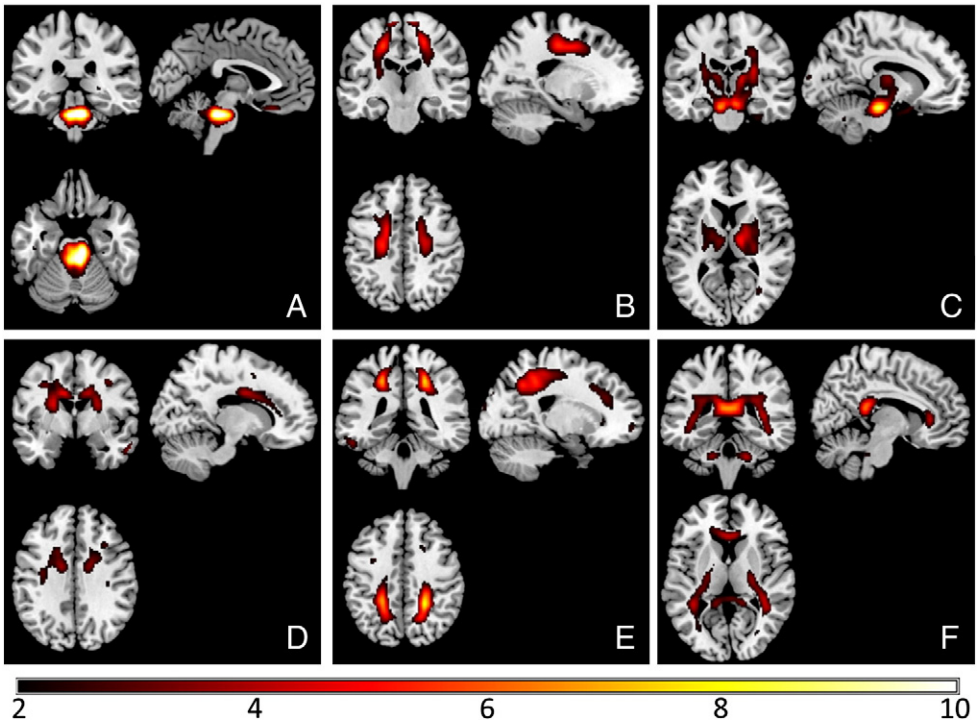

Fig. 1. Threshold map for WM-enriched components identified by ICA on multi-site DTI data (A–F). Color scale represents the Z value for each voxel's ICA score. The higher scoring voxels from each biologically relevant component tend to be distributed symmetrically and along regions of white matter tracts.

patients with schizophrenia after multiple comparison correction (corrected $p \geq 0.05$, Supplementary Table 1).

2.6. Calculation of CPZ dose-year equivalent values in patient sample

Medication information was also obtained for each patient and converted to lifetime CPZ equivalence values based on the expert consensus guideline presented by Kane et al. (2003). Dose year equivalent values of antipsychotics were calculated using the formula described by Abbott et al. (2011):

$$\begin{aligned}\text{Dose year} = &[(\text{dose (mg)} * 100\,\text{CPZ})/\text{drug equivalent}] \\ &* [\text{days on dose}/365.25] * [1\ \text{year}/(100\,\text{CPZ} * 1\ \text{year})].\end{aligned}$$

2.7. Statistical analysis

All statistical tests were performed using R (R Core Team, 2012). Patients and controls were compared using a two sample Kolmogorov–Smirnov test for age, a χ^2 test for sex, and a Fisher's exact test for race/ethnicity. A one-way analysis of variance (ANOVA) and a Fligner–Killeen test of homogeneity of variances were both used to evaluate differences in loading coefficients by site.

A linear regression was used to model ICA loading coefficient values for each component as a function of the subjects' disease status, white/non-white racial status, age, and imaging site in order to determine any differences in ICA loading coefficient values between patients and controls. p values for these models were adjusted using a Bonferroni correction for six independent tests. An additional linear regression was performed in the case group alone modeling ICA loading coefficient as a function of CPZ dose-year equivalents with site as a covariate. Linear regressions were also performed by modeling subjects' ICA loading coefficients as a function of positive or negative symptom scores, with site as a covariate.

SNP genotypes were first represented by number of major alleles (0, 1 or 2 for each genotype in each subject). For one SNP, genotypes were later coded based on minor allele presence (0 or 1). Using control subjects alone, case subjects alone, or both groups combined, linear regression was performed modeling ICA loading coefficient values as a function of genotype, site, white/non-white racial status group, and age. All p values for genetic tests were adjusted using a Bonferroni correction for 726 independent tests (121 SNPs by 6 components).

