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# Expression of Oligodendrocyte-Associated Genes in Dorsolateral Prefrontal Cortex of Patients with Schizophrenia

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## Abstract

Prior studies have found decreased mRNA expression of oligodendrocyte-associated genes in the dorsolateral prefrontal cortex (DLPFC) of patients with schizophrenia. However, it is unclear which specific genes are affected and whether the changes occur in the cortical white or grey matter. We assessed the mRNA expression levels of four oligodendrocyte-related genes: myelin-associated basic protein (MOBP), myelin-associated glycoprotein (MAG), 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) and oligodendrocyte-lineage transcription factor 2 (OLIG2) in DLPFC white and grey matter using quantitative-PCR (~70 controls and ~30 patients with schizophrenia). We also examined the effects of high-risk polymorphisms in CNP and OLIG2 on mRNA levels of these genes. We found that genetic polymorphisms in CNP (rs2070106) and OLIG2 (rs1059004 and rs9653711), previously associated with schizophrenia, predicted low expression of these genes. Expression of MAG, CNP and OLIG2 did not differ between patients with schizophrenia and controls in the grey or white matter but MOBP mRNA levels were increased in the DLPFC white matter in patients with a history of substance abuse. MOBP and CNP protein in the white matter was not altered. Although previously reported reductions in the expression of myelin-related genes in the DLPFC were not detected, we show that individuals carrying risk-associated alleles in oligodendrocyte-related genes had relatively lower transcript levels. These data illustrate the importance of genetic background in gene expression studies in schizophrenia.

### Keywords

schizophrenia; myelin; oligodendrocytes; MOBP; CNP; OLIG2; postmortem studies

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Drs. Hyde and Kleinman were involved with procurement of brain tissue, postmortem clinical characterization and manuscript revisions
Drs. Vakkalanka and Kolachana provided genotype data

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## Introduction

Recent studies using various approaches have investigated the hypothesis that oligodendrocyte function and myelination are disrupted in schizophrenia (Davis & Haroutunian 2003, Davis et al. 2003, Kubicki et al. 2005, McInnes & Lauriat 2006, Dwork et al. 2007). The most replicable findings in the post-mortem brain tissue were reduced expression of MAG and CNP (Table 1). However, the same myelin-associated genes were not consistently altered across various studies, although most used tissue from the same two brain collections, Stanley Consortium and Mount Sinai. Two studies assessed expression of oligodendrocyte-related genes separately in the white matter and both found a reduction in CNP in the tissue of patients with schizophrenia (Parbakaran et al. 2004, McCullumsmith et al. 2007) (Table 1). There are also several association studies of polymorphisms in oligodendrocyte-related genes with schizophrenia. MOG, MAG, PLP1, NOGO and CNP have been found to be associated with the disease in certain populations, but not in others (Table 2). Interestingly, a risk-associated allele also predicted low expression of CNP mRNA in the brain tissue of normal controls (Peirce et al. 2006).

To investigate the hypothesis that oligodendrocyte function is altered in schizophrenia, we examined mRNA expression of MOBP, CNP, MAG and OLIG2 genes in the post-mortem DLPFC from the CBDB/NIMH brain collection, a cohort of relatively young patients and normal controls (Lipska et al. 2006) separately in the white and grey matter. Since recent reports have identified allelic variations in CNP and OLIG2, showing associations with the disease (Pierce et al. 2006, Georgieva et al. 2006), we investigated whether the expression of CNP and OLIG2 is predicted by these polymorphic variants.

## Materials and Methods

### Human post mortem tissue

Postmortem dorsolateral prefrontal cortex (DLPFC) grey and white matter tissue samples were collected at the Clinical Brain Disorders Branch, NIMH (Table 3). Grey matter was removed from a 1.5cm thick coronal slab of the frontal cortex anterior to the corpus callosum and the DLPFC dissected using a dental drill according to the guidelines described by Rajkowska et al. 1995. White matter was dissected from the region just below the superior and middle frontal gyri, adjacent to the cortical grey matter ribbon. A smaller subgroup of 47 controls and 26 patients was used for protein studies in the white matter.

