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The association of white matter volume in psychotic disorders with genotypic variation in *NRG1*, *MOG* and *CNP*: a voxel-based analysis in affected individuals and their unaffected relatives

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We investigated the role of variation in putative psychosis genes coding for elements of the white matter system by examining the contribution of genotypic variation in three single-nucleotide polymorphisms (SNPs) neuregulin 1 (*NRG1*) SNP8NRG221533, myelin oligodendrocytes glycoprotein (MOG) rs2857766 and CNP (rs2070106) and one haplotype HAP_{ICE} (deCODE) to white matter volume in patients with psychotic disorder and their unaffected relatives. Structural magnetic resonance imaging and blood samples for genotyping were collected on 189 participants including patients with schizophrenia (SZ) or bipolar I disorder (BDI), unaffected first-degree relatives of these patients and healthy volunteers. The association of genotypic variation with white matter volume was assessed using voxel-based morphometry in SPM5. The *NRG1* SNP and the HAP_{ICE} haplotype were associated with abnormal white matter volume in the BDI group in the fornix, cingulum and parahippocampal gyrus circuit. In SZ the *NRG1* SNP risk allele was associated with lower white matter volume in the uncinate fasciculus (UF), right inferior longitudinal fasciculus and the anterior limb of the internal capsule. Healthy G-homozygotes of the *MOG* SNP had greater white matter volume in areas of the brainstem and cerebellum; this relationship was absent in those with a psychotic disorder and the unaffected relatives groups. The *CNP* SNP did not contribute to white matter volume variation in the diagnostic groups studied. Variation in the genes coding for structural and protective components of myelin are implicated in abnormal white matter volume in the emotion circuitry of the cingulum, fornix, parahippocampal gyrus and UF in psychotic disorders.

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Introduction

Bipolar I disorder (BDI) and schizophrenia (SZ) share a number of common genetic risk loci and susceptibility genes.[1] Several of these converge on pathways that regulate oligodendrocytes proliferation, assembly, protection and degeneration. These include the genes coding for neuregulin 1 (NRG1)[2] and myelin oligodendrocytes glycoprotein (MOG)[3,4] and in SZ the 2′,3′-cyclic nucleotide 3′ phosphodiesterase enzyme (E.C. 3.1.4.37, CNPase) genes.[5] Postmortem gene expression and mRNA levels support a regional specificity for the effects of these risk alleles in SZ and to some extent in BD. In particular, differential expression for several genes involved in myelination has been reported in the dorsolateral prefrontal cortex of postmortem brains from individuals with an ante-mortem diagnosis of SZ.[6] However, brain-wide analyses of such relationships are not yet available.

Non-invasive *in vivo* structural magnetic resonance imaging (MRI) provides a means by which we can examine the regional effects of these risk polymorphisms that code for structural and protective elements of white matter, on the volume of white matter throughout the brain. Moreover, incorporating groups of affected and related individuals who are unaffected provides the potential to implicate genetic markers potentially responsible for white matter dysfunction that contribute to the development of SZ and BD, and those that confer endophenotypic risk.

***NRG1* single-nucleotide polymorphism (SNP)8NRG221533 and the HAP_{ICE} (*deCODE*) haplotype.** The *NRG1* gene is located on chromosome 8p13 within the SZ susceptibility loci identified at 8p22–p12. The 8p region[7–9] and several loci in the *NRG1* gene[2] confer risk for SZ. In particular, the C-allele of the *NRG1* SNP, SNP8NRG221533 (rs35753505) located in the noncoding 5′-flanking region of the *NRG1* gene, was implicated as giving the best uncorrected single marker association in the Icelandic study and is part of a core haplotype consisting of five SNPs and two microsatellites termed hereafter the HAP_{ICE} haplotype.[10] *NRG1* SNP8NRG221533 has been associated with SZ in Icelandic, Scottish, British/Irish, Dutch, African American, South African and Han Chinese populations[10–14] but not in one Finnish[15] and one Irish population,[16] and in a meta-analysis.[17]

The *NRG1* gene codes for six types of neuronal NRG1, of which types 1 and 4 are implicated in SZ. NRG1 binds the epidermal growth factor receptor family and receptor

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tyrosine-protein kinase erbB-3 and is involved in axonal myelination,[18–20] and NMDA receptor signaling[21] among a number of other functions.[2] In the prefrontal cortex in SZ and BD, altered expression and protein levels of the NRG1-ErbB signaling system have been detected but are not consistent. These reports include increased expression of ErbB4,[22] reduced expression of ErbB3, the alpha isoform of NRG1 (which is increased in hippocampus[23]) and NRG1 mRNA,[24] and no change in expression or protein levels of NRG1.[25–27] However, these studies may be confounded by the effects of antipsychotics medication.[28] Finally, in animal studies, *NRG1* heterozygous mice have a behavioral phenotype that overlaps with the signs and symptoms of SZ,[11] and in *NRG1* hypomorphs these are partially reversed by clozapine administration.[11,29,30] The functional implications of altered expression, protein levels or signaling via NRG1-ErbB include abnormal neuronal growth, neurodegeneration and reduced glutamatergic signaling among other effects.[2,27,31,32]