3. Results

3.1. Sample

Table 1 provides a summary of the demographics for our sample. This sample represents a subset of the cases reported in two previous DTI studies of the MCIC cohort (White et al., 2011, 2013) as our study required all subjects to have both neuroimaging and genetic data. The mean age and age range, handedness, and male to female ratios were not statistically different between the patient group and the control group. In contrast, the racial and ethnic make-up of our patient and control groups differed significantly ($p < 0.01$, Fisher's exact test). Because of the small size of each of the non-white groups, we included white/non-white status as a covariate in all of our models that compared the patient group with the control group.

3.2. Independent component analysis

ICA was used to analyze DTI data from both patients and control subjects. Six of the 20 original ICs were determined to be white matter enriched as more than 60% of their suprathreshold voxels mapped to any of the 20 major white matter regions described in the MRI Atlas of Human White Matter (Fig. 1, Table 2). These components will hereafter be referred to as components A–F. We used SPM8 to map suprathreshold voxels of our WM-enriched components to the Johns Hopkins's DTI atlas (Table 2). Tracts that were especially represented in white matter enriched components include the anterior thalamic radiation (ATR), the corticospinal tract (CST), the forceps major (FMAJ), the forceps minor (FMIN), and the superior longitudinal fasciculus

(SLF). As can be seen in Fig. 1 and Table 2, left-right symmetry was largely maintained in each component.

3.3. Site differences

We assessed the differences in DTI data in each of the four sites using a one-way ANOVA for both cases and controls in each component (Supplementary Fig. 1). We found that the means of ICA loading coefficients for the controls differed significantly ($p < 0.05$) in each site, and that the means of the ICA loading coefficients in the schizophrenia patients differed significantly by site in four out of the six components ($p < 0.05$ for components A, B, E and F and $p > 0.1$ for components C and D). In addition, using the non-parametric Fligner–Killeen test of homogeneity of variances, we found that the variances of components A, B, C, and F differed significantly by site in the control group, while the variances of components A, B, D, and F differed significantly by site in the patient group ($p < 0.05$). Because of these differences all of our models include site as a covariate.

3.4. Differences in ICA loading coefficients in patients versus controls

We also tested the interaction of ICA loading coefficients and diagnosis and found that ICA loading in the patient group was significantly lower than that in the control group for components D and E (Bonferroni corrected $p = 3.36 \times 10^{-4}$ for component D and 5.11×10^{-5} for component E). As shown in Table 2, component D contains a disproportionate portion of the ATR, while component E contains a wide variety of WM tracts, including the ATR, the CST, the CGC, the SLF, and the right IFO. Given that component E contains a large number of tracts, the significant difference seen in WM integrity in this component between patients and controls may be reflective of a difference in global WM integrity. Furthermore, analysis of medication effects on FA values revealed a significant correlation of ICA loadings in components B and E and lifetime chlorpromazine-equivalents (Fig. 2), and this correlation was significant for component E even when the analysis excluded the three patients with doses higher than 200 mg-year. In contrast, no significant correlations were observed between ICA loading coefficients in any components with the severity of positive and negative symptoms (data not shown).

3.5. Effect of genotype on ICA loading coefficients

After characterizing the ICs and their association with disease state, we next sought to establish whether any of the six components showed an association with the genotypes of 121 myelin and schizophrenia related SNPs (Supplementary Table 1) either in the patient group or in the control group. To do so, we performed linear regressions in the patient group alone, in the control group alone, and in both groups combined, modeling ICA loading coefficients as a function of genotype, site, race (white/non-white), and age. The allele frequencies of these 121 SNPs did not differ significantly between the patient group and the control group (corrected $p \geq 0.05$, Supplementary Table 1).

While we found no significant associations of genotype and ICA loadings in the control group or in the combined group, we report one highly significant association in the patient sample alone. The relationship between rs7808623, located in an intronic region of *GRM3*, and ICA loading in component C was found to be significant even after the stringent Bonferroni correction for 726 independent tests (corrected $p = 0.0363$, uncorrected $p = 5.00 \times 10^{-5}$) (Fig. 3). The minor allele of rs7808623 was associated with higher white matter integrity in component C, which contains portions of the ATR as well as the corticospinal tract (Table 2). By mapping the top-contributing voxels in component C slice by slice and comparing these slices with known WM tracts, we observed that component C overlaps with a series of tracts connecting the frontal cortex to the cerebellum (Fig. 4).

The allele frequency for rs7808623 did not differ by site (χ^2 test, $p = 0.211$) or diagnosis (Fisher's exact test, $p = 0.858$) when considering all the subjects or when considering just the patient group ($p = 0.0641$). Since the MAF of this SNP is different in individuals from European descend and African descent (dbSNP), we also analyzed the effect of race on ICA loadings and performed an analysis using only white subjects. No differences were found in ICA loading coefficients for component C between white vs. non-white groups. Also, we found that the association between rs7808623 and ICA scores in white subjects was still significant (uncorrected $p = 0.00194$) but due to the smaller sample size it did not pass a Bonferroni correction. Finally, because there was only one patient with a homozygous minor allele for this SNP, we also performed a new analysis wherein we grouped this homozygous case with the heterozygous cases. Using this model, we found that the

Table 2
Percentage of each tract covered by each component and percentage of each component covered by each tract. SPM was used to map each component to 20 different tracts contained in the John Hopkins DTI atlas.