All brain tissue used in this study was obtained with informed consent from the legal next of kin under NIMH protocol #90-M-0142 (Lipska et al. 2006). Normal controls had no known history of psychiatric symptoms or substance abuse and negative toxicological results. Diagnoses of patients with schizophrenia were determined using DSM-IV criteria. Total daily, lifetime and last dose of neuroleptic medication was calculated for each schizophrenic subject and converted to chlorpromazine equivalents (as described in Lipska et al. 2006). Smoking and substance abuse history was also recorded. Toxicological analysis of blood, vitreous humor fluid, occipital pole and/or urine was conducted for every control and schizophrenia case. Non-psychiatric cases with toxicology screenings positive for ethanol (blood levels greater than 0.05g/dL; American Medical Laboratory Inc.) or positive for any over-the counter or prescription medication above therapeutic levels or any detectable level of illicit drugs were excluded from the control group. Positive toxicology was not an exclusion criterion for schizophrenic cases.

### RNA extraction and reverse transcription

Tissue was pulverized and stored at −80°C. Total RNA was extracted from 300 mg of tissue using TRIZOL Reagent (Life Technologies Inc., Grand Island, NY, USA). RNA from the DLPFC white matter was isolated with TRIZOL and purified using RNeasy spin columns (Qiagen Inc.). The yield of total RNA was determined by absorbance at 260 nm. RNA quality was assessed with a high resolution capillary electrophoresis (Agilent Technologies, Palo Alto, CA, USA). Total RNA (4 μg) was used in 50 μl of reverse transcriptase reaction to synthesize cDNA, by using a SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA).

### Q-PCR Probes and Primers

Taqman probes/primer sets from Applied Biosystems were used for MOBP (Hs00379220_m1), CNP (Hs00263981_m1, which recognizes both CNP isoforms), MAG (Hs00159000_m1) and OLIG2 (Hs00377820_m1) as well as for three endogenous housekeeping genes, beta-actin (Hs99999903_m1), β2-microglobulin (B2M) (Hs99999907_m1) and β-glucuronidase (GUSB) (Hs99999908_m1).

### Quantitative Real-time PCR

Expression levels of mRNAs were measured by quantitative real-time PCR, using ABI Assays-on-Demand (see above) and an ABI Prism 7900 sequence detection system with 384-well format (Applied Biosystems, Foster City, CA, USA). Each 10 μl reaction contained 900 nM of primer, 250 nM of probe and Taqman Universal PCR Mastermix (Applied Biosystems) containing Hot Goldstar DNA Polymerase, dNTPs with dUTP, uracil-N-glycosylase, passive reference and 100 ng of cDNA template. PCR cycle parameters were 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15s, and 59°C or 60°C for 1 min. PCR data were acquired from the Sequence Detector Software (SDS version 2.0, Applied Biosystems) and quantified by a standard curve method using serial dilutions of pooled cDNA derived from RNA obtained from DLPFC grey or white matter of 10-12 normal control subjects. In each experiment the $R^2$ value of the curve was more than 0.99, the amplification efficiency 96 – 101 % and no-template cDNA controls gave no detectable signal. All measurements were performed in triplicate and the gene expression levels calculated as an average of the three samples. Data analysis was based on normalization of target mRNAs to three endogenous control genes (a geometric mean of three genes = normalizing factor, NF).