In vivo imaging in the human brain has provided further evidence for the role of the NRG1 SNP8NRG221533 in white matter pathophysiology in SZ. C-carriers with SZ had reduced microstructural uniformity in the organization (fractional anisotropy (FA)) of their white matter on the left anterior thalamic radiation[33] and the subcortical medial frontal region[34] relative to the TT-genotype group. However, a contrasting study reported reduced FA in the anterior cingulum in the T-carriers relative to the CC-genotype.[35] The risk genotype (TT) of a *NRG1* promoter region SNP8NRG243177 has been associated with reduced FA and white matter volume in the anterior limb of the internal capsule (ALIC)[36] and in the anterior thalamic radiation[33] relative to the C-carriers. Using functional MRI, the latter risk genotype has additionally been associated with reduced medial prefrontal (BA9) and temporo–occipital junction (BA39 and BA19) activation while performing a sentence completion task, and the development of psychotic symptoms, as well as a lower premorbid IQ.[37] This relationship was not present with the SNP examined in this study, SNP8NRG221533.

***MOG* rs2857766.** Oligodendroglia abnormalities have been observed in both SZ and BD[38] with increasing frequency in recent years. In SZ a structural component of myelin, MOG has been found to be differentially expressed in the dorsolateral prefrontal cortex,[39] thalamus[40] and anterior temporal lobe,[41] and MOG mRNA levels were lower in several regions examined.[42] However, MOG mRNA levels did not differ in one study in individuals with BD.[42] Negative findings for association of *MOG* gene variation and SZ also exist: four polymorphisms in *MOG* ((CA)n, (TAAA)n, C1334T and C10991T) were examined in a family study of 111 probands and transmission frequency was not significantly associated with disease.[43] Weak but positive associations with SZ have been detected for three *MOG* microsatellites in a Han Chinese population.[44] Despite these inconclusive data, myelin as a structural axonal component restricting diffusion, have been indirectly implicated in studies detecting reduced diffusion parameters that represent the orientation and microstructural organization of white matter in SZ and BD.[45,46] In the human *MOG* gene (6p21.3) a missense variation in a SNP rs2857766 (511G>C, V142L, constitutively spliced exon-3 coding for a transmembrane segment of the MOG protein[47]) has been implicated as an independent MS susceptibility-modulating factor in the histocompatibility complex class I region, suggesting a possible role in structural degradation of myelin.[48] However, rs2857766 has not previously been examined for association with SZ or BD or with white matter abnormalities in psychosis.

CNP rs2070106. The 2′,3′-cyclic nucleotide 3′ phosphodiesterase enzyme (E.C. 3.1.4.37, CNPase) resides in the oligodendrocytes membrane sheath, catalyzes 2′,3′-nucleotides hydrolysis to form 2′-nucleotides and shows high activity in myelinated regions, reduced levels around plaques in the brain and elevated levels in cerebrospinal fluid during worsening periods in MS sufferers (for historical review see Vogel *et al.*[49]). It is involved in a signaling cascade responsible for an increase in the number and size of microtubule/CNPase-like structures among other changes and thereby the elongation of oligodendrocytes processes, and expansion of membrane sheaths and may thereby ultimately have a role in the assembly and/or maintenance of myelin.[50,51] In mice, the absence of CNPase results in axonal degradation but myelin assembly or the physical stability of myelin appears to be intact. These mice extend smaller outgrowths with less arborized processes[52] and such oligodendrocyte dysfunction may be sufficient to result in secondary axonal loss.[53] Reductions in CNPase levels in animal models have been associated with reduced learning ability.[49]

CNPase and in particular the SNP rs2071006 has been implicated in SZ by a number of types of evidence including case–control[54] and family[55] association, and postmortem gene expression studies but not consistently in all populations[56–58] and not by genome-wide analyses.[59–62] In addition, the risk-conferring allele is in question and this 'flip-flop' phenomenon[63] may be the result of another functionally linked locus acting together to contribute to disease, differences in linkage disequilibrium between the two populations studied or a false-positive in one or other study.[55] The *CNPase* gene SNP rs2071016 has been shown to be functional, affecting the expression of CNPase in a transcript-specific manner,[5] with the presence of the polymorphism (A-allele) predicting low expression of the gene[64] without resulting in an amino acid change in CNPase.[54] Accordingly, reduced *CNPase* gene expression is associated with the A-allele of rs2070106 in SZ (the more common allele in affected individuals[54]) in the dorsolateral prefrontal cortex,[6,39] anterior temporal lobe[41] and anterior cingulate cortex[65] but not in all studies.[64] In major depressive disorder (MDD), *CNPase* expression was reduced in the temporal lobes compared with controls.[66] In concordance with reduced *CNPase* expression, lower mRNA levels were reported in SZ, but not BD or MDD, compare with controls along with mRNA for GALC, MAG and MOG.[42] CNPase dysfunction appears to be regionally specific and certainly the anterior cingulate cortex is repeatedly implicated where both mRNA and protein expression have been reported to be reduced in contrast to the putamen where neither was altered in an elderly schizophrenic group.[67]

Given the evidence implicating each of these myelination genes in psychotic illness, this study aims to explore whether genotypic variation potentially conferring risk for psychosis

was associated *in vivo* with white matter volume deviation in three groups of individuals at varying levels of genetic risk for psychosis: in those individuals who actually developed psychotic illness, in their unaffected first-degree relatives who are likely to be carrying susceptibility genes for illness and in healthy volunteers.

