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ARTICLE

Myelination-related genes are associated with decreased white matter integrity in schizophrenia

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Disruptions in white matter (WM) tract structures have been implicated consistently in the pathophysiology of schizophrenia. Global WM integrity – as measured by fractional anisotropy (FA) – is highly heritable and may provide a good endophenotype for genetic studies of schizophrenia. WM abnormalities in schizophrenia are not localized to one specific brain region but instead reflect global low-level decreases in FA coupled with focal abnormalities. In this study, we sought to investigate whether functional gene sets associated with schizophrenia are also associated with WM integrity. We analyzed FA and genetic data from the Mind Research Network Clinical Imaging Consortium to study the effect of multiple oligodendrocyte gene sets on schizophrenia and WM integrity using a functional gene set analysis in 77 subjects with schizophrenia and 104 healthy controls. We found that a gene set involved in myelination was significantly associated with schizophrenia and FA. This gene set includes 17 genes that are expressed in oligodendrocytes and one neuronal gene (*NRG1*) that is known to regulate myelination. None of the genes within the gene set were associated with schizophrenia or FA individually, suggesting that no single gene was driving the association of the gene set. Our findings support the hypothesis that multiple genetic variants in myelination-related genes contribute to the observed correlation between schizophrenia and decreased WM integrity as measured by FA.
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INTRODUCTION

Schizophrenia is a chronic disabling disorder that affects approximately 1% of the population worldwide with devastating personal, social, and economic effects. Schizophrenia is highly heritable and multiple genes with small-to-modest effect size in combination with unknown environmental factors are involved in its pathogenesis.[1–4] Recent reports suggest the involvement of the neuronal calcium signaling pathway,[3] as well as the involvement of specific glial functions such as those related to oligodendrocyte function.[5] The latter is of particular interest when placed in the context of brain imaging results for schizophrenia.

Oligodendrocytes are glial cells responsible for the synthesis of the myelin sheath, which supports and isolates neuronal axons forming the white matter (WM) in the brain. Disruptions in WM tract structures have been consistently implicated in the pathophysiology of schizophrenia; a number of diffusion tensor imaging (DTI) studies in schizophrenia have implicated WM abnormalities in various brain regions.[6–10] Although there is a lack of consistency in the spatial localization of the brain regions showing reduced fractional anisotropy (FA),[11] in this regard it has been found that WM abnormalities are not localized to a specific brain region but instead reflect a diffuse process with widely dispersed focal reductions in FA that vary spatially among individuals.[12–14] Of all the DTI parameters examined so far, FA of water diffusion has the highest reported heritability.[15] It is unclear how WM integrity abnormalities relate to the underlying genetic architecture of Schizophrenia, nevertheless the endophenotypic importance of FA for schizophrenia is further supported by recent studies showing that many brain regions showing significant decrease in FA in subjects with schizophrenia (including childhood-onset schizophrenia) were also decreased similarly but with smaller effects in their relatives, with a continuous FA decrease from healthy subjects to relatives to subjects with schizophrenia.[16,17]

Thus global WM integrity (as measured by FA) may provide a good biological endophenotype to explain genetic differences in the risk for schizophrenia.[18,19] In this study, we sought to investigate whether oligodendrocyte gene sets associated with schizophrenia are also associated with WM integrity.

METHODS

Gene set definition

When performing a gene set association analysis, the most critical step is the definition of the gene sets. For this study, we used a modified version of the

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oligodendrocyte gene sets derived from expert-curated glial gene lists described in detail by Goudriaan *et al*.[5] The main goal of that study was to create list of genes that were mainly expressed in glial cells. The authors of that study defined their gene sets based on an in-depth literature study and selected glial genes based on microarray gene expression patterns. The microarray sources included (1) microarray studies comparing different mouse central nervous system cell types; (2) microarray studies of whole human brain material; (3) microarray studies of upregulated genes in humans; (4) mouse microglia after stimulation of these cells with pro-inflammatory stimuli; and (5) a group of oligodendrocyte transcription factors derived from a promoter-based analysis of co-expressed genes in myelinated mouse tissue. To strengthen the association of genes in these lists with specific astrocyte, oligodendrocyte, or microglia functioning, genes were removed if found in more than one of these cell types or if present in a curated exclusion list of general neuronal genes.[5] An enrichment analysis using gene ontology (GO) biological processes was performed on the final, filtered astrocyte, oligodendrocyte, microglia, and neuronal lists to see whether processes associated with specific cell-type functions were uniquely enriched within each list. For each glial cell-type (oligodendrocytes, astrocytes, and microglia), functional gene sets were created based on GO biological process annotations. Importantly, gene sets were built according to the hierarchical structure of GO (ie, higher-level parental nodes were subdivided into more specific child nodes), resulting in an organization of related gene sets over a maximum of three levels and substantial overlap of genes between gene sets.[5]

