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# Diffusion tensor imaging data reveals GRM3 polymorphism's association with white matter integrity in schizophrenia

Joanna Mounce

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**DIFFUSION TENSOR IMAGING DATA REVEALS GRM3 POLYMORPHISM'S  
ASSOCIATION WITH WHITE MATTER INTEGRITY IN SCHIZOPHRENIA**

**by**

**Joanna Mounce**  
**B.S. Biology, New Mexico State University, 2008**

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## **ABSTRACT**

While the functional disconnectivity hypothesis of schizophrenia has been the subject of much study, very little is known about the contribution of individual genotypes to connectivity between brain regions in either schizophrenia patients or in healthy controls.

In this study, we obtained diffusion tensor imaging (DTI) maps and genome-wide SNP data from 74 cases and 87 age- and gender-matched controls. Correlations were performed between loading coefficients obtained from fractional anisotropy (FA) values in networks of regions representing 6 maximally independent components and 134 SNPs in genes that have been found to be important in myelination and/or schizophrenia. By using independent component analysis (ICA) to analyze the FA data we move beyond single voxels (voxel based morphometry) to a source based morphometry. In doing so, we can obtain networks of FA values that covary in a similar way among subjects, and we can study the relationship between these networks and genotype. We report one SNP located in the intronic region of the metabotropic glutamate receptor 3 gene *GRM3* that showed a significant correlation with connectivity in patients but not in controls ( $p < 1.0 \times 10^{-4}$ ). This SNP, rs7808623, has not been previously shown to be associated with schizophrenia, although association has been shown with several SNPs in *GRM3*.

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## CHAPTER 1: INTRODUCTION

Schizophrenia is a debilitating psychiatric disease that affects approximately 1% of the population across the world. Psychiatrists characterize schizophrenia by the presence of both positive and negative symptoms. Positive symptoms include hallucinations and delusions, whereas negative symptoms include flat affect and asociality. Schizophrenia heritability is thought to be approximately 50% based on twin studies, with an apparently complex interplay occurring between genes and environment (Brown, 2011; O'Connell et al., 2011). Because many of the risk factors for schizophrenia occur during prenatal life, schizophrenia is widely thought to be a developmental disorder. Adding strength to this hypothesis is the fact that the age of onset for schizophrenia is usually in late adolescence.

The functional disconnectivity hypothesis of schizophrenia states that symptoms of schizophrenia can at least in part be explained by a loss of connectivity between dispersed brain regions. Studies investigating this hypothesis usually focus on myelin integrity and oligodendrocyte development which are necessary for proper signaling of the many myelinated tracts in the central nervous system. This hypothesis is attractive because myelination of some tracts in the limbic system occurs in late adolescence, which is the same time as the usual age of onset for schizophrenia (Benes, 1989). In addition, both schizophrenia and myelination have been found to be affected by an interplay between genes and the environment, with adverse environmental conditions decreasing myelination and increasing risk for developing schizophrenia (Brouwer et al., 2010). The functional disconnectivity hypothesis has been experimentally supported by several post-mortem microarray analyses that have identified several genes in myelin and



oligodendrocyte related pathways that are differentially expressed in the brains of schizophrenia patients (e.g. Aston et al., 2004; Barley et al., 2009; Hakak et al., 2001; Haroutunian et al., 2007). In most instances, myelin and oligodendrocyte genes have been found to be expressed at a lower level in schizophrenia patients compared to controls. In addition, pure demyelinating disorders, such as metachromatic leukodystrophy, can result in psychosis when the age of onset occurs in late adolescence (Walterfang et al., 2005).

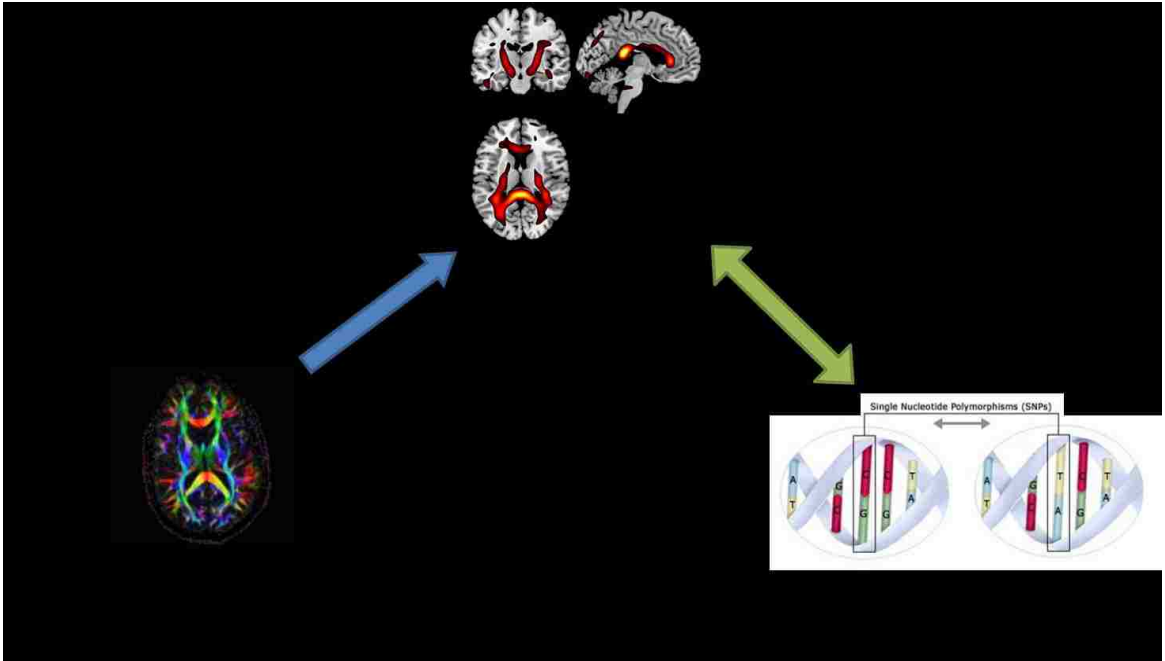
While most support for the functional disconnectivity hypothesis has come from post-mortem studies, there have been several studies showing specific SNPs in myelin related genes that are over-represented in people with schizophrenia compared to controls (e.g. Buxbaum et al., 2008; Qu et al, 2008). For this study, we investigated the presence of 134 SNPs from genes that have been previously shown to be involved in myelin and oligodendrocyte function (Jungerius et al., 2008), as well as from genes that have been shown to be associated with schizophrenia in general (<http://www.szgene.org>, see Appendix A for a list of all SNPs).

Diffusion tensor imaging (DTI) is an application of magnetic resonance imaging that uses magnetic gradients to infer information about water diffusion in living tissue (Basser et al., 1994). In most tissues, including grey matter and ventricles in the brain, water shows random, isotropic diffusion. However, fibrous tissues like myelinated axonal tracts in the brain only allow water to diffuse along the axon of the fiber in an anisotropic fashion. A measure of anisotropy of diffusion, called fractional anisotropy (FA), can be used as a measure of the functional connectivity in each voxel of the DTI image. Functional connectivity and FA change depending on axon myelination, axon diameter, axon density, and/or directional coherence of axons (Basser, 1995). Although more than

one factor can affect FA, it has generally been used in the literature as a marker of white matter integrity and myelination. In agreement with post-mortem gene expression studies, most studies have reported that patients with schizophrenia have lower FA values in at least one white matter tract. In particular, the interhemispheric fibers, anterior thalamic radiation (ATR), inferior longitudinal fasciculi, inferior frontal occipital fasciculi, cingulum and fornix have all been implicated in schizophrenia by a recent meta-analysis (Bora et al., 2011).

Following image acquisition, we used independent component analysis (ICA) to process DTI data into maximally independent components (Arfanakis, 2002). ICA is a blind source separation technique that is widely used in fMRI studies. Although ICA has not been widely used in DTI studies, it may offer several advantages over traditional tract-based data analysis techniques (Li et al., 2011). Firstly, as a data-driven technique, ICA does not automatically treat FA values across whole tracts as constant averages. Secondly, ICA does not assume independence of individual whole-tracts, an assumption that both Wahl et al. (2010) and Li et al. (2011) showed to be untrue. Thirdly, as a hypothesis free technique, ICA reduces dimensionality in a logical way without discarding information across the brain. Finally, it has been suggested that ICA may be more appropriate to discover group differences between patient and control groups because individual components are determined by the variation of white matter across subjects (Li et al., 2011). However, while our ICA results yielded 6 anatomically and functionally relevant components, interpretation of these components requires care as it is not as straightforward as tract based techniques.

After obtaining biologically relevant independent components, we performed multiple correlations of component loading coefficients with the genotypes of patients and controls at 134 different SNP loci to ascertain any relationship between genotype and white matter integrity (Figure 1). Because myelin integrity has been shown to be decreased in patients with schizophrenia, we hypothesized that the genotypes of individual SNPs in genes associated with white matter integrity or schizophrenia would correlate with FA more strongly in our patient sample. We report one SNP, rs7808623, which correlates significantly (corrected  $p < 0.05$ ) with white matter integrity in our patient sample only. This correlation occurred in a component that contains a large portion of the cortico-cerebellar-thalamic-cortical circuit, a circuit which has been proposed to account for many of the diverse symptoms in schizophrenia (Andreasen et al., 1998). Rs7808623 is located in an intronic region of *GRM3*, the gene encoding metabotropic glutamate receptor 3. While polymorphisms in *GRM3* have been shown to be associated with schizophrenia (Cherlyn et al., 2010), any relationship between *GRM3* genotype and myelination has not yet been investigated. This study supports others that have shown a relationship between the glutamatergic system and white matter development and function (Matute 2011; Wake et al., 2011) and is the first to investigate the relationship between schizophrenia, functional connectivity, and genotype in many of the original 134 SNPs.



**Figure 1: Experimental design**

Diffusion tensor images were obtained for a sample of schizophrenia patients and controls from 4 different sites. ICA was then performed to obtain 20 independent components, six of which were determined to be biologically meaningful. The loading coefficients of these components for each subject were then analyzed for case/control differences, medication effects, symptom severity effects, and associations with genotype in 134 myelin and schizophrenia related SNPs.