Materials and methods

Participants. Patients affected with SZ or BDI and unaffected first-degree relatives of these individuals were recruited through voluntary support groups or through direct psychiatric referral. All members of the BDI group had additionally experienced at least one psychotic episode involving delusions and hallucination as described previously.[68] In addition, all patients had at least one first- or second-degree relative affected with a psychotic disorder while none of the healthy volunteers had a personal or family history of psychosis or as personal history of any other psychiatric disorder. Related pairs of unaffected SZ or BD relative were included to preserve power and findings qualified by examining the effect of removing related subjects *post hoc*. The healthy volunteers were recruited from the community through advertisements in local newspapers or from staff. All of the 189 subjects (Table 1) were aged 16–69 years, and their first language was English. The participants were excluded if they had experienced organic brain disease, head trauma resulting in loss of consciousness for >5 min, or DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) substance or alcohol dependence in the 12 months before assessment. The study was approved by the relevant Local Ethics Committees and informed written consent obtained. Structural MRI brain scans were obtained for 189 subjects (Table 1). The recruitment and assessment of these participants has previously been described in detail.[68,69] There was no overlap between the subjects included in this study and those included in our previous region-of-interest studies of families affected with SZ.[70,71]

Clinical assessments. Structured diagnostic interviews were performed on all subjects with the Schedule for Affective Disorders and SZ—Lifetime Version,[72] and additional clinical information was collected to enable lifetime DSM-IV diagnoses to be made. For relatives not assessed directly, information regarding psychiatric diagnoses was obtained from the most reliable informants with the Family Interview for Genetic Studies[73] and supplemented by medical notes where available. The patients were also assessed with the Positive and Negative Syndrome Scale (PANSS).[74] the schedule for schizotypal personalities[75] was used to assess nonpsychotic relatives and comparison subjects for schizotypal traits. Three unaffected SZ relatives and no other unaffected subjects reached criteria for schizotypal personality disorder.

MRI acquisition and analyses. Coronal 1.5-mm-thick contiguous T1-weighted MRI images of the entire brain were obtained by using a three-dimensional spoiled gradient recall echo sequence on a 1.5-T GE N/Vi Signa System scanner (General Electric, Milwaukee, WI, USA) and the following protocol: TR = 13.1, TI = 450 TE = 5.8 ms, number of excitations = 1, flip angle = 20° and acquisition matrix = 256 × 256 × 128. Images were processed for voxel-by-voxel-based analysis including reorientation to align images along the anterior commissure–posterior commissure axis in the sagittal plane and along the interhemispheric fissure in the coronal and axial planes using an automated reorientation matlab script by Carlton Chu and SPM5.[76] Tissue class segmentation and smoothing to a full-width half-maximum kernel of 8 mm^3 as performed using SPM 5.[76] The resulting white matter images were grouped into two genotype-based groups, (Table 1) and voxel-based analysis using the flexible factorial design and a single covariate to account for age-based changes in white matter was performed. Analyses involved a relative threshold that discarded the lower 10% of voxel intensities and a classical model. Modulation was performed and age covaried for, therefore the outcome parameter used refers to relative white matter volume changes that cannot be explained by age.[77,78]

Genotyping. The SNPs, *MOG* rs2857766 and *CNP* rs2070106, were genotyped using KBiosciences (http://www.kbioscience.co.uk), with a competitive allele-specific PCR system. As described in Williams *et al*.,[79] SNP8NRG221533 was genotyped using the primer extension SNuPe and the genotyping platform Megabace (Amersham Bioscience, Buckinghamshire, UK), and the microsatellites were genotyped using a fluorescently labeled primer PCR assay, and were analyzed by the ABI 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA). As defined by Stefansson *et al*.,[10,11] the core NRG1 at-risk haplotype (the HAP_{ICE} haplotype) consists of one SNP marker (SNP8NGR221533) and two microsatellites (478 B14-848 and 420 M9-1395).

Statistics. The Hardy–Weinberg equilibrium was calculated using Haploview version 4.1[80] (Table 2). Analyses of the results of the voxel-based analysis included using T-contrasts between the two allele frequency-based genotype groups (Table 2) within each diagnostic group covarying for age. A height threshold of $T = 2.35$ (0.05/5, $P < 0.01$) was used for each T-contrast to correct for that comparison within each of five diagnostic groups in addition to voxel-based corrections for multiple comparisons using random field theory.[81] Clusters with a corrected P-value < 0.0125 (0.05/4) were reported to correct for comparison across four genotypes. Pearson's χ^2 test was performed to examine the frequency of distribution of gender across genotype groups. Non-parametric Mann–Whitney U-tests were used to confirm that PANSS total score and the age at symptoms onset did not differ between genotype groups within each diagnostic group in these three significant findings (Table 3). Statistical tests were carried out using SPSS version 15 (http://www.spss.com).