For the present study, few adaptions were made to the sets as described in Goudriaan *et al*[5] in order to reduce the number of oligodendrocyte functional gene sets and reduce multiple testing. Most importantly, gene sets were grouped together into fewer, overarching functional sets (Supplementary Table S1). Specifically, the metabolism sets were grouped together into 'metabolism-related genes', the cell signaling groups into 'cell communication-related genes', the intracellular sets into 'cell process-related genes', and the cell development and immune system sets into a set of 'cell development- and health-related genes'. Genes that were not annotated into GO biological processes were grouped together in a 'miscellaneous oligodendrocyte set'. Sets of genes regarded to be especially important for oligodendrocyte myelination-related processes were kept in separate gene sets, and the myelination and node genes were not added to the general group of 'cell process-related genes' but included as a set of 'oligodendrocyte-specific processes'.

In addition, in a secondary analyses we included the *NRG1* gene to the myelination gene set because *NRG1* gene has well-documented roles in myelination in animal models,[20–22] and others have also included the NRG1 gene in previous analyses of myelination genes and WM integrity in schizophrenia.[23] In the glial gene sets reported by Goudriaan *et al*,[5] this gene was not included (as it is believed to be axonally expressed), and the paper by Goudriaan *et al*[5] intended to focus on cell-type-specific functions. As our main goal was to test the role of genes involved in myelination (including genes that regulate expression of genes in oligodendrocytes but are not necessarily expressed in oligodendrocytes themselves), we show results for cell-type-specific gene sets as well as an expanded gene set including the *NRG1* gene as a known regulator of myelination processes.[20–22]

Participants

The sample used for this study has been described in detail elsewhere.[24] In summary, the curated diffusion tensor imaging (DTI) sample consisted of 114 subjects with schizophrenia and 138 controls with available genetic data. Subjects were recruited from four sites: Massachusetts General Hospital in Boston (MGH) ($N=60$; 29 cases and 31 controls), University of Iowa (UI) ($N=92$; 38 cases and 54 controls), University of Minnesota (UMN) ($N=53$; 25 cases and 28 controls), and University of New Mexico (UNM) ($N=47$; 22 cases and 25 controls). Healthy volunteers were recruited from the community; the healthy control subjects were matched within site to the patient cohort for age, sex, handedness, and parental education. Controls were excluded from the study if they had any Axis I psychiatric disorder, including substance abuse/dependence or a history of a schizophrenia or bipolar spectrum disorder in a first-degree relative. Additional exclusion criteria for both patients and controls included a neurological disorder affecting brain function (ie, head injury with loss of consciousness and seizure disorder) or active substance abuse/dependence. Written informed consent was obtained from all subjects prior to participation, and the institutional review boards at each of the four sites approved the study. All the clinical and imaging data used in this study is publically available through the neuroinformatics suite COINS (Collaborative Informatics Neuroimaging Suite) at www.coins.mrn.org.

Imaging

The image acquisition protocols used for this study have also been described previously.[24] In summary, structural MRI data were acquired with either a Siemens (Erlangen, Germany) 1.5-Tesla (MGH, UI, and UNM) or a Siemens 3-Tesla (UMN) MR scanner. The T_1-weighted structural brain scans at each of the four sites were acquired with an in-plane resolution of 0.625×0.625 mm^2, a slice thickness of 1.5 mm, and a flip angle of 7 degrees. MGH and UNM used a Siemens 1.5-Tesla scanner with repetition time (TR) = 12 ms, echo time (TE) = 4.76 ms, and number of excitations (NEX) = 1. UI used a GE (Waukesha, WI, USA) 1.5-Tesla Genesis Sigma scanner with TR = 20 ms, TE = 6 ms, and NEX = 3. UMN used a Siemens 3-Tesla scanner with TR = 2530 ms, inverse time (TI) = 1100 ms, TE = 3.79 ms, and NEX = 1.