Tract	A	B	C	D	E	F
	Percent tract/percent component	Percent tract/percent component	Percent tract/percent component	Percent tract/percent component	Percent tract/percent component	Percent tract/percent component
ATR-L	4.05/10	0.19/0.34	13.05/14.53	8.36/12.43	4.59/6.41	4.24/3.44
ATR-R	6.98/15.87	0.29/0.46	17.76/18.18	7.46/10.21	2.67/3.44	1.91/1.42
CST-L	9.05/17.17	14.64/19.55	13.93/11.9	2.36/2.69	7.79/8.36	13.77/8.58
CST-R	9.94/19.25	12.94/17.65	28.53/24.87	0.67/0.78	8.22/8.99	14.51/9.23
CGC-L	0/0	0/0	0/0	6.79/5.59	9.79/7.56	3.94/1.77
CGC-R	0/0	0/0	0.72/0.32	6.15/3.7	11.1/6.27	2.53/0.83
CGH-L	0/0	0/0	0.21/0.05	0/0	0/0	0.77/0.12
CGH-R	0/0	0/0	1.33/0.29	0/0	1.26/0.35	1.89/0.3
FMAJ	0.03/0.07	1.66/2.84	0.42/0.46	0/0	0.89/1.22	16.65/13.27
FMIN	0.63/1.77	0.1/0.19	0/0	3.99/6.76	5.12/8.14	10.26/9.47
IFO-L	0/0	0.07/0.1	0.06/0.05	1.76/1.95	2.11/2.2	11.7/7.09
IFO-R	0/0	0.54/0.96	1.54/1.74	1.47/2.22	3.96/5.62	11.68/9.63
ILF-L	1.14/2.76	0/0	0/0	0/0	2.07/2.84	7.9/6.3
ILF-R	0.23/0.48	0.34/0.5	2.5/2.37	0.54/0.68	1.01/1.2	4.67/3.22
SLF-L	0.01/0.03	3.85/13.99	0.36/0.85	3.54/11.01	3.93/11.46	3.7/6.27
SLF-R	0.5/2.42	1.63/5.55	3.24/7.09	1.73/5.05	5.61/15.4	2.6/4.14
UF-L	0/0	0.67/0.22	0.07/0.02	2.02/0.55	0/0	0.37/0.06
UF-R	0/0	0/0	6.78/1.46	0.79/0.23	0/0	1.78/0.28
SLFt-L	0/0	0/0	0/0	0/0	0.4/0.02	2.38/0.07
SLFt-R	0/0	2.88/0.34	0/0	0.41/0.04	0/0	21.81/1.19

Abbreviations: ATR = anterior thalamic radiation, CST = corticospinal tract, CGC = cingulum (cingulate gyrus), CGH = cingulum (hippocampus), FMAJ = forceps major, FMIN = forceps minor, IFO = inferior fronto-occipital fasciculus, ILF = inferior longitudinal fasciculus, SLF = superior longitudinal fasciculus, UF = uncinate fasciculus, SLFt = superior longitudinal fasciculus (temporal part), L = left, and R = right.