## CHAPTER 2: METHODS

### *Sample*

The subjects for this study were participants in the Mind Clinical Imaging Consortium (MCIC), a multi-site study involving investigators from New Mexico (UNM), Iowa (IA), Massachusetts General Hospital (MGH), and Minnesota (MINN) (Abbott et al., 2011). The cross section of individuals from the MCIC study with both genetic and DTI data consisted of 75 cases and 87 controls matched for age and sex. Unfortunately, these groups were not matched for race, and white/non-white status was included as a covariate for all linear regression analyses that compared cases and controls with no change in results. One case subject had to be excluded because of poor DTI image quality, leaving a final sample of 74 subjects and 87 controls (Table 1).

|                 | Schizophrenia<br>(n=74) | Controls<br>(n=87) | P value (test)                                |
|-----------------|-------------------------|--------------------|---|
| Age             |                         |                    |   |
| Mean (SD)       | 34.8 (11.1)             | 32.7 (11.2)        | 0.41 (Two sample<br>Kolmogorov-Smirnov test)  |
| Range           | 18-60                   | 18-57              |   |
| Sex (M/F)       | 53/21                   | 52/35              | 0.16 (X <sup>2</sup> test)                    |
| Race/Ethnicity  |                         |                    | 8.1x10 <sup>-4</sup><br>(Fisher's exact test) |
| White           | 50                      | 77                 |   |
| Black           | 15                      | 3                  |   |
| Asian           | 4                       | 3                  |   |
| Native American | 1                       | 0                  |   |
| Unknown         | 4                       | 4                  |   |

**Table 1:** Demographic information for subjects used in this study. All subjects were members of the Mind Clinical Imaging Consortium (MCIC)

All participants provided written informed consent, and the institutional review board at each of the four sites approved this project. Participants in the control group were negative for any medical, neurological, or psychiatric illnesses. All participants in the patient group had received a diagnosis of schizophrenia, schizoaffective disorder, or

schizophreniform disorder. This diagnosis was confirmed upon their entry into the study using the Structured Clinical Interview for DSM-IV-TR Disorders (First et. al., 1997) 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 inhalant use, showed substance abuse or dependence within the past month, or had an intelligence quotient equal to or less than 70.

#### *Diffusion tensor imaging data acquisition and processing*

Diffusion tensor imaging data was acquired at each of four sites: UNM, IA, MGH and MINN. All sites except UNM used a Siemens 3 Tesla Trio Scanner. UNM used a 1.5 Tesla Sonata. All sites used a field of view of 256mmx256mm. UNM and MINN used 12 gradient directions with  $b=1000 \text{ s/mm}^2$ , whereas IA used 6 gradient directions with  $b=1000 \text{ s/mm}^2$ , and MGH used 60 gradient directions with  $b=700 \text{ s/mm}^2$ . All sites imaged 64 slices, except MGH which imaged 60 slices. DTI experiments were repeated twice to increase signal-to-noise ratio.

The dicom2nii program found at [www.sph.sc.edu/comd/rorden/dicom.html](http://www.sph.sc.edu/comd/rorden/dicom.html) was used to convert dicom files to nifti format. Dicom2nii also outputs gradient direction tables after image slice orientation correction and a b-value table. Both DTI experiments were combined into one 4D nifti file with a concatenated table of corresponding b-value and gradient direction tables. Correction for Eddy currents was performed by registering all the images to a  $b=0 \text{ s/mm}^2$  diffusion image using FLIRT (FSL,

www.fmrib.ox.ac.uk/fsl) with a mutual-information cost function. This algorithm registers images of both the DTI measurements to a common image.

The diffusion tensor, scalar diffusion parameters (including FA) were calculated using DTIFIT (FSL). The data was not averaged for this calculation. FNIRT (FSL), a non-linear registration algorithm, was used to normalize the FA image of each subject to a  $1 \times 1 \times 1 \text{mm}^3$  FA template (FMRIB58\_FA\_1mm) in the Montreal Neurological Institute (MNI) space. This normalized data was then down-sampled to a  $2 \text{mm} \times 2 \text{mm} \times 2 \text{mm}$  resolution and smoothed with a Gaussian kernel of 88 mm full width half maximum prior to independent component analysis performance.

### *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 break data into maximally independent components in order to ascertain sources within very large data sets. ICA was performed in Matlab using Group ICA fMRI Toolbox (GIFT) software (<http://icatb.sourceforge.net>) to extract 20 independent components from a subject-by-voxel FA matrix (Calhoun et al., 2001; Erhardt, et al., 2010; Li, et al. 2007; Xu, et al. 2009). We chose to set the number of components at 20 based on previous observations by our lab group that this number works well with imaging data and returns biologically meaningful components. Components were visualized using MRICron, a visualization tool freely available online at <http://www.cabiatl.com/mricro/mricron/index.html>.

### *White matter characterization of biologically relevant independent components*

The white matter tracts in each component were determined in three different ways. Quantitatively, SPM8 was used to map each component to the John Hopkins DTI atlas (Mori, et al., 2005). This allowed us to view the percent of 20 different tracts in each component, as well as the percent of each component which was composed of each of the 20 different tracts (see Table 2 for white matter tracts). Qualitatively, we also used Mori et al.'s 2005 MRI Atlas of Human White Matter and <http://www.dtiatlas.org> (based on Wakana et al., 2004) to map the regions in component B's threshold map (Figures 2 and 9) to previously defined white matter tracts. This map represents the voxels that contribute the most to the make-up of component B. The threshold was determined by viewing which score was able to best eliminate artifacts while maintaining information about white matter tracts across all slices of all six biologically relevant maps. The qualitative analysis has the advantage of allowing us to visualize more than just the 20 white matter tracts from the SPM analysis, as well as to see the extent of connectivity between these tracts.

### *Determination of symptom severity and white matter association in patient sample*

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). Linear regressions were performed in R by modeling subjects' ICA loading coefficients as a function of positive, negative, or disorganized symptom scores, with site set up as a series of indicator variables.



| Name of tract  | Abbreviation for tract |
|--|------------------------|
| Anterior thalamic radiation left                       | Left ATR               |
| Anterior thalamic radiation right                      | Right ATR              |
| Corticospinal tract left                               | Left CST               |
| Corticospinal tract right                              | Right CST              |
| Cingulum (cingulate gyrus) left                        | Left CGC               |
| Cingulum (cingulate gyrus) right                       | Right CGC              |
| Cingulum (hippocampus) left                            | Left CGH               |
| Cingulum (hippocampus) right                           | Right CGH              |
| Forceps major  | FMAJ                   |
| Forceps minor  | FMIN                   |
| Inferior fronto-occipital fasciculus left              | Left IFO               |
| Inferior fronto-occipital fasciculus right             | Right IFO              |
| Inferior longitudinal fasciculus left                  | Left ILF               |
| Inferior longitudinal fasciculus right                 | Right ILF              |
| Superior longitudinal fasciculus left                  | Left SLF               |
| Superior longitudinal fasciculus right                 | Right SLF              |
| Uncinate fasciculus left                               | Left UF                |
| Uncinate fasciculus right                              | Right UF               |
| Superior longitudinal fasciculus (temporal part) left  | Left SLFt              |
| Superior longitudinal fasciculus (temporal part) right | Right SLFt             |

**Table 2:** List of 20 tracts that were mapped to each of the biologically relevant components using SPM. Atlas used for map was taken from John Hopkin's DTI atlas (Mori et al., 2005)

*Calculation of CPZ dose-year equivalent values in patient sample and regression with ICA loading coefficients*

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 of antipsychotics were calculated using the formula described by Abbott et al., 2011:

$$\text{Dose year} = \left[ \frac{\text{dose(mg)} * 100\text{CPZ}}{\text{drugequivalent}} \right] * \left[ \frac{\text{daysondose}}{365.25} \right] * \left[ \frac{1\text{year}}{(100\text{CPZ} * 1\text{year})} \right]$$

Following calculation of dose year equivalents, linear regression was once again performed in R modeling ICA loading coefficient as a function of CPZ dose-year equivalents with site as a covariate.

*Statistical analysis of components prior to integration of genotype data*

After components were determined, any differences based on site were assessed using a one way ANOVA. Differences in components between cases and controls were assessed using the following linear regression model:

$$Y = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5$$

Where Y is the subjects' ICA loading coefficient values for each component,  $\alpha$  is the intercept,  $X_1$  is disease state, and  $X_2$ - $X_4$  are a series of indicator variables representing the four different sites, and  $X_5$  is white/non-white status for each patient. P-values were adjusted using a Bonferroni correction for 6 independent tests.

Effects of medication analysis and symptom severity on ICA loading coefficient values in the patient sample were assessed using the following linear regression analyses:

$$Y = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4$$

Where Y is the subjects' ICA loading coefficient value for each component,  $\alpha$  is the intercept,  $X_1$  is either lifetime medication use or symptom severity, and  $X_2$ - $X_4$  are a series of indicator variables representing the four different sites. P-values were adjusted using a Bonferroni correction for 6 independent tests.

*Genetic analysis*

All subjects were genotyped using an Illumina's HumanOmni Quad Duo Chip. A list of 288 myelin and/or schizophrenia related SNPs for further analysis was compiled

using <http://www.szgene.org>, Jungerius et al. (2008) and various other literature sources. Out of these 288, 140 were available from the Illumina Chip, although one failed quality control (genotyping rate < 95%), 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 134 myelin and/or schizophrenia-related SNPs (Appendix A). Allele frequency differences between cases and controls was determined using a  $X^2$  analysis, and no genotype was present at differing rates in either sample (uncorrected  $p > .001$ ). Linkage disequilibrium units for Table 3 were determined using the HapMap project's data within Applied Biosystems SNPbrowser software ([http://marketing.appliedbiosystems.com/mk/get/snpb\\_landing](http://marketing.appliedbiosystems.com/mk/get/snpb_landing)).

#### *Linear regression analyses of genotype*

Genotypes were either represented as number of major alleles (i.e., 0, 1 or 2 for each SNP in each subject), or they were coded by presence of a minor allele (i.e., either 1 or 2). Pearson correlation coefficients and significance were then determined for each SNP-component combination using R. Because of the need to correct for site, linear regression was also performed modeling ICA loading coefficient value as a function of genotype and site by fitting the following model:

$$Y = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4$$

Where Y is the subjects' ICA loading coefficient values for each component,  $\alpha$  is the intercept,  $X_1$  is the number of major alleles for each genotype, and  $X_2$ - $X_4$  are a series of indicator variables representing the four different sites. This type of test assumes an additive model for major allele presence, although similar results were obtained when a one-way ANOVA was performed for each SNP/component combination (without

correcting for site). P values for coefficients of interest were adjusted using a Bonferroni correction for 804 independent tests (134 SNPs \* 6 components).