All DTI were obtained at each site with a 2-mm isotropic resolution. MGH used a Siemens Sonata 1.5-Tesla scanner with TR = 8900 ms, TE = 80 ms, *B* values of 0 and 700, NEX = 1, and 60 directions. UI used a Siemens TRIO 3-Tesla scanner with TR = 9500 ms, TE = 90 ms, *B* values of 0 and 1000, NEX = 4, and 6 directions. UNM used a Siemens Sonata 1.5-Tesla scanner with TR = 9800 ms, TE = 86 ms, *B* values of 0 and 1000, NEX = 4, and 12 directions. UMN used a Siemens TRIO 3-Tesla scanner with TR = 10 500 ms, TE = 86 ms, *B* values of 0 and 1000, NEX = 2, and 12 directions.

The diffusion-weighted images were analyzed using the GTRACT.[25] Scalar measures for FA were calculated on the DTI images for all subjects; measurements of FA were calculated in coronal Talairach sections from the anterior to the posterior region along the whole brain. The mean FA within each coronal slice for all subjects were calculated, and a within-site *z*-transformation was performed prior to pooling the data; this is a crucial step as each site had large FA differences, so standardization was done prior to pooling the data in order to control for those differences.

In addition to obtaining coronal slices, regions from the Johns Hopkins University WM atlas (http://www.dtiatlas.org/) were applied to the FA maps to extract mean FA values for each individual, as this atlas selects only the major WM tracts, and evaluating FA within the mask provides a global mean DTI value of the major WM tracts.

Genotyping

Whole blood was collected from subjects for DNA extraction; whole-genome genotyping (1 million SNPs) was done using HumanOmni1 Quad Beadchip Kits (Illumina, San Diego, CA, USA). Genotyping data quality control (QC) was performed in order to assess the failure rate per individual and per SNP, the degree of relatedness between individuals, and to identify ancestral outliers, following the standard protocol for data QC in genetic case–control association studies by Anderson *et al*.[26] We removed 37 cases and 34 controls in the QC process. The vast majority of subjects who were removed from the study failed ancestry clustering; after removing these subjects, the genomic inflation factor (based on median chi-squared) was 1.0153, and the mean chi-squared statistic is 1.0047. After QC, 710 224 SNPs remained for association analysis (the total genotyping rate in remaining individuals was 0.9989). As previously published,[24] owing to consortium agreements and IRB restrictions, the raw genotypic data used in the study is not publically available, but genome-wide association study (GWAS) analysis results obtained as part of the gene set analysis are publically available at GWAS Central: http://www.gwascentral.org/study/HGVST1829.

Statistics

The gene set association analyses were performed using a statistical software package developed by our group: the Joint Association of Genetic Variants (JAG) software (http://ctglab.nl/software/), the details of the statistical methods used by this software are described elsewhere.[27] In summary, all SNPs that survived QC were mapped to genes on the basis of NCBI (National Center for

Biotechnology Information) human assembly build 36.3 and dbSNP release 129. For the definition of the gene boundaries, we downloaded the 'seq_gene.md' file from the FTP website of NCBI. From this list of records, we deleted genes coded as pseudo in the column 'feature_type'. Subsequently, we selected the records with gene as 'feature_type' and reference as 'group_label'. For these records, we assigned SNPs to genes when annotated between 'chr_start' (transcription start site) and 'chr_stop' (transcription stop site). We then conducted SNP association analyses using additive models of allele counts. Cochran–Mantel–Haenszel tests implemented in PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/)[28] were used for the association analyses. These single SNP outcomes were then used to conduct a self-contained test by summing the logarithm of the reciprocal of the *P*-values (denoted as $\Sigma - \log_{10}(P)$ method), and permutation was used to determine the significance of the combined effect of all SNPs in a gene set. For gene sets with a significant empirical *P*-value, we conducted competitive tests to evaluate whether the gene set was more significantly associated with the trait than 500 randomly composed, matched control gene sets.

RESULTS

Descriptive

After removing subjects who failed ancestry clustering, the total sample consisted of 181 subjects: 77 cases (76% male, mean age 35.65 years[18–60]), and 104 controls (57% male, mean age 32.92 years[19–58]). After removing subjects who did not survive DTI QC, total brain FA was available for 129 subjects: 48 cases and 81 controls. In this subsample, total brain FA was significantly decreased in cases compared with controls (ANOVA $F=15.6$, $P=0.0001$), which compares well to results reported previously for the entire sample.[14]

Single SNP association analysis

SNP association analyses using additive models of allele counts were calculated in order to generate the association *P*-values needed for the gene set association analyses; a GWAS was performed in the final curated sample. As expected, we found that no single SNP reached genome-wide significant association for schizophrenia (lowest $P=5.195e\text{-}06$ for SNP rs2028122 on chromosome 15) or for FA (lowest $P=4.08e\text{-}06$ for SNP rs1422121 on chromosome 5) (Manhattan plots for GWAS results are presented in Supplementary Figure S1.). Additionally, when looking at the individual *P*-values for each SNP included in each oligodendrocyte gene set (data not shown), none of the SNP survived multiple testing correction for the number of SNPs in each gene set (see number of SNPs per gene set in Table 1).