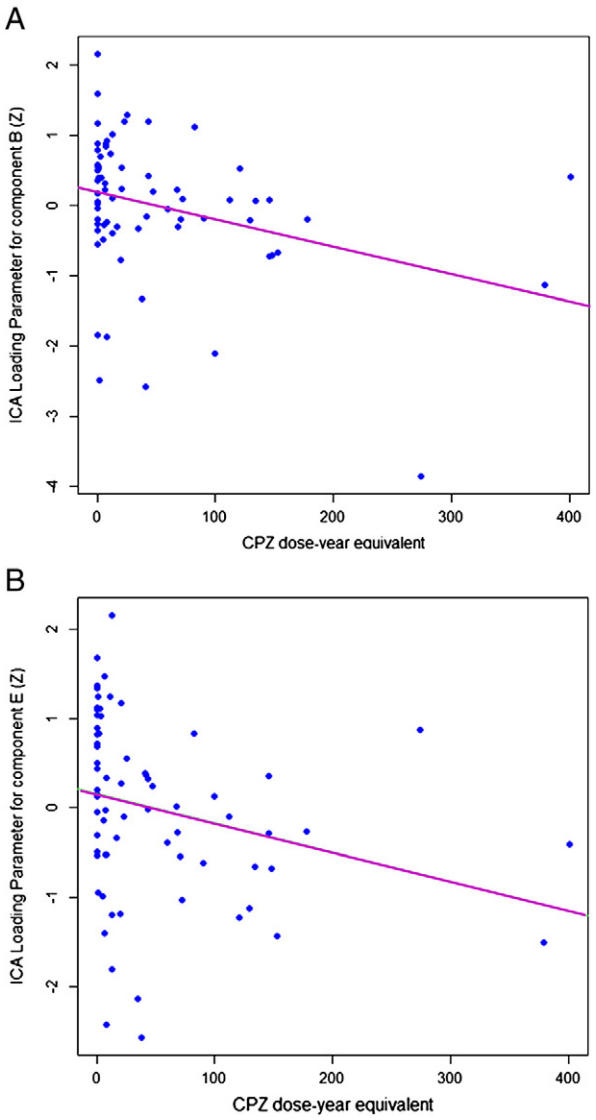

Fig. 2. Correlation of ICA loading coefficients with lifetime CPZ dose-year equivalent use. ICA loading coefficients in components B (panel A) and E (panel B) showed a significant association with lifetime CPZ use in patients when including site as a covariate (component B corrected $p = 0.0131$; component E corrected $p = 0.00599$).

association between rs7808623 and component C was still highly significant, but no longer passed a Bonferroni correction at the $p < 0.05$ level (uncorrected $p = 1.01 \times 10^{-4}$, corrected $p = 0.0730$).

4. Discussion

In this study, we obtained both DTI data and genotypes of 121 myelin and schizophrenia related SNPs in a sample of 74 schizophrenia patients and 87 age- and gender-matched controls. To our knowledge, this is the first study to use ICA of DTI data to investigate any effect of genotype on FA. We report one SNP, rs7808623 located in an intronic region of the metabotropic glutamate receptor 3 gene, *GRM3*, to be significantly associated with white matter integrity in an IC that contains a large proportion of the cortico–cerebellar–thalamic–cortical circuit that has long been associated with higher cognitive processes and schizophrenia symptomology (Andreasen et al., 1998). Notably, the relationship between rs7808623 and ICA loading in this component was found only in the patient group, indicating an interaction of FA, this SNP and diagnosis.

4.1. GRM3

GRM3 modulates the effects of glutamate spillover by decreasing further glutamate or GABA release, thereby either decreasing or increasing glutamate-mediated excitation. While our study did not find a difference in any allele frequencies between schizophrenia and control subjects, an association between allele frequency and schizophrenia has been demonstrated with other polymorphisms in *GRM3* (Harrison et al., 2008). Although no other study to date has investigated a relationship between *GRM3* polymorphisms and DTI either in schizophrenia patients or in healthy controls, other studies have reported associations between *GRM3* polymorphisms and schizophrenia symptom severity and/or presentation (Bishop et al., 2011) (Egan et al., 2004; Mossner et al., 2008), response to medication (Bishop et al., 2005; Fijal et al., 2009), and brain activation patterns (Egan et al., 2004). Among these *GRM3* SNPs, the only one that is in significant linkage disequilibrium ($LD > 0.8$) with rs7808623 is rs1476455, which was found to be associated with symptom severity in treatment refractory schizophrenia patients (Bishop et al., 2011).

Association of *GRM3* genotype and FA could be explained by a number of different mechanisms. Most obviously, FA decreases with decreased myelination by oligodendrocytes. Cultured rodent oligodendrocyte progenitor cells (OPCs) and mature oligodendrocytes (Deng et al., 2004) and adult human OPCs (Luyt et al., 2004) all express *GRM3*, and in primary oligodendrocyte cultures *GRM3* expression appears to be developmentally regulated (Deng et al., 2004). However, the role of this metabotropic glutamate receptor in myelination and OPC development is still unclear. The *GRM3* association that we observed with FA in our patient sample could also be caused by a decreased number of axons or axonal damage in patients with major allele of rs7808623. Finally, it has been shown that astrocytic *GRM3* expression is necessary to protect neurons from NMDA-induced neurotoxicity (Corti et al., 2007). This implies that reduced functionality of GRM3, which itself could cause hyperglutamatergic signaling, could also lead to increased neuronal damage as a result of the aberrant signaling seen in schizophrenia. Supporting this idea, a recent report demonstrated a correlation of cortical thickness and areas of decreased white matter integrity ("potholes") in the same MCIC cohort of patients (Ehrlich et al., in press).