### Immunoblotting

Human DLPFC white matter tissue samples (100-130 mg; 1 g tissue : 10 mL buffer) were homogenized in a protease inhibitor-Tris-glycerol extraction buffer (AEBSF 0.024%, aprotinin 0.005%, leupeptin 0.001%, pepstatin A 0.001%, glycerol 50%, Tris 0.6%). Protein concentration was determined using the Bradford assay. Homogenates (4 μg of total protein/ 10 μl) were diluted with water, XT sample buffer and 1X antioxidant (Bio-Rad Laboratories Inc, Hercules, CA), and heated to 80°C for 5 minutes. Ten μl of sample was loaded onto precast 10% Bis-Tris polyacrylamide gels (Bio-Rad Laboratories Inc, Hercules, CA), and proteins were separated by electrophoresis at 200 V for one hour. Each gel contained a molecular weight marker ladder SeeBlue Plus 2 (Invitrogen, Carlsbad, CA), a pooled sample from 10 normal controls at three concentrations (2, 4, 6 μg of total protein content per 10 μl), and samples from patients with schizophrenia and controls. A total of four separate gels were used in one experiment (n = 73 samples). Gels were transferred onto nitrocellulose membranes at 85 V for thirty minutes, membranes blocked for an hour in 10% goat serum in Tris buffered saline (TBS) with 0.1% Tween-20 (TBS-T), and incubated with primary antibodies. Anti-CNP antibody (Chemicon International Inc, Temecula CA, 1:10,000 dilution), anti-MOBP (Santa Cruz Biotechnology Inc., Santa Cruz, CA, 1: 2000 dilution) and anti-GAPDH (Chemicon

International Inc, Temecula CA,1:1,000 dilution) and anti-actin (Chemicon International Inc, Temecula CA, 1:10,000 dilution) antibodies were used. Blots were rinsed in TBS-T, incubated in a peroxidase-conjugated mouse secondary antibody (1:20,000 dilution, Chemicon International Inc, Temecula, CA) for 2 hours in 10% normal goat serum in TBS-T, rinsed in TBS-T and developed in ECL-plus (Amersham). The values were expressed as ratios to housekeeping proteins.

### CNP and OLIG2 genotype determination

DNA for genotyping was extracted from cerebellar tissue using standard methods. Based on previous reports of association, four SNPs in CNP (rs2070106, rs11079028, rs11296, rs4796751) and four in OLIG2 (rs2834070, rs762178, rs1059004, rs9653711) were genotyped in all subjects. Only the exonic CNP SNP rs2070106 has been associated with schizophrenia and with reduced expression of CNP mRNA in a Caucasian population (Peirce et al. 2006). All four OLIG2 SNPs chosen for genotyping have been previously associated with schizophrenia (Georgieva et al. 2006). Each PCR reaction contained 10 ng of DNA, 1 μM of each primer, 100 nM of each probe and 5 μl of Taqman Universal PCR Mastermix (Applied Biosystems) containing AmpliTaq Gold DNA polymerase, AmpErase UNG, dNTPs, with dUTP passive reference and optimized buffer components. The PCR cycling conditions were at 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15s, and 60°C for 1 min. Genotype reproducibility was routinely assessed by re-genotyping all samples for selected SNPs and was generally >99%.

### Statistical Analyses

Statistical analyses were conducted using Statistica version 7.1. www.statsoft.com. Multiple regression analyses were used for determining the contribution of demographic, tissue-and disease-related variables to gene expression levels. Comparisons between diagnostic groups were made using ANCOVA for each mRNA with diagnosis as an independent variable and potentially confounding variables as covariates. The same three genes (B2M, β-actin and GUSB) were used for normalization of the data in the white and grey matter of the DLPFC. For immunoreactivity, the data were normalized to actin or GAPDH. Effects of genotype on gene expression were examined using ANCOVA with genotype and race or diagnosis as independent variables. Effects of race were restricted to analyses of African American and Caucasian individuals due to the small sample size in other ethnic groups. For all SNPs examined in this study, the observed genotype frequencies were within expected distribution according to Hardy-Weinberg equilibrium (evaluated by the chi-squared test, p values > 0.05). Because for some DNA samples, an unequivocal assignment of genotype was not possible, the subjects with missing genotypes were not included in the genotypic analysis. In the entire sample, we eliminated outliers (points > ±2 SD from the mean) for each expression variable (n=0-3).

## Results

### Analysis of mRNA Expression

RNA quality (RIN) significantly influenced the non-normalized expression levels of all four genes, MOBP, CNP, MAG and OLIG2 in the white matter as well as the normalizing factors (NF) in the white and grey matter of the DLPFC (all p values < 0.01, Table 4). Importantly, normalization of the data appeared to correct for RNA quality as there was no significant effect of RIN on normalized levels of any of the target genes. PMI and pH had a lesser impact, weakly affecting only raw expression levels of MAG and OLIG2 in the grey matter. Although PMI did not affect expression of non-normalized MOBP levels or the NF, it was found to be a significant variable in the normalized MOBP expression in the white matter (p=0.02). Increasing age predicted lower expression of all four genes in the DLPFC white matter (Table

4). No other tissue-related or demographic parameters had a significant effect on the expression of myelin-related genes in the DLPFC.