Table 1 Results of gene set association analysis of the oligodendrocyte gene sets with schizophrenia

Oligodendrocyte gene sets	*Number of genes*	*Number of SNPs*	*Self-contained test P-value*
Oligodendrocytes: all genes	1893	42 692	0.84
Subgroup 1: Metabolism	544	9979	0.76
Subgroup 2: Cell communication	281	8234	0.50
Subgroup 3: Cell processes	434	11 278	0.83
Subgroup 4: Cell development and health	141	2638	0.49
Subgroup 5: Oligodendrocyte-specific functions	24	908	**0.04**
5.1 Myelination	17	222	**0.004**
5.2 Node Processing	8	399	0.97
Subgroup 6: Miscellaneous	469	9655	0.52

Statistically significant *P*-values are shown in bold.

Gene set association analysis

Based on our prior knowledge of schizophrenia being associated with glial gene sets,[5] and in order to reduce the number of tests and avoid multiple testing errors, we first analyzed whether any of the six primary oligodendrocyte gene sets were associated with schizophrenia in our sample. We found that only the gene set of oligodendrocyte-specific functions was nominally associated with schizophrenia ($N=181$, self-contained test $P=0.04$; and competitive test $P=0.05$; Table 1). The oligodendrocyte-specific function gene set was divided into two subgroups as previously described by Goudriaan *et al*[5]: (i) the myelination gene set, and (ii) the node-processing gene set. Second, we tested whether any of these two gene sets were also associated with schizophrenia; we found that the myelination gene set was significantly associated with Schizophrenia ($N=181$, self-contained test $P=0.004$; and competitive test $P=0.006$; both *P*-values surviving multiple testing correction); additionally, we performed the same analysis including only the subjects for which FA was available ($N=129$), when found that, in this smaller group, also the myelination gene set was significantly associated with Schizophrenia using the competitive test ($P=0.006$) but not with the self-contained test ($P=0.06$). Third, we tested whether the myelination gene set that was significantly associated with schizophrenia was also associated with FA; the results showed no association of the myelin gene set with FA ($N=129$, self-contained test $P=0.2$; Table 2).

As we did not want to limit ourselves to only one cell type (ie, only looking at genes predominantly expressed in oligodendrocytes), in a secondary analysis, we added a known regulator of myelination (the *NRG1* gene) to the myelination gene set, which itself is not expressed in oligodendrocytes. Rerunning our gene set analyses for schizophrenia and FA showed that the expanded myelination gene set was again significantly associated with schizophrenia ($N=181$, self-contained test $P=0.001$; and competitive test $P=0.001$); this was also the case for the smaller sample including only the subjects with FA data available ($N=129$, self-contained test $P=0.005$; and competitive test $P=0.002$) and now also with total brain FA (self-contained test $P=0.04$; and competitive test $P=0.024$) (Table 2).

When testing the association between the myelination gene set and schizophrenia while correcting for FA, the association remains significant (Table 2 and Figure 1b), indicating that there is additional variance in schizophrenia liability explained by the myelin gene set that was also explained by FA ($N=129$, self-contained test $P=0.012$; and competitive test $P=0.008$). On the other hand, when testing the association between the myelination gene set and FA while correcting for schizophrenia status, this association was also no longer statistically significant (Table 2 and Figure 1b).