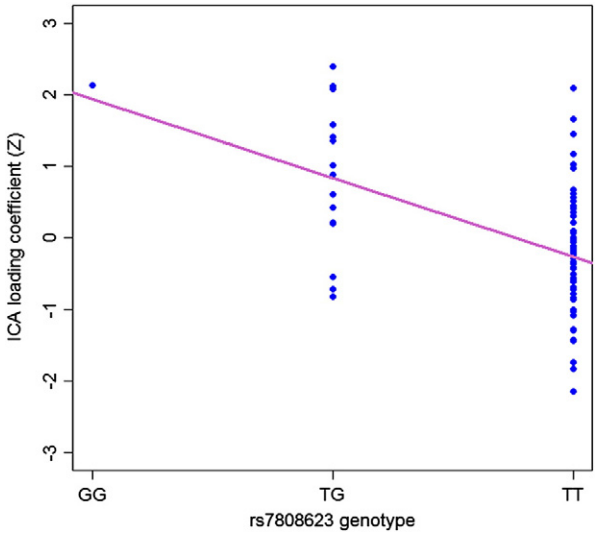

Fig. 3. Effect of rs7808623 genotype on component C ICA loading coefficients. ICA loading coefficients for each subject in the patient group plotted as a function of rs7808623 genotype and corrected p with site as a covariate ($p = 0.0252$).

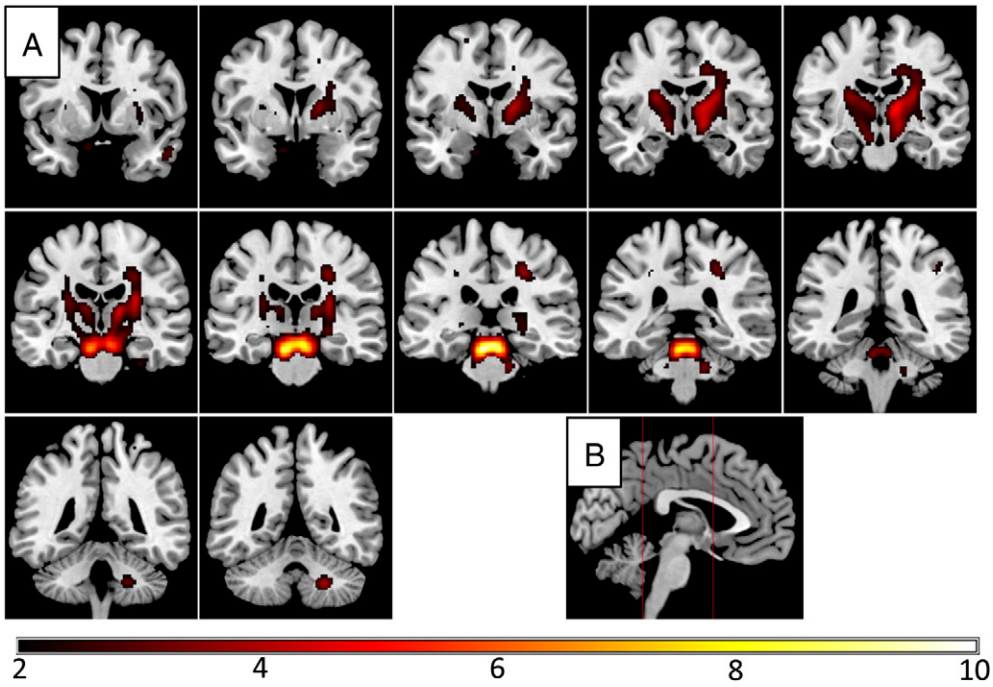

Fig. 4. Threshold maps of component C. Coronal slices in fixed intervals showing threshold map starting anteriorly in the top right (A). Sagittal section without threshold map overlay showing interior and posterior boundaries of coronal sections (B). Color scale represents the Z value for each voxel's ICA score.

4.2. Component C: cortico–cerebellar–thalamic–cortical circuit

The genotype/FA association that we observed occurred in an IC that was dominated by tracts connecting the internal capsule and the thalamus to the cerebellar peduncles (Fig. 4). Although the threshold map of this component does not extend into the prefrontal cortex, component C is reminiscent of the cortico–cerebellar–thalamic–cortical circuit (CCTC) that was proposed by Andreasen et al. (1998) to be responsible for cognitive dysmetria in schizophrenia patients. The cerebellum has been shown to be active independent of its traditional motor function during many activities that are impaired in schizophrenia, including emotion attribution, directed attention, and working memory (Andreasen and Pierson, 2008). Additionally, cerebellar lesions can lead to complex symptomology affecting higher cognitive functions (Schmahmann et al., 2007). Therefore, an altered CCTC could be a potential source of many of the cognitive defects seen in schizophrenia.

Further evidence for a loss of connectivity between the cortex and the cerebellum in schizophrenia comes from other DTI studies that have reported decreased FA in WM tracts within the cerebellum and cortical–cerebellar tracts (Magnotta et al., 2008; Liu et al., 2011). Additionally, several studies have reported reduced cerebellar volume in schizophrenia (Andreasen and Pierson, 2008), and others have reported reduced blood flow during working memory tasks in regions involved in cortical–subcortical–cerebellar connectivity (Kim et al., 2009).