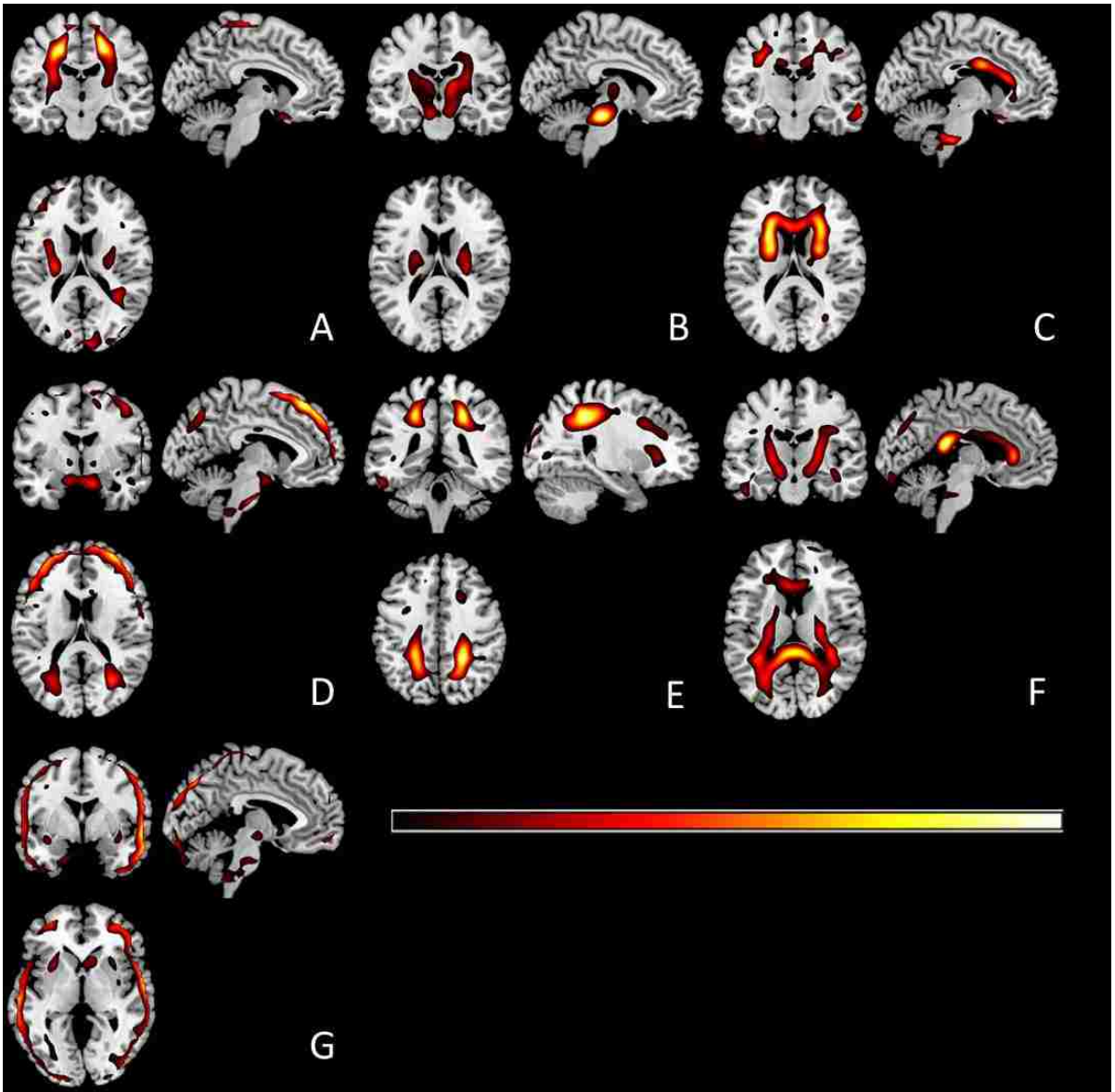
Additionally, ICA loading coefficient values were also modeled as a function of genotype, medication level and site, as well as a function of genotype, age, sex, handedness, race and site.

## CHAPTER 3: RESULTS

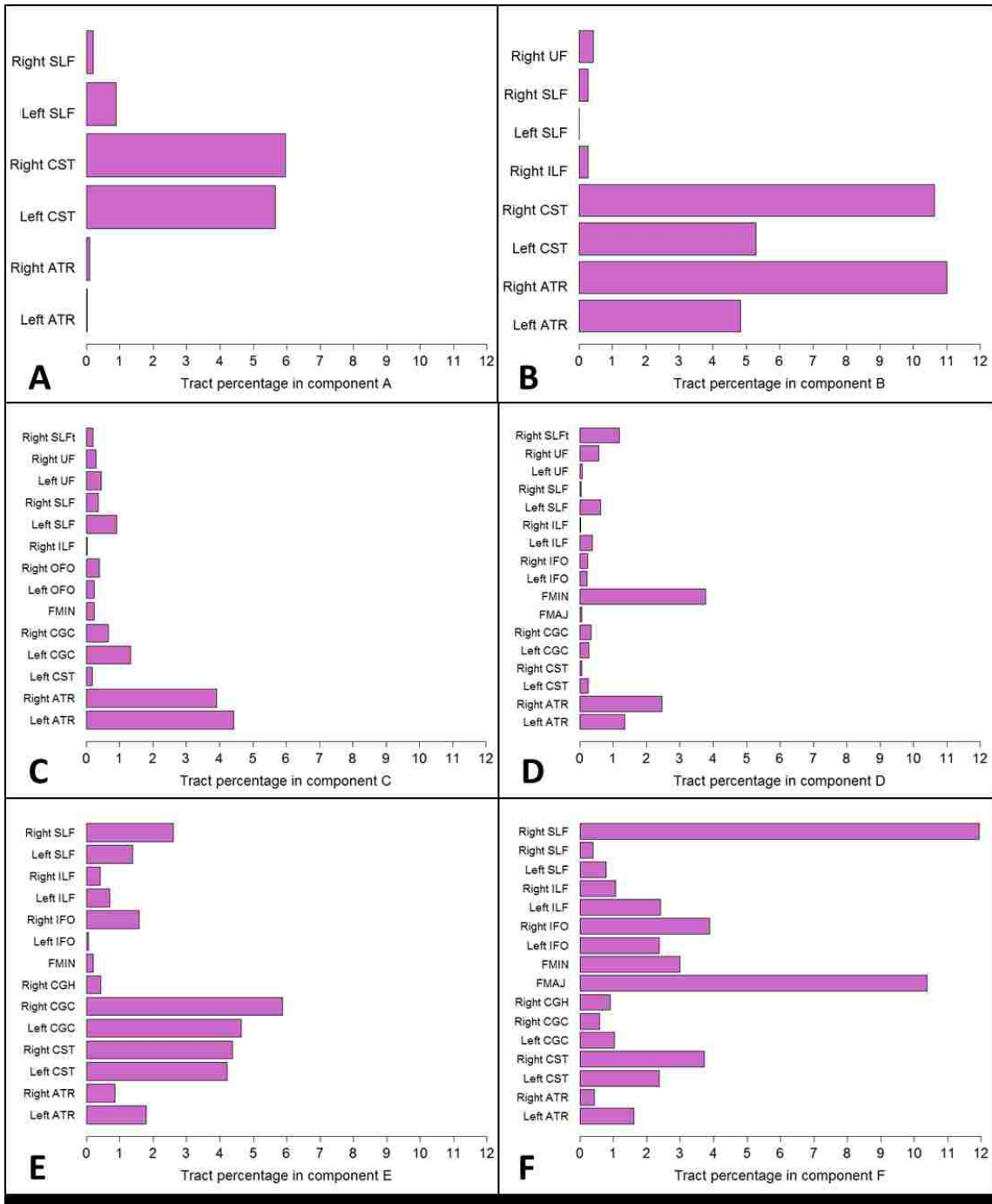
### *Independent Component Analysis*

Out of the 20 independent components obtained, six were qualitatively determined to be biologically meaningful (Figure 2 A-F). These components will hereafter be referred to as components A-F corresponding with the order of their appearance in Figure 2. The remaining 14 were excluded from further analysis on the basis of having large portions of brain regions which appeared to be artifacts of different brain/ventricle size among participants (Figure 2G). Having such a large number of excluded components helps to assure that most of the noise caused by variation in size along the edge of the brain is excluded from our actual analysis. Additionally, the edge of the brain does not contain any large myelinated white matter tracts, so we could exclude these components without fear of losing meaningful data.

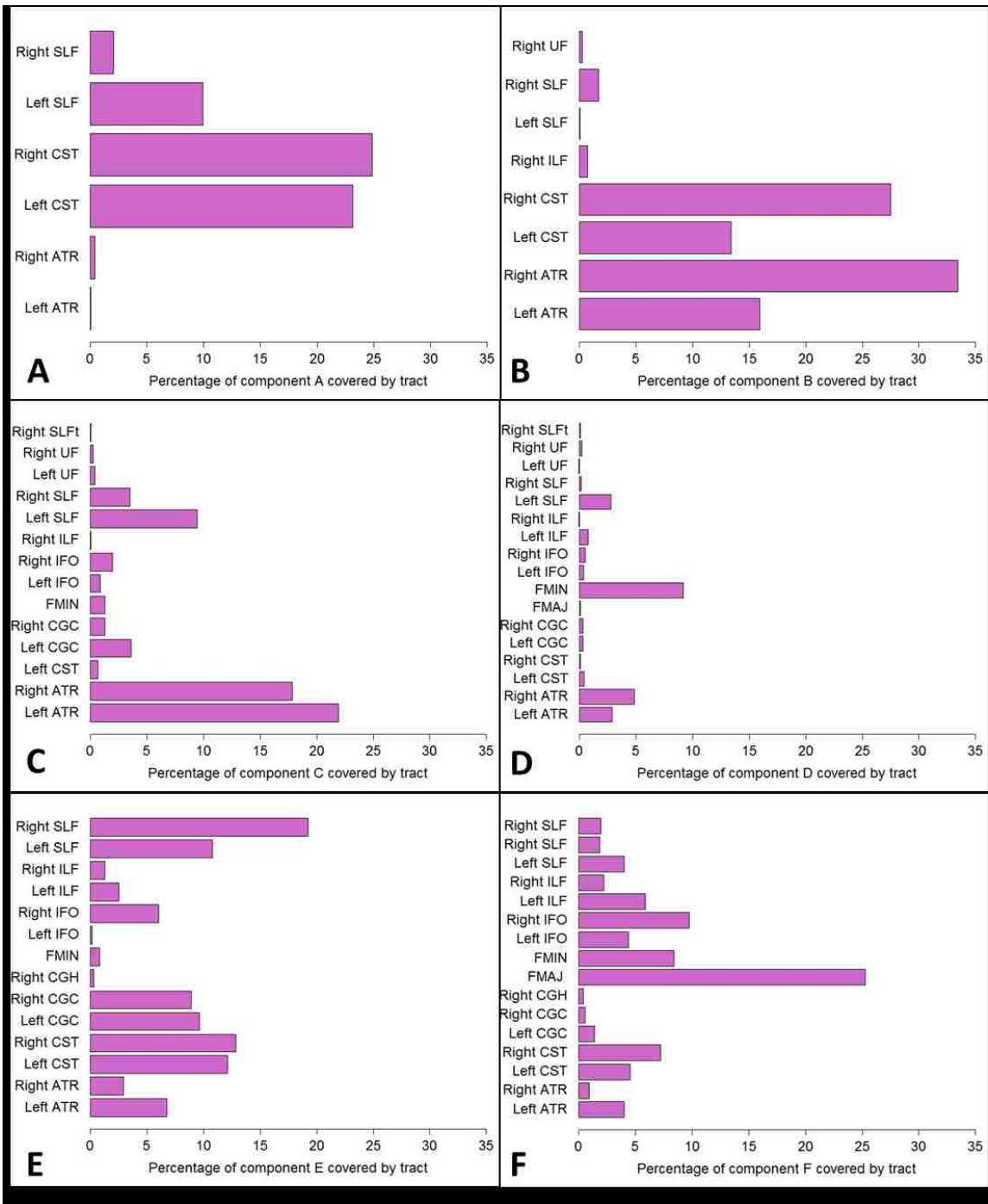
We used SPM8 to quantitatively map the biologically meaningful components to 20 tracts defined in Johns Hopkins's DTI atlas (Table 2, Mori et al., 2005). This allowed us to view both the percentage of each tract in each component (Figure 3) as well as the percentage of each component occupied by each tract (Figure 4). Tracts which are especially represented in the biologically relevant components include the anterior thalamic radiation (ATR), the corticospinal tract (CST), the forceps major (FMAJ), forceps minor (FMIN), and the superior longitudinal fasciculus (SLF). Also, as can be seen in Figures 2-4, left-right symmetry was largely maintained in each component. This symmetry would be expected given that the same tracts on opposite sides of the brain should covary with each other.