MOBP mRNA expression in the white matter of the DLPFC was modestly increased in patients with schizophrenia as compared with controls (by 16%, t-value = 2.42, p = 0.017, Fig. 1). This difference remained significant after the data were co-varied by age, sex, smoking, agonal state, pH, PMI and RNA quality ($F(1,93) = 3.84$, $p = 0.03$). To check whether an increase in MOBP mRNA in patients with schizophrenia was related to other factors than the disease itself, such as antipsychotic medication or drug abuse, we performed multiple regression analysis, which revealed that a history of substance abuse significantly predicted MOBP expression levels in the DLPFC white matter of schizophrenia patients (standardized regression beta coefficient = 0.497, $p = 0.025$, Fig. 2). Further analysis revealed that toxicology-positive (n=8) and toxicology-negative (n=7) schizophrenia patients, who all had history of substance abuse, did not differ in MOBP mRNA levels ($p=0.42$, data not shown), suggesting that history-based drug abuse was the main determinant of high MOBP expression in this study. Other parameters included in the analysis (age at the onset of the disease, age at first hospitalization, duration of illness, estimated daily, life and last dose of neuroleptics) had no effect on MOBP expression in patients. In the DLPFC grey matter, there was no difference in MOBP mRNA levels between patients with schizophrenia and normal controls (Fig. 1) and, in contrast to the white matter, there was no effect of substance abuse. Expression of other genes, CNP, MAG and OLIG2, in the DLPFC white and grey matter did not show significant differences between patients with schizophrenia and unaffected controls either without (t values < 1.5, p values > 0.1) or with co-varying for potentially confounding factors, including substance abuse (F values < 1.2, p values >0.2).

## Protein Analysis

We performed protein analysis only in DLPFC white matter. Immunoblotting produced a prominent 12kD band for MOBP protein (Fig. 3) and a 46-48 kD double band for CNP, corresponding to two CNP isoforms, CNP1 (46kD) and CNP2 (48kD) (Fig. 3). B-Actin antibody which produced a 43kD band and GAPDH antibody, which gave a 36kD band corresponding to the reduced monomer of GAPDH, were used as loading controls for MOBP and CNP, respectively (Fig. 3). No statistically significant differences in MOBP (t-value = 0.01, $p = 0.7$) or CNP (t-value = 0.01, $p = 0.6$) immunoreactivities were found between patients with schizophrenia and controls (Fig. 3). Co-varying for potentially confounding factors also did not reveal differences between the diagnostic groups (F values < 1.0, p values > 0.5). Furthermore, substance abuse did not have a significant effect on MOBP or CNP protein expression in patients (F values < 1.0, p values > 0.5).

## Genotype Effects

For CNP, ANCOVA with genotype and race/ethnicity as independent variables and RIN, pH and age as continuous predictors showed a significant effect of a synonymous exonic SNP rs2070106 on CNP gene expression in the DLPFC grey matter ($F=4.7$, $p=0.03$). There was no genotype by race interaction ($F=1.7$, $p=0.2$) and no race effect ($F=0.2$, $p=0.6$). However, consistent with the finding of Peirce et al. 2006, Caucasian individuals carrying the risk-associated allele (A) had significantly lower levels of CNP mRNA compared with Caucasian subjects homozygous for the G allele (by 25%, $p=0.02$). There was no difference in expression between these two alleles in African American subjects ($p=0.5$, Fig. 4). A-carriers (i.e., A/G and A/A subjects) were combined into one group, as there were only two subjects homozygous for an A allele (one in each racial group), and compared with G/G individuals. There was no effect of genotype on CNP expression in white matter. No other CNP control SNP examined here (negative in previous association studies) showed an effect on expression in either grey or white matter of the DLPFC (all F values < 1.5, all p values >0.3). Moreover, there was no

effect of any genotype, including the high risk SNP rs2070106, on CNP immunoreactivity (all F values < 1.5, all p values >0.3).