Results

In total, 70 individuals with a psychotic disorder (37 SZ and 33 BDI) and 119 unaffected subjects (39 HC, 40 with a relative with SZ, 40 with a relative with BDI) were studied. Of the

Table 1 Demographic description of the participants for each pair of genotype groups compared

	HC		*SZ*		*SZrel*		*BD*		*BDrel*	
NRG rs35753505	**C-carriers**	**TT**	**C-carriers**	**TT**	**C-carriers**	**TT**	**C-carriers**	**TT**	**C-carriers**	**TT**
n	27	10	25	8	27	9	18	9	22	17
% Female (*n*)	41 (11)	80 (8)	20 (5)	13 (1)	48 (13)	56 (5)	61 (11)	67 (6)	55 (12)	41 (7)
Mean age ± s.d.	40 ± 15	44 ± 15	37 ± 9	33 ± 7	50 ± 14	51 ± 12	42 ± 11	37 ± 11	41 ± 17	46 ± 15
PANSS total (mean ± s.d.)	0 ± 0.7	0	25 ± 12	28 ± 9	1 ± 2	1 ± 2	5 ± 5	5 ± 5	1 ± 1	1 ± 2
Current exposure[a]										
Medication free	27	10	0	0	27	9	3	0	21	16
Atypical APs	0	0	18	6	0	0	3	2	0	0
Other APs	0	0	5	3	0	0	1	0	0	0
Mood stabilizers	0	0	2	1	0	0	14	8	0	0
SSRIs	0	0	6	0	0	0	4	4	1	0
Other Ads	0	0	4	3	0	0	0	1	0	1
HAP_{ICE} haplotype	Arh0	Arh1	Arh0	Arh1	Arh0	Arh1	Arh0	Arh1	Arh0	Arh1
n	26	11	22	11	24	10	23	8	30	10
% Female (*n*)	58 (15)	36 (4)	23 (5)	18 (2)	50 (12)	70 (7)	61 (14)	63 (5)	47 (14)	60 (6)
Mean age ± s.d.	43 ± 16	36 ± 13	35 ± 9	36 ± 8	50 ± 13	52 ± 16	41 ± 12	44 ± 11	42 ± 16	48 ± 17
PANSS total (mean ± s.d.)	0 ± 0.5	0 ± 0.6	26 ± 9	26 ± 15	1 ± 2	1 ± 2	5 ± 4	7 ± 7	1 ± 2	1 ± 2
Current exposure[a]										
Medication free	26	11	0	0	24	10	3	0	27	9
Atypical APs	0	0	15	9	0	0	4	2	0	0
Other APs	0	0	7	1	0	0	4	3	0	0
Mood stabilizers	0	0	2	1	0	0	18	7	0	0
SSRIs	0	0	3	4	0	0	7	3	1	0
Other Ads	0	0	6	1	0	0	4	0	1	1
MOG rs2857766	GG	C-carriers	GG	C-carriers	GG	C-carriers	GG	C-carriers	GG	C-carriers
n	21	15	21	12	20	10	19	11	28	11
% Female (*n*)	48 (10)	47 (7)	29 (6)	17 (2)	65 (13)	40 (4)	68 (13)	55 (6)	61 (17)	27 (3)
Mean age ± s.d.	45 ± 12	34 ± 16	35 ± 9	36 ± 9	51 ± 13	43 ± 17	41 ± 12	40 ± 10	46 ± 15	38 ± 18
PANSS total (mean ± s.d.)	0 ± 0.7	0 ± 0.5	27 ± 10	21 ± 14	2 ± 3	0 ± 0.3	6 ± 5	6 ± 6	1 ± 2	1 ± 1
Current exposure[a]										
Medication free	21	15	0	0	20	10	4	0	25	11
Atypical APs	0	0	13	11	0	0	5	3	0	0
Other APs	0	0	7	1	0	0	0	0	0	0
Mood stabilizers	0	0	3	0	0	0	15	10	0	0
SSRIs	0	0	1	5	0	0	6	3	1	0
Other Ads	0	0	6	1	0	0	2	1	2	0
CNP rs2070106	GG	A-carriers	GG	A-carriers	GG	A-carriers	GG	A-carriers	GG	A-carriers
n	21	16	7	21	9	24	16	14	12	24
% Female (*n*)	43 (9)	50 (8)	29 (2)	24 (5)	89 (8)	46 (11)	63 (10)	64 (9)	75 (9)	38 (9)
Mean age ± s.d.	39 ± 14	45 ± 17	42 ± 8	35 ± 9	53 ± 14	49 ± 14	40 ± 12	41 ± 12	42 ± 17	44 ± 16
PANSS total (mean ± s.d.)	0 ± 0.5	0 ± 0.9	25 ± 6	24 ± 13	1 ± 2	1 ± 2	7 ± 6	4 ± 4	1 ± 3	1 ± 1
Current exposure[a]										
Medication free	21	16	0	0	9	24	1	3	12	21
Atypical APs	0	0	3	17	0	0	5	1	0	0
Other APs	0	0	3	3	0	0	1	0	0	0
Mood stabilizers	0	0	1	2	0	0	14	11	0	0
SSRIs	0	0	2	4	0	0	5	4	0	1
Other Ads	0	0	1	5	0	0	2	0	0	2