**Figure 2:** Threshold map for all biologically meaningful components that were observed as a result of ICA on multi-site DTI data (A-F) as well as an example of a non-biologically meaningful component (G). Color represents the extent that each voxel contributes to the map of each component. The higher contributing voxels from each biologically relevant component tend to be distributed symmetrically and along regions of white matter tracts. The voxels above threshold in the non-biologically relevant components tended to be present along the edge of the brain and may represent variation in brain size or in grey matter structure.



**Figure 3:** SPM was used to map each component to a DTI atlas containing 20 different tracts (table 2). For components A-F the percentage of each tract that is present in each component is shown.



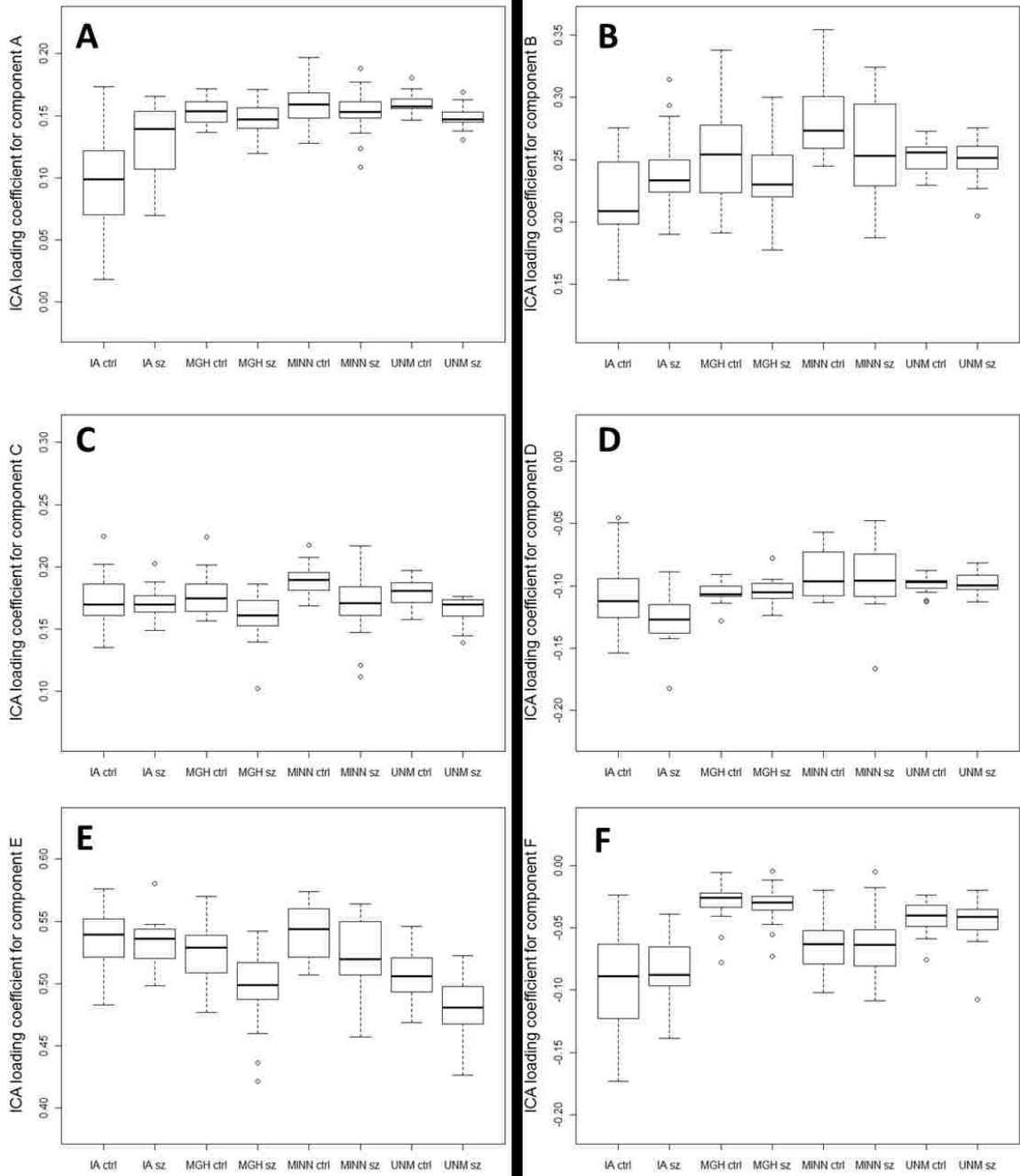
**Figure 4:** SPM was used to map each component to a DTI atlas containing 20 different tracts (table 2). For components A-F the percentage of each component that is covered by each tract is shown.



### *Site differences*

We assessed the differences in DTI data between sites using a one-way ANOVA for both cases and controls in each component (Figure 5). 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 for four out of the six sites ( $p > 0.1$  for components B and C). In addition, using the non-parametric Fligner-Killeen test of homogeneity of variances, we found that the variances at components A, B, D, and F differed significantly both in the patient and the control group. Because of these differences, all tests subsequently performed included a correction for site. Because our results did not appear to change whether site was treated as a fixed or a random effect, we treated site as a fixed effect for ease of interpretation of our regression coefficients of interest (Bates, 2006).

Using a  $X^2$  analysis we assessed for any differences in allele frequencies between sites. Twelve SNPs were determined to differ significantly (unadjusted  $p > 0.05$ ) by site, none of which played a role in our results. These SNPs are marked with an asterisk in Appendix A.



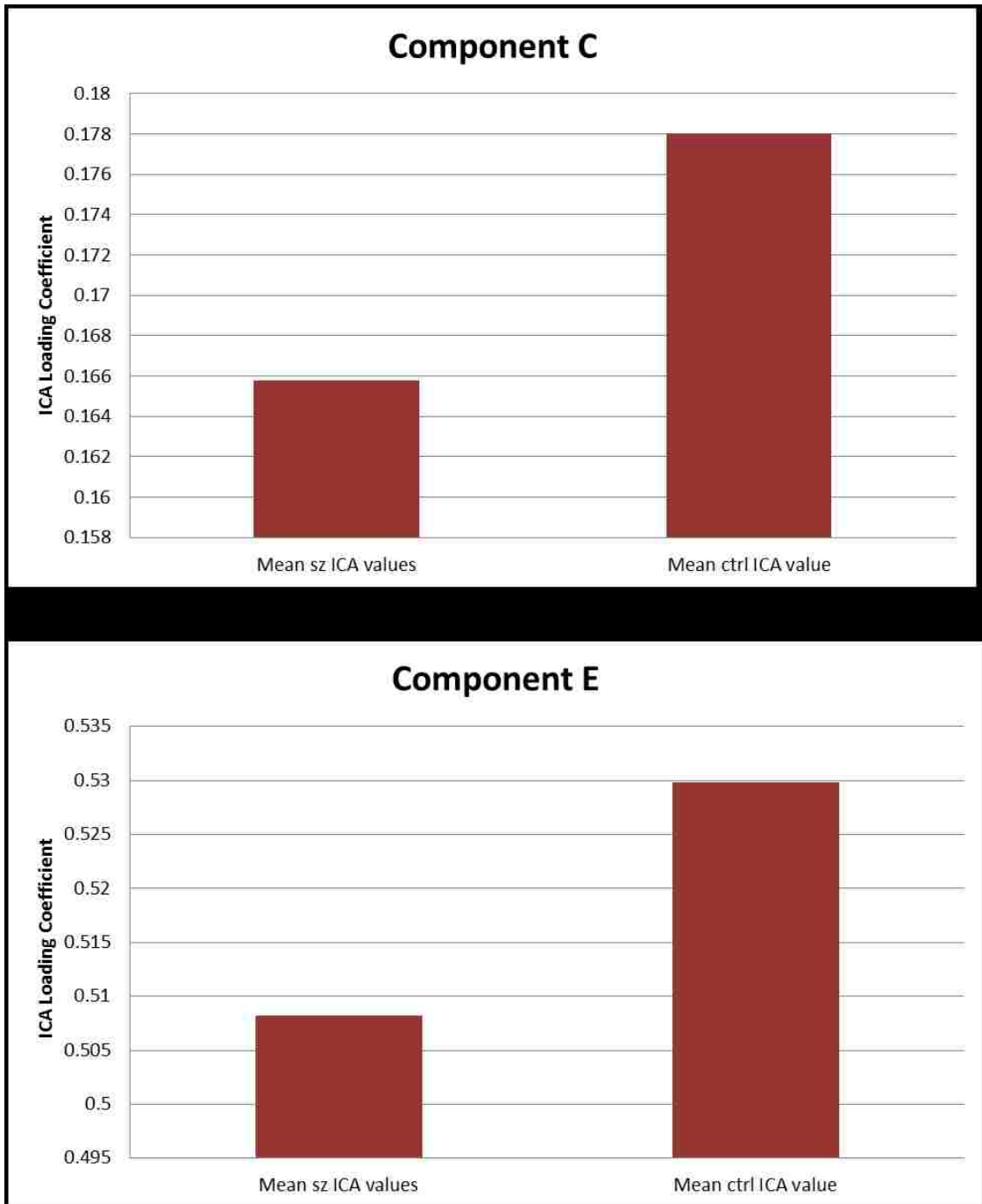
### *Differences in ICA loading coefficients in patients versus controls*

ICA loading coefficients in both components C and E were determined to significantly differ between the patient group and the control group following a Bonferroni correction for 6 independent tests (Figure 6,  $p=5.16 \times 10^{-5}$  for component C and  $p=3.68 \times 10^{-5}$  for component E). As shown in Figures 3 and 4, component C contains a disproportionate portion of the ATR, while component E contains a wide variety of white matter 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 between white matter integrity in this component between patients and controls may be reflective of a difference in global white matter integrity.