4.3. Study limitations

Multi-site studies allow for larger sample sizes, but may cause the risk of inter-site variability. We found that both the variances and the means among the four sites varied significantly, and so we included a site correction for all our analyses. However, we lacked the sample size at any individual site to attempt to reproduce our findings in any site by itself. Also, our methods required us to correct for 726 independent tests in the genotype–DTI association study, increasing the possibility of a type II error. Thus, although the correlation between the rs7808623 and ICA loading coefficients in component C of the patients was significant even after multiple comparison corrections using three genotypes, this no longer passed a Bonferroni correction at the $p < 0.05$ level when the single minor allele homozygous subject was grouped with heterozygous cases. The other limitation of our study is that it was based on SNP association and not gene association using 121 SNPs available in the chip for 81 myelin- and schizophrenia-related genes. Therefore, each SNP was used as an independent variable. Although our study investigated several SNPs in genes that have previously been shown to be risk factors for schizophrenia and to be associated with WM integrity such as those in *ERBB4*, *CNP*, *MAG* and *OLIG2* (Konrad et al., 2009; Voineskos et al., 2013; Supplementary Table 1), due to the large number of comparisons we found no association in our sample. Finally, while other groups reported associations between WM integrity in specific tracts and symptom severity (Shin et al., 2006; Skelly et al., 2008), we did not observe any significant correlations of ICA loading in any of the six components in our patient sample.

Abbreviations

WM	white matter
FA	fractional anisotropy
ICA	independent component analysis
DTI	diffusion tensor imaging
SNP	single nucleotide polymorphism

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Contributors

VC and NPB designed the study, AC, LL JL and JM undertook the statistical analysis and data processing, NPB and JM managed the literature search and analysis, and JM wrote the

first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

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References

Abbott, C., Juarez, M., White, T., Gollub, R.L., Pearson, G.D., Bustillo, J., Lauriello, J., Ho, B., Bockholt, H.J., Clark, V.P., Magnotta, V., Calhoun, V.D., 2011. Antipsychotic dose and diminished neural modulation: a multi-site fMRI study. Prog. Neuropsychopharmacol. Biol. Psychiatry 35 (2), 473–482.

Anderson, C.A., Pettersson, F.H., Clarke, G.M., Cardon, L.R., Morris, A.P., Zondervan, K.T., 2010. Data quality control in genetic case–control association studies. Nat. Protoc. 5 (9), 1564–1573.

Andreasen, N.C., 1983. The Scale for the Assessment of Negative Symptoms (SANS). The University of Iowa Press, Iowa City IA.

Andreasen, N.C., 1984. The Scale for the Assessment of Positive Symptoms (SAPS). The University of Iowa Press, Iowa City IA.

Andreasen, N.C., Pierson, R., 2008. The role of the cerebellum in schizophrenia. Biol. Psychiatry 64 (2), 81–88.

Andreasen, N.C., Flaum, M., Arndt, S., 1992. The Comprehensive Assessment of Symptoms and History (CASH). An instrument for assessing diagnosis and psychopathology. Arch. Gen. Psychiatry 49 (8), 615–623.

Andreasen, N.C., Paradiso, S., O'Leary, D.S., 1998. "Cognitive dysmetria" as an integrative theory of schizophrenia: a dysfunction in cortical–subcortical–cerebellar circuitry? Schizophr. Bull. 24 (2), 203–218.

Bishop, J.R., Ellingrod, V.L., Moline, J., Miller, D., 2005. Association between the polymorphic GRM3 gene and negative symptom improvement during olanzapine treatment. Schizophr. Res. 77 (2–3), 253–260.

Bishop, J.R., Miller del, D., Ellingrod, V.L., Holman, T., 2011. Association between type-three metabotropic glutamate receptor gene (GRM3) variants and symptom presentation in treatment refractory schizophrenia. Hum. Psychopharmacol. 26 (1), 28–34.

Bora, E., Fornito, A., Radua, J., Walterfang, M., Seal, M., Wood, S.J., Yücel, M., Velakoulis, D., Pantelis, C., 2011. Neuroanatomical abnormalities in schizophrenia: a multimodal voxelwise meta-analysis and meta-regression analysis. Schizophr. Res. 127 (1–3), 46–57.

Caprihan, A., Abbott, C., Yamamoto, J., Pearlson, G., Perrone-Bizzozero, N., Sui, J., Calhoun, V.D., 2011. Source-based morphometry analysis of group differences in fractional anisotropy in schizophrenia. Brain Connectivity 1 (2), 133–145.

Chen, J., Calhoun, V.D., Pearlson, G.D., Ehrlich, S., Turner, J.A., Ho, B.C., Wassink, T.H., Michael, A.M., Liu, J., 2012. Multifaceted genomic risk for brain function in schizophrenia. Neuroimage 61 (4), 866–875.