Abbreviations: AD, antidepressant; AP, antipsychotic; LSD, lysergic acid diethylamide; MOG, myelin oligodendrocytes glycoprotein; PANSS, Positive and Negative Syndrome Scale; SSRI, selective serotonin reuptake inhibitor.
[a]Dependence constituted an exclusion criteria for the study, this item refers to regular use at some point in their lives meeting criteria for abuse.

37 individuals in the SZ group, 3 had schizoaffective disorder, 16 were undifferentiated, 17 of the paranoid and 1 of the disorganized subtype and none were related to any member of the BD group. There was no significant difference in gender distribution between the genotype or haplotype groups within any of the diagnostic groups with three exceptions. In NRG8SNP221533, the HC group has a lower proportion of males in the TT-group ($n=2$). In *MOG* rs2857766, the unaffected group with a BD relative had a lower proportion of females among the C-carriers group ($n=3$) compared with the GG-genotype group. In the *CNP* rs2070106, GG-genotype had a lower proportion of males ($n=1$ and $n=3$) among the unaffected individuals with a SZ relative and those with a BD relative, respectively, compared with the A-carriers. The SZ and BD groups had significantly greater total PANSS scores than the three unaffected groups. At time of scanning a number of members of the SZ and BD groups were receiving medication (Table 1).

***NRG1* rs35753505 (NRG8SNP221533).** No effect of the NRG8SNP221533 genotype was detected in the HC group, nor among unaffected individuals with a relative with a psychotic disorder on white matter volume. In the SZ group, the NRG8SNP221533 risk allele, C-carrier genotype was associated with reduced white matter volume relative to the TT-genotype group in the region of the right inferior longitudinal fasciculus, ALIC (partially overlapping the anterior thalamic radiations and corticopontine tracts) and right

uncinate fasciculus (Figure 1). In the BDI-group, the risk allele C-carriers was associated with greater white matter relative to those of the TT-genotype in several regions including the cingulum/parahippocampal gyrus (Figure 1b) and the callosal body. The PANSS total, the age at symptom onset nor the presence of atypical antipsychotic medication in the SZ group differed significantly between C-carriers and the T-homozygotes. Similarly, in the BDI group C-carriers and T-homozygotes did not differ significantly on PANSS total, the age at symptom onset not the presence of mood stabilizing medication.

***HAP*$_{ICE}$ haplotype.** There were no significant differences in white matter volume between groups possessing one or two copies of the *HAP*$_{ICE}$ haplotype versus having no copies in the HC or SZ groups or in the unaffected group with a BD relative. Those unaffected individuals with a relative with SZ and one or two copies showed a trend toward lower white matter volume (29–33%) in the superior cerebellar peduncle and the fornix relative to those with no copies. Removing all related individuals (25–46% lower) or removing one of each pair of related participants (preferring first the minor allele and then the first recruited subject, 22–28% lower) did not change the magnitude or direction of this finding. In the BDI group those carrying one or two copies of the haplotype had greater white matter than those carrying none in the fornix, caudate and cingulum (Figure 1c). The PANSS total, the age at symptom onset nor the presence of mood stabilizing or antidepressant medication in the BDI-group differed significantly between those having no copies versus those with one or two copies of the arh haplotype.

Table 2 Sample size for each genotype or haplotype investigated

Genotype/ haplotype sample size	*HC*	*SZ*	*SZrel*	*BD*	*BDrel*	*Total*	χ^2 (P)	*HWp*
NRG1 rs35753505								
C-carriers	27	25	27	18	22	119		
TT	10	8	9	9	17	53	4.5 (0.34)	0.24
HAP$_{ICE}$ *haplotype*								
Arh0	26	22	24	23	30	125		
Arh1	11	11	10	8	10	50	0.77 (0.94)	NA
MOG rs2857766								
GG	21	21	20	19	28	109		
C-carriers	15	12	10	11	11	59	1.59 (0.81)	0.26
CNP rs2070106								
GG	21	7	9	16	12	65		
A-carriers	16	21	24	14	24	99	12.1 (0.017)	1.00
Total number of subjects	39	37	40	33	40	189		

Abbreviations: MOG, myelin oligodendrocytes glycoprotein; NA, not applicable; NRG, neuregulin.