### *Effects of medication on ICA loading coefficient value in patient sample*

We were able to collect information on lifetime medication use in 72 out of our 74 patient subjects and convert that information into lifetime CPZ-equivalent use in dose years (Kane et al., 2003; Abbott et al., 2011). Because one subject had a lifetime CPZ-equivalent dose-year value that was more than 6 standard deviations above the mean, we excluded that subject from our analysis.

We found that lifetime medication use was significantly associated with ICA loading coefficients following a Bonferroni correction for 6 multiple comparisons in components A and E (Figure 7, corrected  $p=0.0131$  for component A and 0.00599 for component E). Interestingly, component E had also been found to differ between patients and controls, indicating that the white matter in this component may be a target of medication. As shown in Figures 3 and 4, component E contains a wide variety of white matter tracts, whereas the CST comprises a large portion of component A.

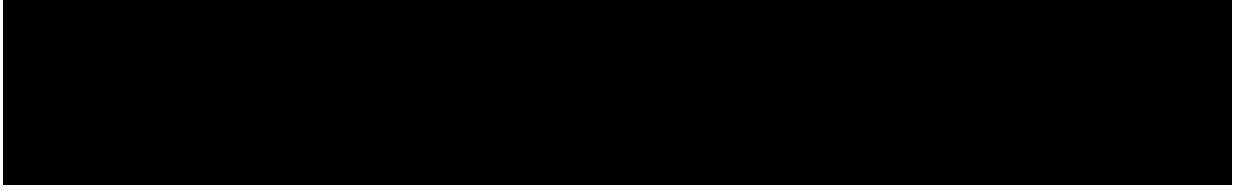
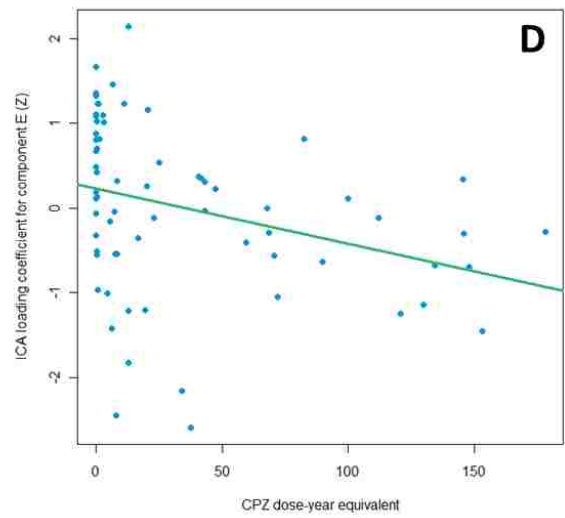
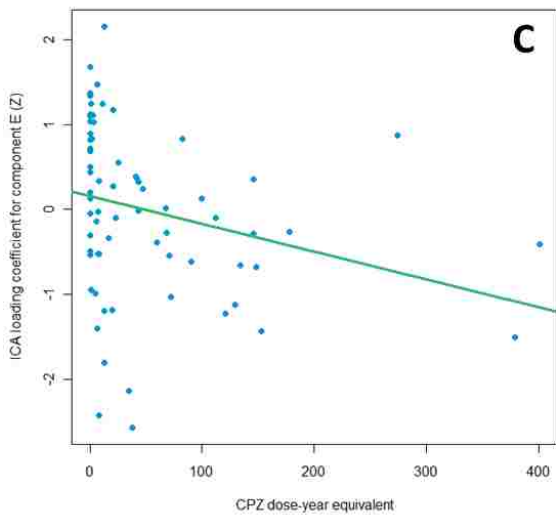
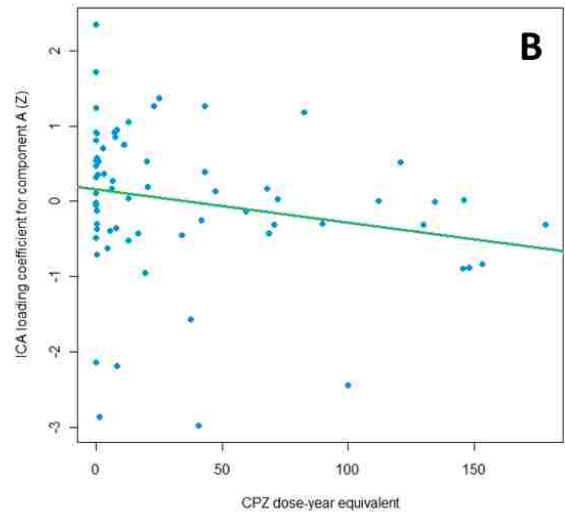
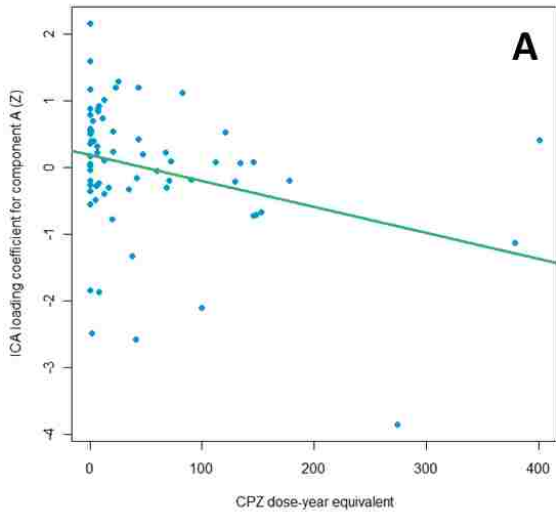


**Figure 6:** Mean ICA loading coefficients in both components C and F differed significantly between patients and controls when controlling for site (corrected  $p=5.16 \times 10^{-5}$  for component C; corrected  $p=3.68 \times 10^{-5}$  for component E).

Because 200 mg-years represented a natural break in our data (i.e., only three patients reported medication levels above 200 mg-years), we ran the same linear regression model but excluded patients whose lifetime CPZ equivalency dose-years were higher than 200 to observe whether these three subjects were driving our findings. We found that in component E medication levels were still associated with ICA loading coefficient values at a threshold-significance level (Figure 7D, uncorrected  $p = 0.0156$ , corrected  $p = 0.0936$ ). A significant association between medication level and ICA loading coefficient was no longer seen in component A (Figure 7B, corrected  $p = 0.451$ ).

*Effects of symptom severity scores on ICA loading coefficient values in patient sample*

For our patient sample we had access to both the SAPS and SANS ratings of positive, disorganized, and negative symptom severity (Andreasen, 1983; Andreasen, 1984). We performed three linear regression analyses to assess any association between ICA loading coefficient values and symptom severity scores. These models were similar to the ones used to ascertain case/control differences and medication effects, but with  $X_1$  representing positive, negative or disorganized symptom severity score. Surprisingly, we did not find any association between ICA loading coefficient values or symptom severity scores in any of the six biologically relevant components.



### *Effect of genotype on white matter integrity*

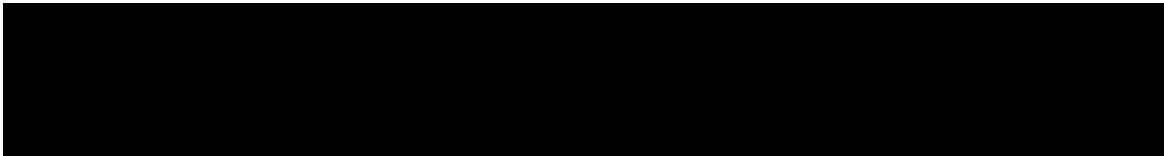
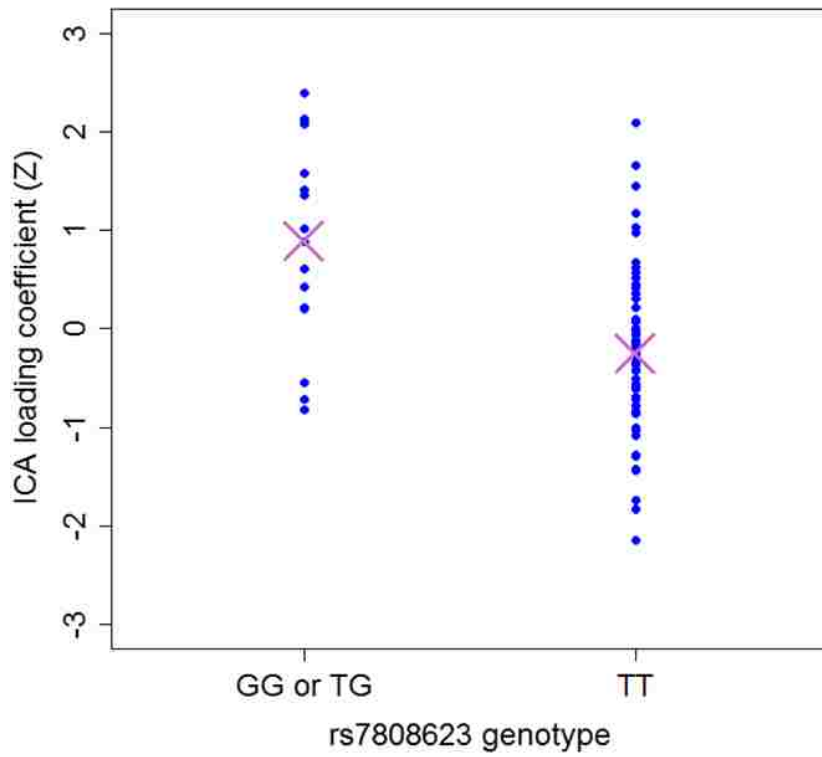
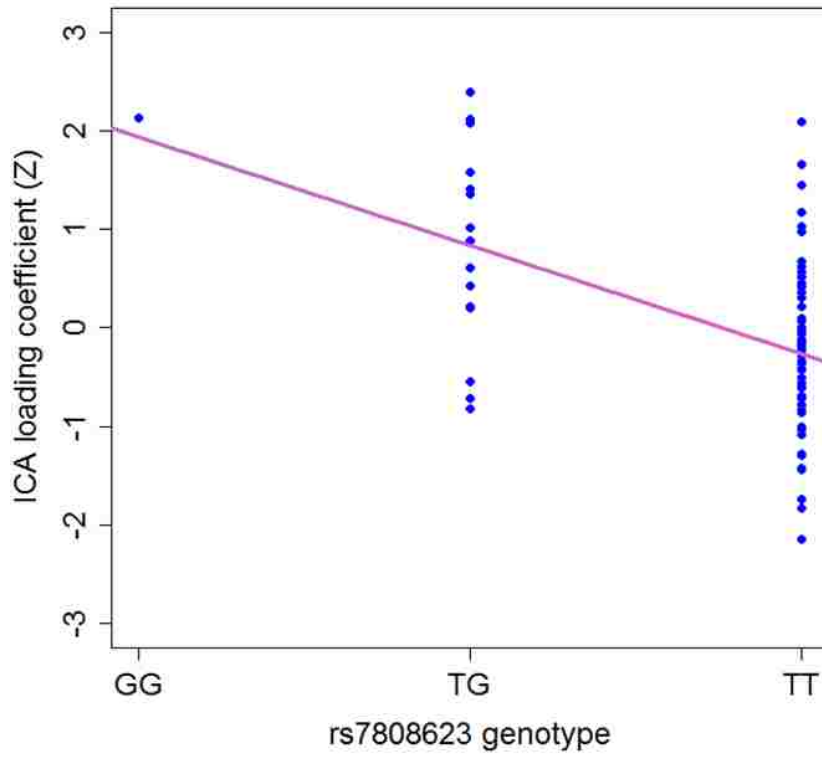
After characterizing the independent components and their association with disease state, medication level, and symptom severity, we next sought to establish whether any of the six components showed an association with the genotypes of 134 myelin and schizophrenia related SNPs (Appendix A) in either the patient group or the control group.