Gene-based analyses of all genes in the myelination gene set

The final myelination gene set was composed of 18 genes: oligodendrocyte-expressed genes and *NRG1* gene (Table 3). As the genotyping platform did not include any SNPs for the *POU3F1* gene, this gene was not included in any of the analyses. We used JAG to perform a gene-based association analysis of all genes included in the myelination gene set to investigate whether one of these genes was independently associated with schizophrenia or total FA and was driving the gene set association. We found nominal weak associations of *MOBP* and *OMG* with schizophrenia, *MAG* with FA, and *NRG1* with both schizophrenia and FA. None of these associations would have survived multiple testing correction for the number of gene-based tests performed, suggesting that the association of the gene set with schizophrenia and FA was not driven by singleton genes, but

Table 2 Results of gene set association analysis of the myelination gene set with schizophrenia and total brain fractional anisotropy (FA)

		Glial myelination gene set		*Glial myelination gene set+NRG1*	
	N	*Self-contained test* P-*value*	*Competitive test* P-*value*	*Self-contained test* P-*value*	*Competitive test* P-*value*
Schizophrenia	181	**0.007**	**0.005**	**0.001**	**0.001**
FA available[a]	129	0.061	**0.043**	**0.005**	**0.002**
Schizophrenia (FA as covariant)	129	**0.049**	**0.038**	**0.012**	**0.008**
Total brain FA	129	0.201	NA	**0.040**	**0.024**
Total brain FA (SZ as covariant)	129	0.279	NA	0.146	NA

[a]Association analysis including only subjects for which FA was available. Statistically significant *P*-values are shown in bold.

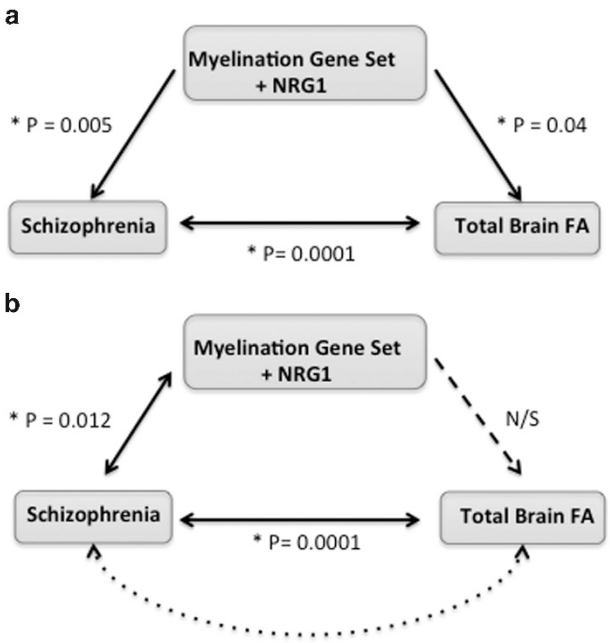

Figure 1 Combined effect of genetic variants of myelination genes is associated with schizophrenia and decreased total brain FA. Model A shows how the myelination gene set may explain part of the link between total brain FA and Schizophrenia; under this model it is not possible to establish the direction of causation, as is not possible to determine whether genetic variants in the myelination gene set impact on FA, which in turn increases the risk for SZ or vice versa. Model B shows the effect when testing the association between the myelination gene set and schizophrenia while correcting for total brain FA and vice versa (denoted by the dotted line). Under this model is possible to identify a possible direction of causation; while correcting for FA the association between the myelination gene set and schizophrenia remains significant, but the association between the myelination gene set and total brain FA while correcting for schizophrenia is no longer significant (discontinuous line) indicating that the association between the myelination gene set and total brain FA is mediated by schizophrenia diagnosis status. NS, not significant. A full color version of this figure is available at the *European Journal of Human Genetics* journal online.

instead indicating that the association was due to the combined effect of multiple genes within the gene set.

DISCUSSION

Global WM integrity alterations have been consistently found in patients with schizophrenia.[12,29] Whereas the cause of these alterations is still unknown, there is significant evidence for heritability of WM integrity of specific brain regions in subjects with schizophrenia and their unaffected relatives.[16–18] These findings suggest that WM integrity measures may be useful endophenotypes for genetic studies of schizophrenia.[13] To date, none of the significant genome-wide genetic variations associated with schizophrenia have been found to explain the differences in WM integrity seen in schizophrenia patients.[30] Individual genetic variants associated with schizophrenia have small effect sizes.[3] Thus it has been suggested that the additive effect of multiple genetics variants with small effects may explain complex phenotypes.[31] Neuroimaging-based intermediate phenotypes have emerged as particularly promising, because they map risk-associated gene effects onto physiological processes in the brain that are altered in patients.[32] In this study, we sought to investigate whether glial gene sets associated with schizophrenia were also associated with WM integrity.