***MOG* rs2857766.** The genotypic frequency for rs2857766 among the sample of 168 Europeans was 0.65 for G-homozygotes and 0.33 for C-carriers. Consistent with the frequency previously reported for C-homozygotes (0.086), we detected a total of three C-homozygotes (0.018). Among Europeans, the genotypic frequency is reported to be 0.483, 0.431 and 0.086 for the G-homozygotes, heterozygotes and C-homozygotes, respectively. Healthy controls who were G-homozygotes of the *MOG* SNP rs2857766 had greater white matter volume in the middle and inferior cerebellar peduncles and in medulla-level corticopontine/corticospinal tracts relative to C-carriers (Figure 1d). In contrast, the BD or SZ group, or in the unaffected individuals with a SZ or BD relative, no white matter differences were detected between the G-homozygotes and C-carriers of the *MOG* SNP rs2857766.

***CNP* rs2070106.** None of the groups showed any significant differences in white matter across the CNP rs2070106 genotype.

Discussion

The major finding from this study is the association between genotypic variation in NRG, previously linked to psychotic

Table 3 White matter variation across genotype or haplotype detected by voxel-based analysis

Genotype, group and direction	*Maxima (MNI, T)*	*Cluster* K_E (P)
NRG SNP8NRG221533		
BDI c-carriers > tt		
Cingulum/PHG, splenium and anterior CC	−16 −12 −28 (4.66), 18 −42 14 (4.34), 20 26 36 (4.32)	17862 (0.0001)
SZ c-carriers < tt		
Right UF, right ILF, right ALIC (atr, cpt)	12 14 −16 (3.61), 38 −16 −2 (3.54), 22 4 −10 (3.52)	5004 (0.0001)
HAP$_{ICE}$ *haplotype*		
Unaffected individuals with a relative with SZ arh0 > arh1		
SCP and fornix	−2 −34 −4 (3.48), −12 −48 −18 (3.46), 6 −28 14 (3.26)	903 (0.006)
BDI arh0 < arh1		
Fornix, caudate, posterior cingulum bundle	24 −42 8 (4.34), −8 6 12 (4.23), 28 −40 −10 (3.14)	1625 (0.0001)
MOG rs2857766		
Healthy controls gg > c-carriers		
Corticopontine/spinal tract, MCP, ICP	6 −28 −40 (3.46), 12 −44 −50 (3.34), 6 −52 −52 (3.25)	1305 (0.001)

Abbreviations: ALIC, anterior limb of the internal capsule; BDI, bipolar I disorder; ICP, inferior cerebellar peduncle; ILF, inferior longitudinal fasciculus; MCP, middle cerebellar peduncle; NRG1, neuregulin 1; PHG, parahippocampal gyrus; SCP, superior cerebellar peduncle; SZ, schizophrenia; UF, uncinate fasciculus.
K_E is the cluster size (number of voxels) and the *P*-value refers to the cluster level-corrected *P* value (cutoff $P<0.0125$).