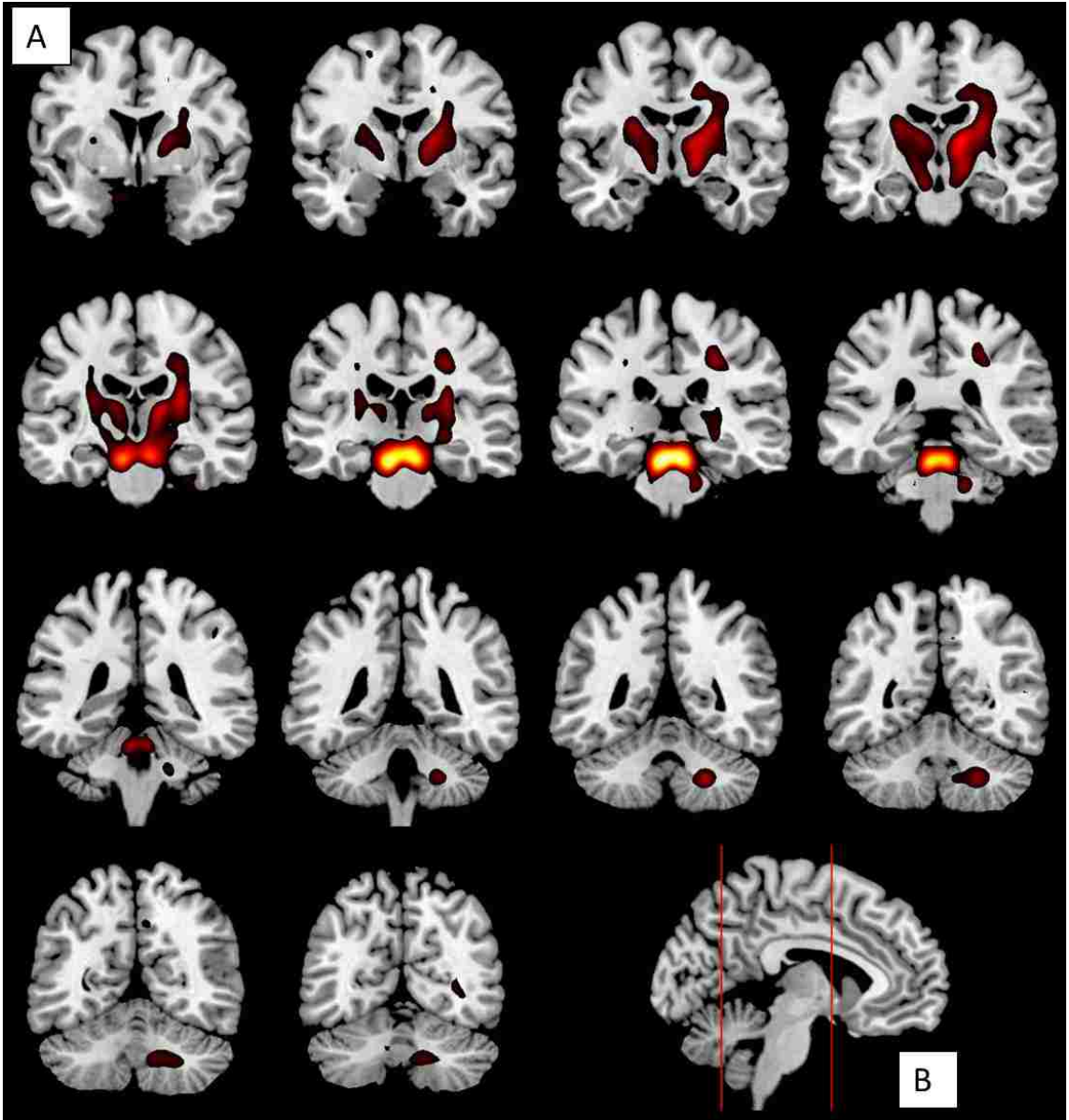
While there were no significant effects of genotype on FA found in the control group, we report one highly significant association in the patient sample. The relationship between rs7808623, located in an intronic region of *GRM3*, and component B was found to be significant even after the stringent Bonferroni correction for 804 independent tests (corrected  $p=0.0252$ , uncorrected  $p=3.14 \times 10^{-5}$ ). The minor allele of rs7808623 was associated with higher white matter integrity in component B (Figure 8A), which contains portions of the ATR as well as the corticospinal tract (Figure 3B and Figure 4B). The allele frequency for rs7808623 did not differ by site ( $X^2$  test,  $p=0.211$ ). By mapping the top-contributing voxels in component B slice by slice (Figure 9) and comparing these slices with two different white matter atlases, we could see that component B represents a series of tracts connecting the frontal cortex to the cerebellum and that these tracts correspond to the cortico-cerebellar-thalamic-cortical circuit that has been implicated in schizophrenia pathology (Andreasen and Pierson, 2008).

Because there was only one patient with a homozygous minor genotype (Figure 8A), we also performed a regression analysis wherein we grouped the homozygous minor case with the heterozygous cases (Figure 8B). This design no longer represents an additive model, but looks at the difference in mean ICA loading coefficient between

subjects with or without at least one minor allele. We found that with this model the association between rs7808623 and component B was still quite significant (uncorrected  $p=6.75 \times 10^{-5}$ ), although it no longer passed a Bonferroni correction at the  $p < 0.05$  level (corrected  $p=0.0542$ ). This association also remained significant when medication levels were used as an additional covariate (corrected  $p=0.0469$ ). Finally, this association remained significant when using the demographic factors age, sex, handedness, and race ('non-hispanic white/other' due to sample size) as covariates (corrected  $p=0.0249$ ).







## CHAPTER 4: DISCUSSION

In this study, we obtained both diffusion tensor images and genotypes of 134 myelin and schizophrenia related SNPs in a sample of 74 schizophrenia patients and 87 age- and gender-matched controls. We hypothesized that SNPs in myelin and schizophrenia-related genes would correlate with FA values in biologically relevant independent components obtained from ICA of our DTI data. 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, to be significantly associated with white matter integrity in an independent component that contains a large proportion of the cortico-cerebellar-thalamic-cortical circuit (Figure 9) that has long been associated with higher cognitive processes and schizophrenia symptomology (Andreasen et al., 1998).

### *GRM3*

GRM3, also known as metabotropic glutamate receptor 3 (mGluR3), is one of eight metabotropic glutamate receptors (GRM1-8), which are in turn subdivided into three groups based on sequence homology and signaling properties (Nakanishi, 1992; Niswender and Conn, 2010). The metabotropic glutamate receptors appear to be responsible for fine-tuning the actions of ionotropic glutamate receptors (Conn and Pin, 1997; Niswender and Conn, 2010). GRM3, along with GRM2, is a type II metabotropic glutamate receptor and is expressed on the extrasynaptic membranes of presynaptic glutamatergic (Mateo and Porter, 2007) and GABAergic (Mitchell and Silver, 2000) neurons. GRM3 modulates the effects of glutamate spillover by decreasing further

glutamate or GABA release, thereby either decreasing or increasing glutamate-mediated excitation. Binding of GRM3 by glutamate activates  $G_{i/o}$  receptors which act to inhibit further neurotransmitter release both by inhibition of adenylyl cyclase and by uncoupling with  $G_{\beta\gamma}$  subunits which then directly activate  $K^+$  channels and inhibit  $Ca^{2+}$  channels (Niswender and Conn, 2010). Because of its role as an inhibitory receptor on glutamatergic neurons, GRM3 modulates firing of many different kinds of neurons that are innervated by glutamatergic synapses. This includes dopaminergic and serotonergic neurons, both of which are targets of atypical antipsychotics.