Clemm von Hohenberg, C., Wigand, M.C., Kubicki, M., Leicht, G., Giegling, I., Karch, S., Hartmann, A.M., Konte, B., Friedl, M., Ballinger, T., Eckbo, R., Bouix, S., Jager, L., Shenton, M.E., Rujescu, D., Mulert, C., 2013. CNTNAP2 polymorphisms and structural brain connectivity: a diffusion-tensor imaging study. J. Psychiatr. Res. 47 (10), 1349–1356.

R Core Team, 2012. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Australia.

Corti, C., Battaglia, G., Molinaro, G., Riozzi, B., Pittaluga, A., Corsi, M., Mugnaini, M., Nicoletti, F., Bruno, V., 2007. The use of knock-out mice unravels distinct roles for mGlu2 and mGlu3 metabotropic glutamate receptors in mechanisms of neurodegeneration/neuroprotection. J Neurosci 27 (31), 8297–8308.

Deng, W., Wang, H., Rosenberg, P.A., Volpe, J.J., Jensen, F.E., 2004. Role of metabotropic glutamate receptors in oligodendrocyte excitotoxicity and oxidative stress. Proc. Natl. Acad. Sci. U. S. A. 101 (20), 7751–7756.

Egan, M.F., Straub, R.E., Goldberg, T.E., Yakub, I., Callicott, J.H., Hariri, A.R., Mattay, V.S., Bertolino, A., Hyde, T.M., Shannon-Weickert, C., Akil, M., Crook, J., Vakkalanka, R.K., Balkissoon, R., Gibbs, R.A., Kleinman, J.E., Weinberger, D.R., 2004. Variation in GRM3 affects cognition, prefrontal glutamate, and risk for schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 101 (34), 12604–12609.

Ehrlich, S., Geisler, D., Yendiki, A., Panneck, P., Roessner, V., Calhoun, V.D., Magnotta, V.A., Gollub, R.L., White, T., 2014. Associations of white matter integrity and cortical thickness in patients with schizophrenia and healthy controls. Schizophr. Bull. (in press).

Erhardt, E.B., Rachakonda, S., Bedrick, E.J., Allen, E.A., Adali, T., Calhoun, V.D., 2011. Comparison of multi-subject ICA methods for analysis of fMRI data. Hum. Brain Mapp. 32 (12), 2075–2095.

Feng, Y., 2008. Convergence and divergence in the etiology of myelin impairment in psychiatric disorders and drug addiction. Neurochem. Res. 33 (10), 1940–1949.

Fijal, B.A., Kinon, B.J., Kapur, S., Stauffer, V.L., Conley, R.R., Jamal, H.H., Kane, J.M., Witte, M. M., Houston, J.P., 2009. Candidate-gene association analysis of response to risperidone in African-American and white patients with schizophrenia. Pharmacogenomics J. 9 (5), 311–318.

Fitzsimmons, J., Kubicki, M., Shenton, M.E., 2013. Review of functional and anatomical brain connectivity findings in schizophrenia. Curr. Opin. Psychiatry 26 (2), 172–187.

Gollub, R.L., Shoemaker, J.M., King, M.D., White, T., Ehrlich, S., Sponheim, S.R., Clark, V.P., Turner, J.A., Mueller, B.A., Magnotta, V., et al., 2013. The MCIC collection: a shared repository of multi-modal, multi-site brain image data from a clinical investigation of schizophrenia. Neuroinformatics 11 (3), 367–388.

Harrison, P.J., Lyon, L., Sartorius, L.J., Burnet, P.W., Lane, T.A., 2008. The group II metabotropic glutamate receptor 3 (mGluR3, mGlu3, GRM3): expression, function and involvement in schizophrenia. J. Psychopharmacol. 22 (3), 308–322.

Jungerius, B.J., Hoogendoorn, M.L.C., Bakker, S.C., Van't Slot, R., Bardoel, A.F., Ophoff, R.A., Wijmenga, C., Kahn, R.S., Sinke, R.J., 2008. An association screen of myelin-related genes implicates the chromosome 22q11 PIK4CA gene in schizophrenia. Mol. Psychiatry 13 (11), 1060–1068.

Kane, J.M., Leucht, S., Carpenter, D., Docherty, J.P., 2003. The expert consensus guideline series. Optimizing pharmacologic treatment of psychotic disorders. Introduction: methods, commentary, and summary. J. Clin. Psychiatry 64 (Suppl. 12), 5–19.

Kim, D.I., Manoach, D.S., Mathalon, D.H., Turner, J.A., Mannell, M., Brown, G.G., Ford, J.M., Gollub, R.L., White, T., Wible, C., Belger, A., Bockholt, H.J., Clark, V.P., Lauriello, J., O'Leary, D., Mueller, B.A., Lim, K.O., Andreasen, N., Potkin, S.G., Calhoun, V.D., 2009. Dysregulation of working memory and default-mode networks in schizophrenia using independent component analysis, an fBIRN and MCIC study. Hum. Brain Mapp. 30 (11), 3795–3811.