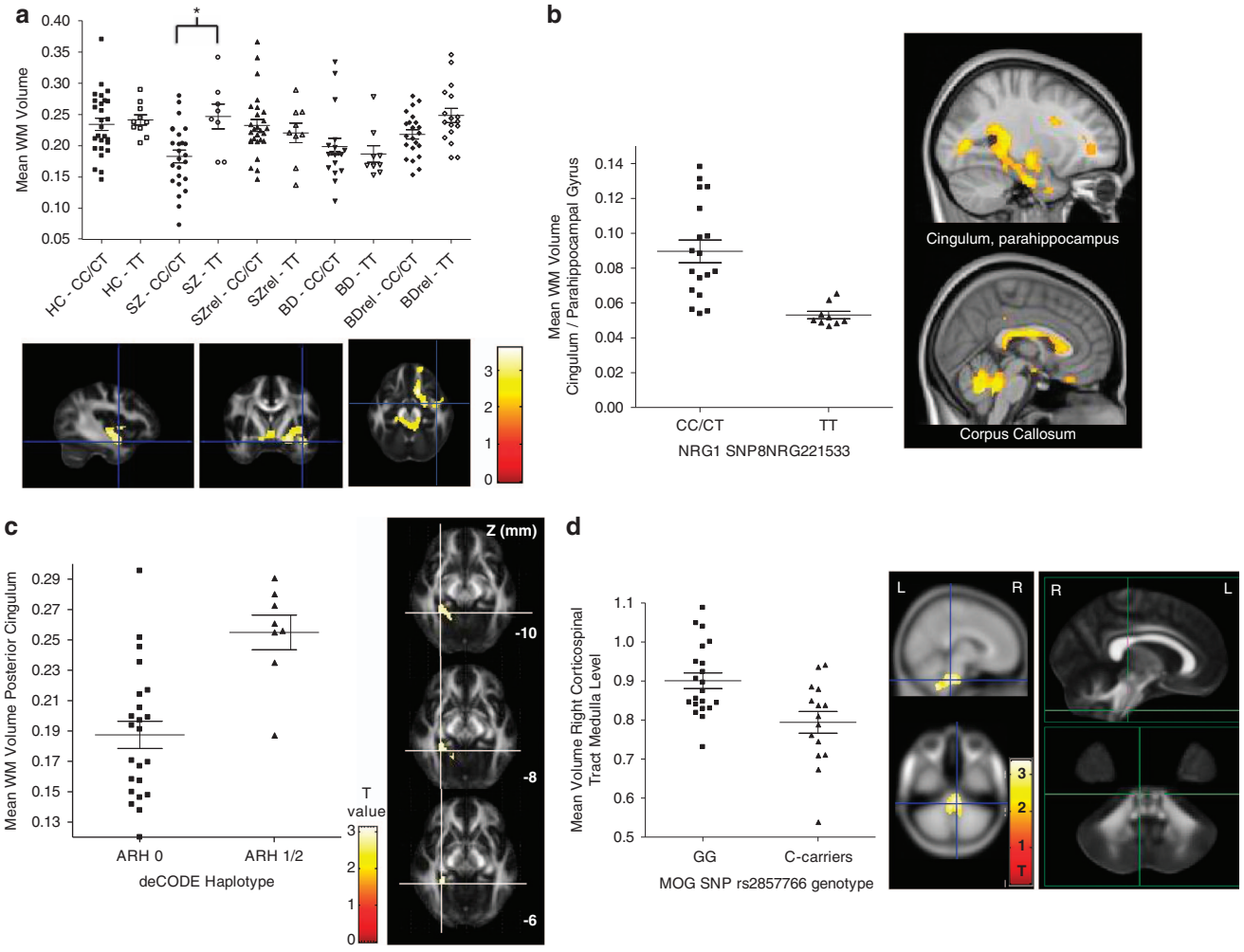

Figure 1 The association of white matter (WM) volume in psychotic disorders with genotypic variation in neuregulin 1 (NRG1) and myelin oligodendrocytes glycoprotein (MOG). (**a**) C-allele carrier genotype of the *NRG1* single-nucleotide polymorphism (SNP), SNP8NRG221533 is associated with lower white matter volume in the region of the uncinate fasciculus (UF) in patients with schizophrenia (SZ). Scatter plot of mean (±s.e.m.) white matter volume across the TT versus C-carriers genotype groups in all five diagnostic categories in a sphere of 2 mm radius centered on the cluster peak (12 14 −16) implicating the UF in SZ. Beneath is the cluster T-map overlaid on sections of the MNI152 T1 template. (**b**). The risk genotype (TT) of the *NRG1* SNP8NRG221533 and bipolar I disorder (BD) is associated with reduced white matter volume in the posterior inferior portion of the cingulum, parahippocampal gyrus and in the callosal body. Scatter plot of mean (±s.e.m.) white matter volume in a sphere of 2 mm radius centered on the posterior cingulum/parahippocampal gyrus cluster peak (−16 −12 −28). Top right illustrates the cluster extent and magnitude on a T-map, bottom right, the callosal body cluster effect for the finding in the same group and direction. (**c**). BD and having the HAP_{ICE} haplotype is associated with greater white matter volume in the posterior portion of the cingulum bundle. Scatter plot of mean (±s.e.m.) white matter volume in a sphere of 2 mm radius centered on the cluster peak (28 −40 −10) and adjacent, the cluster T-map overlaid on slices (Z −10 to −6 mm) of the functional MRI (fMRI)B58 FA map. (**d**). Psychiatrically healthy controls who are G-homozygotes of the *MOG* SNP rs2857766 have greater corticospinal tract white matter volume at the level of the medulla relative to C-carriers. Scatter plot of mean (±s.e.m.) white matter volume in a sphere of 2 mm radius centered on the cluster peak (6 −28 −40), middle panel illustrated the cluster on a statistical T-map. The cluster peak is at the level of the medulla in the right corticopontine/corticospinal tract and this is indicated by the crosshairs on sagittal and axial views of the fMRIBs mean FA map (FMRIB58_FA). L, left; R, right.

illness, and white matter abnormalities in psychotic patients and their relatives. Individuals with SZ and carrying the risk allele C, of the *NRG1* SNP8NRG221533, had lower white matter volume in the regions of the right uncinate fasciculus, right inferior longitudinal fasciculus and in the ALIC (overlapping portions of the anterior thalamic radiations and the corticopontine tracts). Unaffected relatives of patients with SZ possessing at least one copy of the HAP_{ICE} haplotype had less white matter volume in the fornix and superior cerebellar peduncle compared with those with no copies. In contrast, those BD1 patients carrying the NRG risk allele C of SNP8NRG221533 had greater white matter volume in the posterior cingulum/parahippocampal gyrus and a number of regions of the corpus callosum. Similarly, individuals with BDI and at least one copy of the HAP_{ICE} haplotype, which includes the risk allele of the *NRG1* SNP8NRG221533, possessed greater white matter volume in the cingulum and fornix.