Rs7808623 is located in an intronic region of *GRM3* and has not previously been investigated for any functional consequence or association with schizophrenia or white matter. To our knowledge no other studies looking at *GRM3* polymorphisms and schizophrenia have investigated the rs7808623 genotype in particular. Our report that rs7808623 is associated with FA in schizophrenia subjects could indicate that either this SNP is in linkage disequilibrium with another *GRM3* SNP such as rs1476455, which has been shown to be associated with schizophrenia symptom severity (Bishop et al, 2011, see Table 3), or that rs7808623 itself has functional consequences. Intronic SNPs are likely important in *GRM3* expression because *GRM3* is subject to alternative splicing (Sartorius et al., 2006). While our study did not find a difference in any allele frequencies between schizophrenia and control subjects, a positive association between allele frequency and schizophrenia has been shown with at least three other polymorphisms in *GRM3* (Chen et al., 2005; Egan et al., 2004; Fujii et al., 2003; Mössner et al., 2008; Schwab et al., 2008), although other studies have found no association between *GRM3* polymorphisms and schizophrenia (Albalushi et al., 2008; Bishop et al., 2007; Fallin et

al., 2005; Jönsson et al., 2009; Martí et al., 2002; Tochigi et al., 2006). Also, to the best of our knowledge no other study has investigated a relationship between *GRM3* polymorphisms and DTI in either schizophrenia patients or in healthy controls, though other studies have reported a connection between *GRM3* polymorphisms and schizophrenia symptom severity/presentation (Bishop et al., 2011; Egan et al., 2004; Jablensky et al., 2011; Mössner et al., 2008), response to medication (Bishop et al., 2005; Fijal et al., 2009), brain activation patterns (Egan et al., 2004), and brain structure (Haukvik et al., 2010). Table 3 shows the linkage disequilibrium units (LDUs) determined by SNPbrowser between rs7808623 and selected other *GRM3* SNPs reported in the literature as having an association with schizophrenia. LDUs have been proposed to be superior when representing variation of linkage disequilibrium with physical location (Zhang et al., 2002) and for detecting patterns on a small scale (Ke et al., 2004). A linkage disequilibrium block is defined by SNPbrowser as having an LDU value of less than 0.3 (De La Vega et al., 2006). According to this definition, rs1476455 is the only SNP in linkage disequilibrium with rs7808623 in the Caucasian HapMap sample. Linkage disequilibrium in European-Americans has also been reported to extend approximately 60KB (Reich et al., 2001), and both rs2299225 and rs1468412 were reported by SNPbrowser to be within this range (Table 3). Given that these SNPs have been shown to be associated with schizophrenia (Bishop et al., 2011, Chen et al., 2005, Fujii et al, 2003), future studies will need to evaluate of the significance our genetic-neuroimaging findings in a larger cohort of patients and control cases.

| SNP        | LDUs to rs7808623                      | Distance to rs7808623 | Association with schizophrenia   | Reference                                  |
|------------|--|-----------------------|--|--|
| rs1476455  | 0.00<br>(Caucasian)                    | 61.586 KB             | Symptom severity   | Bishop et al., 2011                        |
| rs2299225  | 0.32<br>(Caucasian)<br>0.17 (Chinese)  | 42.574 KB             | Allele frequency   | Chen et al., 2005                          |
| rs2237562  | 0.47<br>(Caucasian)                    | 67.922 KB             | Allele frequency (trend level)   | Schwab et al., 2008                        |
| rs1468412  | 0.47<br>(Caucasian)<br>1.08 (Japanese) | 56.703 KB             | Allele frequency   | Fujii et al., 2003                         |
| rs2228595  | 0.64<br>(Caucasian)                    | 74.167 KB             | Splice variant   | Sartorius et al., 2008                     |
| rs6465084  | 0.64<br>(Caucasian)                    | 86.679 KB             | Allele frequency, global symptom severity, activation patterns during working memory task, attention | Egan et al., 2004;<br>Mössner et al., 2008 |
| rs274622   | 1.23<br>(Caucasian)                    | 217.214 KB            | Negative symptom improvement with olanzapine treatment   | Bishop et al., 2005                        |
| rs724226   | 1.14<br>(Caucasian)                    | 164.78 KB             | PANSS change following risperidone treatment   | Fijal et al., 2009                         |
| rs2189814  | 1.14<br>(Caucasian)                    | 158.398 KB            | Cognitive deficit  | Jablensky et al., 2011                     |
| rs13242038 | 1.14<br>(Caucasian)                    | 202.734 KB            | Hippocampal volume (not schizophrenia specific)  | Haukvik et al., 2010                       |

**Table 3: Selected *GRM3* polymorphisms' associations with schizophrenia and distances to rs7808623 in LDUs and MB. Distance was determined using Applied Biosystems' SNPbrowser software. Chinese or Japanese population is also reported if appropriate based on reference's sample population.**

*GRM3* has been shown to be expressed both in the prefrontal cortex and at lower levels in dopaminergic neurons in the midbrain, although it does not appear to be differentially expressed between schizophrenia patients and controls either in grey matter or in white matter in the PFC (Ghose et al., 2008). Interestingly, agonists of type II metabotropic glutamate receptors have been shown to be effective antipsychotics. In a stage II clinical trial, an agonist of *GRM3/GRM2* was shown to be statistically as

effective as olanzapine in reducing symptoms of schizophrenia without the weight gain side effect seen with olanzapine use (Patil et al., 2007). These agonists inhibit the large increase in prefrontal cortex glutamate following treatment of mice with phencyclidine (Moghaddam and Adams, 1998), and they inhibit the large increase in dopamine signaling seen following treatment of mice with ketamine (Fell et al., 2011). These findings suggest that GRM3/GRM2 agonists correct the signaling aberrancies that are responsible for the symptoms of schizophrenia. Adding strength to this hypothesis is the finding that both *GRM2* and *GRM3* knockout mice express more high affinity D2 receptors and are hypersensitive to dopamine neurotransmission (Seeman et al., 2009). Although these pharmacological treatments target both GRM2 and GRM3, it has been suggested that GRM3 especially is responsible for the antipsychotic properties based on the fact that GRM3 polymorphisms, but not GRM2 polymorphisms, have been found to be associated with schizophrenia in several studies (Harrison et al., 2008).

Association of *GRM3* and decreased FA can be explained by a number of reasons. Most obviously, FA decreases with decreased myelination by oligodendrocytes. Cultured rodent oligodendrocyte progenitor cells (OPCs), cultured rodent oligodendrocytes (Luyt et al., 2003; Deng et al., 2004), and adult human OPCs (Luyt et al., 2004) all express GRM3, although any functional role in oligodendrocytes development or myelination has not been completely defined (Luyt et al., 2006). In culture, *GRM3* does not appear to be developmentally regulated (Luyt et al., 2006), and more research is needed to determine its functional role on oligodendrocytes. The *GRM3* association that we observed with FA in our patient sample could also be caused by a decreased number of axons in patients with the TT genotype of rs7808623. Unfortunately, the developmental profile of *GRM3*

in the human brain has yet to be determined, though 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 hyper-glutamatergic signaling, could also lead to increased neuronal damage as a result of the aberrant signaling seen in schizophrenia.

#### *Component B/ Cortico-cerebellar-thalamic-cortical circuit*

The genotype/FA association we observed occurred in an independent component that was dominated by white matter tracts connecting the internal capsule and the thalamus to the cerebellar peduncles (Figure 9). Although the threshold map of this component does not extend into the prefrontal cortex, component B is still very reminiscent of the cortical-subcortical-cerebellar circuit that was proposed by Andreasen et al. (1998) to be responsible for “cognitive dysmetria” in schizophrenia patients. This circuit is now more often referred to as the cortico-cerebellar-thalamic-cortical circuit. Although the cerebellum has been traditionally viewed as performing mainly balance and motor tasks, it has become increasingly clear that the cerebellum is also involved in tasks related to emotion and cognition (Andreasen and Pierson, 2008). Indeed, the cerebellum has been shown to be active independent of its normal motor function during many activities that are impaired in schizophrenia, including facial recognition (Andreasen et al., 1996), cognitive problem solving and task improvement (Kim et al., 1994; Seidler et al., 2002), emotion attribution (Schulte-Rüther et al., 2007), directed attention (Allen et al., 1997), and working memory (Hayter et al., 2007). Additionally, cerebellar lesions can lead to complex symptomology affecting higher cognitive functions (Schmahmann et



al., 2007; Tavano et al., 2007). Therefore, an altered cortico-cerebellar-thalamic-cortical circuit 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 diffusion tensor imaging studies that have reported decreased FA in the white matter tracts within the cerebellum and of cortical-cerebellar tracts (Kanaan et al., 2009; Liu et al., 2011; Magnotta, 2008; Solowij et al., 2011). Additionally, several studies have reported reduced cerebellar volume in schizophrenia (Greenstein et al., 2011; Keller et al., 2003; Nopoulos et al., 2009; Thomann et al., 2009), and others have reported reduced blood flow during working memory tasks in regions involved in cortical-subcortical-cerebellar connectivity (Bor et al., 2011; Kiehl et al., 2005; Kim et al., 2009; White et al., 2011).

Although the prominence of the ATR in component B is not apparent from its threshold map, it is worth noting that an analysis mapping each voxel in component B to a DTI atlas mapped 48.4% of component B to either the left or right ATR, a tract that has been reported to have reduced white matter integrity in other DTI and schizophrenia studies (Figure 4, McIntosh et al., 2008; Bora et al., 2011). The ATR is located in the medial portion of the anterior limb of the internal capsule, a tract that is also a part of the cortico-cerebellar-thalamic-cortical circuit. The ATR relays information between the mediodorsal nucleus of the thalamus and the prefrontal cortex (Mori et al., 2005), and loss of integrity of the ATR in particular has been suggested as a partial explanation for some symptoms of schizophrenia (Mamah et al., 2010). In addition to DTI studies in schizophrenia patients, loss of anisotropy in the anterior limb of internal capsule has been reported in temporal lobe epilepsy patients who suffer from psychosis (Sundram et al.,

2010). Interestingly, although we found no statistical difference in white matter integrity between our patients and controls in the independent component that strongly correlated with rs7808623 in our patient sample, we did report a strong difference in anisotropy in another component which contained a large proportion of the ATR (component C, Figures 2C, 3C, 4C, and 6).

### *Negative results*

Our study investigated several SNPs that have previously been shown to be risk factors for schizophrenia (Appendix A), but we failed to show any differences in allele frequencies between our case sample and our control sample. Also, polymorphisms in both *NRG1* (McIntosh et al., 2008A; Winterer et al., 2008) and its receptor *ERBB4* (Konrad et al., 2008; Zuliani et al., 2011) have been shown to be associated with white matter integrity, and we found no such association in our sample. Finally, while many studies have reported an association between white matter integrity and symptom severity (de Weijer et al., 2011; Miyata et al., 2011; Shin et al., 2006; Skelly et al., 2008), we found no such association.

### *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 one site by itself. Also, our methods required us to correct for 804 independent tests

in the genotype-DTI association study, increasing the possibility of a type II error.

Finally, although ICA has several advantages over traditional DTI analysis techniques, a traditional region of interest study would be necessary to confirm a relationship between *GRM3* and the cortico-cerebellar-thalamic-cortical circuit.