Konrad, A., Vucurevic, G., Musso, F., Stoeter, P., Dahmen, N., Winterer, G., 2009. ErbB4 genotype predicts left frontotemporal structural connectivity in human brain. Neuropsychopharmacology 34 (3), 641–650.

Liu, H., Fan, G., Xu, K., Wang, F., 2011. Changes in cerebellar functional connectivity and anatomical connectivity in schizophrenia: a combined resting-state functional MRI and diffusion tensor imaging study. J. Magn. Reson. Imaging 34 (6), 1430–1438.

Luyt, K., Varadi, A., Halfpenny, C.A., Scolding, N.J., Molnar, E., 2004. Metabotropic glutamate receptors are expressed in adult human glial progenitor cells. Biochem. Biophys. Res. Commun. 319 (1), 120–129.

Magnotta, V.A., Adix, M.L., Caprahan, A., Lim, K., Gollub, R., Andreasen, N.C., 2008. Investigating connectivity between the cerebellum and thalamus in schizophrenia using diffusion tensor tractography: a pilot study. Psychiatry Res. 163 (3), 193–200.

Mori, S., Wakana, S., Zijl, P.C.M.V., 2005. MRI Atlas of Human White Matter. Elsevier, Amsterdam.

Mossner, R., Schuhmacher, A., Schulze-Rauschenbach, S., Kuhn, K.U., Rujescu, D., Rietschel, M., Zobel, A., Franke, P., Wolwer, W., Gaebel, W., Hafner, H., Wagner, M., Maier, W., 2008. Further evidence for a functional role of the glutamate receptor gene GRM3 in schizophrenia. Eur. Neuropsychopharmacol. 18 (10), 768–772.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a toolset for whole-genome association and population-based linkage analysis. Am. J. Hum. Genet. 81 (3), 559–575.

Schmahmann, J.D., Weilburg, J.B., Sherman, J.C., 2007. The neuropsychiatry of the cerebellum — insights from the clinic. Cerebellum 6 (3), 254–267.

Shin, Y.-W., Kwon, J.S., Ha, T.H., Park, H.-J., Kim, D.J., Hong, S.B., Moon, W.-J., Lee, J.M., Kim, I.Y., Kim, S.I., Chung, E.C., 2006. Increased water diffusivity in the frontal and temporal cortices of schizophrenic patients. Neuroimage 30 (4), 1285–1291.

Skelly, L.R., Calhoun, V., Meda, S.A., Kim, J., Mathalon, D.H., Pearlson, G.D., 2008. Diffusion tensor imaging in schizophrenia: relationship to symptoms. Schizophr. Res. 98 (1–3), 157–162.

Takahashi, N., Sakurai, T., Davis, K.L., Buxbaum, J.D., 2011. Linking oligodendrocyte and myelin dysfunction to neurocircuitry abnormalities in schizophrenia. Prog. Neurobiol. 93 (1), 13–24.

Voineskos, A.N., Felsky, D., Kovacevic, N., Tiwari, A.K., Zai, C., Chakravarty, M.M., Lobaugh, N.J., Shenton, M.E., Rajji, T.K., Miranda, D., Pollock, B.G., Mulsant, B.H., McIntosh, A.R., Kennedy, J.L., 2013. Oligodendrocyte genes, white matter tract integrity, and cognition in schizophrenia. Cereb. Cortex 23 (9), 2044–2057.

White, T., Magnotta, V.A., Bockholt, H.J., Williams, S., Wallace, S., Ehrlich, S., Mueller, B.A., Ho, B.C., Jung, R.E., Clark, V.P., Lauriello, J., Bustillo, J.R., Schulz, S.C., Gollub, R.L., Andreasen, N.C., Calhoun, V.D., Lim, K.O., 2011. Global white matter abnormalities in schizophrenia: a multisite diffusion tensor imaging study. Schizophr. Bull. 37 (1), 222–232.

White, T., Ehrlich, S., Ho, B.C., Manoach, D.S., Caprihan, A., Schulz, S.C., Andreasen, N.C., Gollub, R.L., Calhoun, V.D., Magnotta, V.A., 2013. Spatial characteristics of white matter abnormalities in schizophrenia. Schizophr. Bull. 39 (5), 1077–1086.

Williams, J.B., Gibbon, M., First, M.B., Spitzer, R.L., Davies, M., Borus, J., Howes, M.J., Kane, J., Pope Jr., H.G., Rounsaville, B., et al., 1992. The structured clinical interview for DSM-III-R (SCID). II. Multisite test-retest reliability. Arch. Gen. Psychiatry 49 (8), 630–636.