There was no association between white matter volume and genotypic variation in SNP8NRG221533 in healthy volunteers, consistent with the study by Winterer *et al* [34] using a similar voxel-based approach (optimized VBM and SPM5). However,

Winterer *et al.*[34] did detect reduced medial frontal FA using diffusion tensor imaging in C-carriers of the SNP8NRG221533. McIntosh *et al.*[36] have additionally reported reduced white matter density and FA in the ALIC associated with the TT-genotype of another *NRG1* SNP in the promoter region of the gene (SNP8NRG243177). Finally, the *NRG1* gene SNP and exact population studied herein has been examined previously for contribution to variance in the volume of the lateral ventricles or hippocampus and no relationship was detected.[82] The present finding implicates the *NRG1* gene SNP, SNP8NRG221533 in white matter-related abnormalities of the emotion circuitry in BD,[83] and in previously implicated regions in SZ including the ALIC. It is not clear why in contrast to the SZ group, the SZ risk genotype is associated with increased white matter in the cingulum and corpus callosum in the BDI group. These data suggest the possibility that another currently unknown factor associated with having BDI may be involved in modulating the relationship between the *NRG1* SNP genotype and white matter volume. In addition, long-term antipsychotic medication exposure may mediate structural changes as has been recently demonstrated.[84] Thus, it remains possible that in the SZ group, an as yet unidentified, interaction between genotype and medication response may contribute to the present findings.

Several models of the neurocircuitry underlying psychiatric disorders have been developed many based on structural and functional evidence from *in vivo* neuroimaging studies. These and similar recent studies[33,36,37,85–87] substantiate a growing body of evidence implicating genetic susceptibility to developing abnormalities within and between (white matter volume and microstructural organization) emotion-related structures. These studies collectively contribute to progress toward the inevitable future clinical application of such *in vivo* knowledge, which may include earlier detection of susceptibility, monitoring progression, presaging treatment response and potentially diagnosing based classification.[88]

It is not known whether *MOG* rs2857766 confers risk for SZ or BD and, in our study we found no relationship between genotype and white matter volume in the brain in these groups. However, we provide preliminary evidence suggesting a possible relationship between G-homozygosity (the ancestral allele) in *MOG* rs2857766 and greater brainstem level white matter volume in psychiatrically healthy individuals that was not detected in psychotic patients or their unaffected relatives. The uniformity of microstructural organization of white matter in this region, the middle cerebellar peduncle has been reported to be disrupted in SZ using diffusion-weighted imaging.[89] However, there is insufficient evidence to directly implicate *MOG* rs2857766 in disruption of the middle cerebellar peduncle.

Despite previous reports of the involvement of *CNPase* SNP rs2070106 in SZ and BD, this study did not detect any relationship between white matter volume and genetic variation in this SNP in any patient or relative group examined or among the healthy controls.

Individual SNPs are unlikely to confer more than a relatively minor proportion of variation in white matter volume, however, such associations may serve to highlight biological processes that are involved in dysfunctional white matter circuits and point to other genetic variants or functional units that are tightly linked to such pathophysiology and that warrant further exploration. A limitation of this study is the small number of subjects in some of the genotype groups and it is possible we were underpowered to detect relationships with white matter that may be detected in the future with more sensitive technology, analysis methods and larger cohorts of subjects. In addition, the inclusion of a related pairs of individuals within the unaffected SZ and BD relative groups to preserve power represents a limitation of this study. Despite their inclusion, however, no positive finding was detected for any genotype examined in the unaffected BD group or for three of the four genotypes examined in the case of the unaffected SZ group. In the unaffected SZ group, those having one or two copies of the HAP_{ICE} haplotype showed a trend toward lower white matter volume in the superior cerebellar peduncle and the fornix relative to those with no copies. Removing all related individuals from this group did not significantly alter the magnitude or direction of this finding. A particular limitation of the voxel-based approach to image analysis is the possibility of errors due to registration and segmentation steps.[77] To minimize these possible sources of error, registration and segmentation performance were visually assessed on an individual basis. A particular strength of this study was the inclusion of subjects who were unaffected but had a relative with a psychotic disorder. In the case of the *MOG* SNP examined, the absence in all affected groups and their relatives of the relationship detected in healthy controls suggests a possible pathological role for this SNP in psychotic disorders. Future linkage, case–control, family and genome-wide studies may further elucidate the role of this *MOG* SNP in psychosis.

In summary, these findings provide support for the theory that genotypic variation in neuregulin may confer risk for psychosis by influencing white matter in areas known to underlie the emotional circuitry of the human brain.[83] The deficits in white matter volume detected in this study have a number of possible underlying explanations including reduced axonal projections or a reduction in the volume of non-axonal white matter components including glia. An optimum ratio of axonal diameter to myelin sheath thickness termed the g-ratio has been described in terms of efficiency of conductivity.[90,91] It is plausible that regional perturbations of this ratio may manifest as signs and symptoms associated with psychotic disorders.[92] Volume changes in white matter are inherently limited in forming the nature or identity of molecular factors involved in abnormalities detected, and future studies examining the uniformity of microstructural organization of white matter using diffusion-weighted MR imaging with genotype among other approaches will aid in the identification of the mechanism by which the NRG risk allele contributes to the signs and symptoms of psychotic disorders.

Conflict of interest

The authors declare no conflict of interest.

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