As previously reported by White *et al*,[14] FA was significantly decreased in schizophrenic subjects compared with controls in the same sample used for this study. WM integrity abnormalities have been found in first-episode antipsychotic-naive schizophrenic patients[33] and are not believed to be a consequence of the disease itself or the antipsychotic treatment.[34] One possible mechanism of FA decrease involves an alteration or weakening of the myelin sheath. This is supported by histopathological studies suggesting a disturbed myelination in schizophrenia.[35]

Table 3 Results of gene-based association analysis of myelination genes with schizophrenia and total brain fractional anisotropy

		Gene-based self-contained test nominal P-values	
Gene	*Number of SNPs*	*Schizophrenia*	*Total brain FA*
C11orf9	13	0.162	0.258
CNP	4	0.491	0.708
ILK	5	0.520	0.559
MAG	9	0.127	0.018
MAL	3	0.372	0.119
MBP	97	0.081	0.320
MOBP	24	0.007	0.784
MOG	11	0.056	0.842
OMG	1	0.027	0.959
CLDN11	4	0.928	0.341
PLP1	1	0.696	0.452
POU3F1	0	NA	NA
KLK6	2	0.239	0.941
TF	24	0.942	0.167
EIF2AK3	11	0.869	0.711
GAL3ST1	3	0.111	0.944
OLIG2	3	0.931	0.720
PLLP	7	0.264	0.383
NRG1	300	0.012	0.041

In our study, we tested the combined effects of all genetic variants available within oligodendrocyte-expressed genes grouped in functional gene sets. As in many other imaging genetic studies, our study was limited by sample size, especially after performing genetic QC we had to remove 37 cases and 34 controls owing to population stratification issues. From the six gene sets tested, only the oligodendrocyte-specific function gene set was associated with schizophrenia; this gene set is composed of two separated subgene sets, of which only the myelination gene set was significantly associated with schizophrenia but not with FA.

In our previous, larger-scale study,[5] we tested for association of 96 astrocyte, oligodendrocyte, and microglia gene sets with schizophrenia. We found an association of six astrocyte gene sets and three oligodendrocyte gene sets. The oligodendrocyte gene sets included lipid metabolism, oxidation–reduction, and gene transcription, yet the myelination gene set did not survive multiple testing in study by Goudriaan *et al*[5] although this gene set showed nominal significance (uncorrected $P = 0.0203$).

A recent study by Voineskos *et al*[23] tested whether individual genetic variants in myelin genes had an effect on WM integrity. They found that one SNP located in the *MAG* gene (SNP rs756796) and one SNP in the *CNP* gene (SNP rs2070106) had an effect on the microstructural integrity of all WM tracts. In our study, using a gene-based analysis, we did not find that any of the genes in our original myelination gene set (including *MAG* and *CNP*) was individually significantly associated with schizophrenia or total brain FA. Voineskos *et al*[23] also included *NRG1* in their analysis of gene variants for myelination, as the myelin genes show epistatic risk for schizophrenia with variants in the *NRG1* gene system; expression of these two gene systems is coordinated,[36] and disruption of the *Nrg1–ErbB4* pathway in oligodendrocytes in animal models leads to alteration of the myelin sheath of major WM tracts, reduced conduction velocity, and cognitive changes.[37]

Therefore, we conducted secondary analyses following Voineskos *et al*[23] by including the *NRG1* gene in the myelin gene set. We found that this expanded gene set was again significantly associated with schizophrenia and now also with total brain FA, even though the *NRG1* gene by itself was only marginally associated with FA. This finding suggests that *NRG1* could modulate WM integrity in the context of the additive effect seen with other myelin genes. Under this model, neuronally expressed *NRG1* likely regulates myelination genes through an effect on oligodendrocyte-expressed specific genes cells.

Altogether, we found a statistically significant association between schizophrenia and FA (confirming previously established links[11–14]), between the myelination gene set and schizophrenia (replicating earlier, independent findings[5]) and between the myelination gene set and FA (novel finding). Additionally, we tested for the association between the myelination gene set and schizophrenia; while correcting for FA, this association remains significant, indicating that the variance in schizophrenia liability is partially explained by the myelin gene set independently of the variance explained by FA. On the other hand, when testing the association between the myelination gene set and FA while correcting for schizophrenia status, this association was no longer statistically significant; this suggests that the variance observed for total brain FA is explained only in part by the myelination gene set, and that there are other genetic variants associated with schizophrenia that have an effect on FA and are not included in the myelination gene set.

In conclusion, our findings support the hypothesis that multiple genetic variants in myelination-related genes contribute to the observed correlation between schizophrenia and decreased WM integrity as measured by FA. These findings warrant further research in using myelination-related proteins as pharmaceutical targets for preventing WM integrity loss as seen in schizophrenia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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