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## APPENDIX A: LIST OF SNPs INVESTIGATED

134 SNPs and their corresponding genes that were investigated for relationship with FA in biologically relevant independent components.

| <b>SNP</b> | <b>GeneSymbol</b> | <b>Gene name</b>  |
|------------|-------------------|---|
| rs1801133  | MTHFR             | 5,10-methylenetetrahydrofolate reductase (NADPH)                      |
| rs6691840  | GRIK3             | glutamate receptor, ionotropic, kainate 3                             |
| rs364482   | EIF2B3            | eukaryotic translation initiation factor 2B, subunit 3 gamma, 58kDa   |
| rs2661319  | RGS4              | regulator of G-protein signaling 4                                    |
| rs6660593  | CNTN2             | contactin 2 (axonal)  |
| rs4951161  | CNTN2             | contactin 2 (axonal)  |
| rs1800896  | IL10              | interleukin 10  |
| rs841865   | PLXNA2            | plexin A2   |
| rs752016   | PLXNA2            | plexin A2   |
| rs7520974  | CHRM3             | cholinergic receptor, muscarinic 3                                    |
| rs2919129  | RTN4              | reticulon 4   |
| rs6715980  | RTN4              | reticulon 4   |
| rs7601625  | RTN4              | reticulon 4   |
| rs10496037 | RTN4              | reticulon 4   |
| *rs2920898 | RTN4              | reticulon 4   |
| rs2421954  | C2ORF86           | chromosome 2 open reading frame 86                                    |
| rs2278718  | MDH1              | malate dehydrogenase 1, NAD (soluble)                                 |
| rs7560571  | MAL               | mal, T-cell differentiation protein                                   |
| rs16944    | IL1B              | interleukin 1, beta   |
| rs1344706  | ZNF804A           | zinc finger protein 804A  |
| rs6435659  | ERBB4             | v-erb-a erythroblastic leukemia viral oncogene homolog 4              |
| rs7588431  | ERBB4             | v-erb-a erythroblastic leukemia viral oncogene homolog 5              |
| rs7598440  | ERBB4             | v-erb-a erythroblastic leukemia viral oncogene homolog 6              |
| rs839523   | ERBB4             | v-erb-a erythroblastic leukemia viral oncogene homolog 7              |
| rs707284   | ERBB4             | v-erb-a erythroblastic leukemia viral oncogene homolog 8              |
| rs6436310  | PAX3              | paired box 3  |
| rs2305234  | ARPP21            | cyclic AMP-regulated phosphoprotein, 21 kD                            |
| rs7372209  | CTDSPL            | CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small |

|             |                     |  |
|-------------|---------------------|--|
|             |                     | phosphatase-like   |
| rs3772917   | GAP43               | growth associated protein 43                                       |
| rs1370807   | GAP43               | growth associated protein 43                                       |
| rs1700      | FSTL1               | follistatin-like 1   |
| rs8177191   | TF                  | transferrin  |
| rs1799852   | TF                  | transferrin  |
| *rs1358024  | TF                  | transferrin  |
| rs1115219   | TF                  | transferrin  |
| rs1477211   | CLDN11              | claudin 11   |
| rs6794467   | CLDN11              | claudin 11   |
| rs2070022   | FGA                 | fibrinogen alpha chain   |
| rs2070016   | FGA                 | fibrinogen alpha chain   |
| rs875462    | DTNBP1              | dystrobrevin binding protein 1                                     |
| rs6913660   | MHC REGION          | major histocompatibility complex region                            |
| rs6932590   | MHC REGION          | major histocompatibility complex region                            |
| rs29228     | MOG                 | myelin oligodendrocyte glycoprotein                                |
| rs2252711   | MOG                 | myelin oligodendrocyte glycoprotein                                |
| rs3130375   | MHC REGION/<br>TCF4 | Major histocompatibility complex region/<br>transcription factor 4 |
| rs3131296   | NOTCH4              | notch homolog 4  |
| rs11759115  | MDGA1               | MAM domain containing<br>glycosylphosphatidylinositol anchor 1     |
| rs2064430   | AHI1                | Abelson helper integration site 1                                  |
| rs1475069   | AHI1                | Abelson helper integration site 1                                  |
| rs2758331   | SOD2                | superoxide dismutase 2, mitochondrial                              |
| rs9456869   | QKI                 | quaking homolog, KH domain RNA binding                             |
| rs6964705   | EGFR                | epidermal growth factor receptor                                   |
| rs6465084   | GRM3                | glutamate receptor, metabotropic 3                                 |
| rs7808623   | GRM3                | glutamate receptor, metabotropic 3                                 |
| rs1045642   | ABCB1               | ATP-binding cassette, sub-family B<br>(MDR/TAP), member 1          |
| rs1128503   | ABCB1               | ATP-binding cassette, sub-family B<br>(MDR/TAP), member 1          |
| rs7341475   | RELN                | reelin   |
| *rs1196475  | PTPRZ1              | protein tyrosine phosphatase, receptor-type, Z<br>polypeptide 1    |
| *rs11136442 | ARHGEF10            | Rho guanine nucleotide exchange factor (GEF)<br>10                 |
| rs4876268   | ARHGEF10            | Rho guanine nucleotide exchange factor (GEF)<br>10                 |
| rs2270641   | SLC18A1             | solute carrier family 18 (vesicular monoamine),<br>member 1        |
| rs35753505  | NRG1                | neuregulin 1   |

|            |             |   |
|------------|-------------|---|
| rs4452759  | NRG1        | neuregulin 1  |
| rs6994992  | NGR1        | neuregulin 1  |
| rs10503929 | NRG1        | neuregulin 1  |
| rs3925     | FGFR1       | fibroblast growth factor receptor 1   |
| rs6996321  | FGFR1       | fibroblast growth factor receptor 1   |
| rs2272648  | NDRG1       | N-myc downstream regulated 1  |
| rs1011784  | HSA-MIR-23B | homo sapiens microRNA-23-B  |
| rs913767   | GSN         | gelsolin (amyloidosis, Finnish type)  |
| rs306761   | GSN         | gelsolin (amyloidosis, Finnish type)  |
| rs306770   | GSN         | gelsolin (amyloidosis, Finnish type)  |
| rs3793753  | PIP5K2A     | phosphatidylinositol-4-phosphate 5-kinase type II-alpha                     |
| *rs885834  | CHAT        | choline acetyltransferase   |
| rs762571   | PSAP        | prosaposin  |
| rs2299939  | PTEN        | phosphatase and tensin homolog; phosphatase and tensin homolog pseudogene 1 |
| rs2797986  | PLCE1       | phospholipase C, epsilon 1  |
| rs4917450  | PLCE1       | phospholipase C, epsilon 1  |
| *rs521674  | ADRA2A      | adrenergic, alpha-2A-, receptor   |
| rs2429511  | ADRA2A      | adrenergic, alpha-2A-, receptor   |
| rs2912787  | FGFR2       | fibroblast growth factor receptor 2   |
| rs2981428  | FGFR2       | fibroblast growth factor receptor 2   |
| rs1800955  | DRD4        | dopamine receptor D4  |
| rs6578993  | TH          | tyrosine hydroxylase  |
| rs1800532  | TPH1        | tryptophan hydroxylase 1  |
| rs6265     | BDNF        | brain-derived neurotrophic factor   |
| rs1602565  | INTERGENIC  | N/A   |
| rs6277     | DRD2        | dopamine receptor D2  |
| *rs6275    | DRD2        | dopamine receptor D2  |
| rs1076560  | DRD2        | dopamine receptor D2  |
| rs2071521  | APOC3       | apolipoprotein C-III  |
| rs12807809 | NRGN        | neurogranin (protein kinase C substrate, RC3)                               |
| *rs3016384 | OPCML       | opioid binding protein/cell adhesion molecule-like                          |
| rs705708   | ERBB3       | v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)           |
| rs4623951  | DAO         | D-amino-acid oxidase  |
| rs6311     | HTR2A       | 5-hydroxytryptamine (serotonin) receptor 2A                                 |
| rs1130233  | AKT1        | v-akt murine thymoma viral oncogene homolog 1                               |
| rs3803300  | AKT1        | v-akt murine thymoma viral oncogene homolog 1                               |

|            |          |   |
|------------|----------|---|
| rs3087454  | CHRNA7   | cholinergic receptor, nicotinic, alpha 7  |
| rs1355920  | CHRNA7   | cholinergic receptor, nicotinic, alpha 7  |
| *rs2135551 | ADAMTSL3 | ADAMTS-like 3   |
| rs4784642  | GNAO1    | guanine nucleotide binding protein (G protein), alpha activating activity polypeptide O |
| rs1801200  | ERBB2    | v-erb-b2 erythroblastic leukemia viral oncogene homolog                                 |
| rs2070106  | CNP      | 2',3'-cyclic nucleotide 3' phosphodiesterase  |
| rs4796751  | CNP      | 2',3'-cyclic nucleotide 3' phosphodiesterase  |
| rs2258689  | MAPT     | microtubule-associated protein tau  |
| rs734194   | NGFR     | nerve growth factor receptor (TNFR superfamily, member 16)                              |
| rs1860985  | PRKCA    | protein kinase C, alpha   |
| rs3813065  | PIK3C3   | phosphoinositide-3-kinase, class 3  |
| rs470826   | MBP      | myelin basic protein  |
| rs4890875  | MBP      | myelin basic protein  |
| rs470131   | MBP      | myelin basic protein  |
| rs509620   | MBP      | myelin basic protein  |
| rs2282557  | MBP      | myelin basic protein  |
| rs4890876  | MBP      | myelin basic protein  |
| rs2301600  | MAG      | myelin associated glycoprotein  |
| rs3746248  | MAG      | myelin associated glycoprotein  |
| rs720308   | MAG      | myelin associated glycoprotein  |
| rs405509   | APOE     | hypothetical LOC100129500; apolipoprotein E   |
| rs7412     | APOE     | hypothetical LOC100129500; apolipoprotein E   |
| rs6140671  | PLCB1    | phospholipase C, beta 1 (phosphoinositide-specific)                                     |
| rs762178   | OLIG2    | oligodendrocyte lineage transcription factor 2  |
| *rs1059004 | OLIG2    | oligodendrocyte lineage transcription factor 2  |
| rs737865   | TXNRD2   | thioredoxin reductase 2   |
| rs4680     | COMT     | catechol-O-methyltransferase  |
| rs701427   | RTN4R    | reticulon 4 receptor  |
| rs1567871  | RTN4R    | reticulon 4 receptor  |
| rs165862   | PIK4CA   | phosphatidylinositol 4-kinase, catalytic, alpha   |
| rs165793   | PIK4CA   | phosphatidylinositol 4-kinase, catalytic, alpha   |
| rs139884   | SOX10    | SRY (sex determining region Y)-box 10   |
| rs475827   | PLP1     | proteolipid protein 1   |
| rs471416   | PLP1     | proteolipid protein 1   |
| *rs6571291 | ABCD1    | ATP-binding cassette, sub-family D (ALD), member 1                                      |
| *rs4129148 | CSF2RA   | colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage)      |

\*Allele frequency for genotype at SNP differed significantly by site ( $p < 0.05$ ,  $X^2$  test)