Analysis of the effects of OLIG2 genotypes on gene expression revealed that two OLIG2 SNPs, rs1059004 and rs9653711, both previously associated with schizophrenia (Georgieva et al. 2006), predicted lower OLIG2 mRNA expression levels in the DLPFC white matter (main effects of genotype: $F(2, 85)=5.19$, $p=0.007$ and $F(2,86)=4.33$, $p=0.01$, respectively). Post-hoc comparisons showed that individuals carrying the minor A allele (enriched in cases according to Georgieva et al 2006) at SNP rs1059004 had lower OLIG2 mRNA levels than subjects homozygous for the C allele (A/A and A/C groups different from C/C, $p<0.01$) (Fig. 5). Also, the minor C allele at SNP rs9653711, which was shown to be enriched in cases in the same study, was found to be associated with decreased OLIG2 expression as compared with the G allele (C/C and C/G groups different from G/G, $p<0.01$, data not shown). This SNP was in perfect linkage disequilibrium ($r^2=1$) with SNP rs1059004 in Caucasians, so these results, being repetitions of the same genetic analysis, are not independent. Even so, the data for either SNP remained significant after correcting for multiple comparisons of all typed SNPs within this gene (i.e., Bonferroni-corrected p values <0.0125). In two-way ANOVAs with genotype (rs1059004 and rs9653711) and race or diagnosis, there were no effects of race or diagnosis and no significant interactions between genotype and race or genotype and diagnosis (all F values <1.0, all p values >0.5). Furthermore, there were no effects of two other previously associated OLIG2 SNPs in either the grey or white matter of the DLPFC.

## Discussion

We investigated the expression of myelin-associated genes in post-mortem human DLPFC. Based on the results of prior studies, we hypothesized that reduced levels of CNP, MAG and OLIG2 (but perhaps not MOBP) will be detected in our large cohort of patients. Contrary to our hypothesis, we did not find changes in CNP, MAG or OLIG2 expression in patients with schizophrenia but a small increase in MOBP mRNA levels in the DLPFC white matter, which appeared to be related to co-morbid substance abuse in patients. The inconsistency between our study and previous reports may be related to the effect size of myelin gene expression within the population or differences in cohort characteristics. The results from our genotype analyses suggest that cohort effects may indeed be important; in particular, genetic background appears to be a significant factor contributing to the expression levels of at least two such genes, CNP and OLIG2. Other possibilities include differences between cohorts in age at death (e.g., the Mount Sinai collection contains much older subjects than our collection), race and the exposure to environmental factors, including substance abuse and medication, as all these factors are likely to affect oligodendrocyte-associated gene expression.

Substance abuse in our group of patients consisted primarily of alcohol, THC, PCP, heroin and/or cocaine and in most cases was a combination of these substances. Dysregulation of myelin-related genes has been previously observed in cocaine abusers and alcoholics (Albertson et al. 2004, Liu et al. 2004). In an imaging study, significant increases in temporal and occipital white matter volume have been found in methamphetamine users, suggesting possible increased myelination in substance abusers (Thompson et al. 2004). Importantly in this context, our normal controls were thoroughly screened for history of substance abuse and those subjects with a history of drug abuse or positive toxicological results were not included in the collection.

Although a study in mice suggested that antipsychotics can reduce expression of myelin-related genes (Narayan et al. 2007), we did not find a reduction in the expression although all our patients were medicated. Moreover, we have not detected correlations between the expression

levels and the doses of neuroleptics (with a caveat that neuroleptic dose estimates are based on medical records and thus may not necessarily reflect actual drug intake).

The most interesting finding of this study is that allelic variation in CNP and OLIG2, both implicated as susceptibility genes in schizophrenia in previous studies, predicted lower expression of these genes in the DLPFC grey and white matter, respectively. For CNP, we found a similar effect of SNP rs2070106 on CNP expression as previously reported (Peirce et al. 2006) (24% vs 25% reduction in our study), although a different method was used. There are several caveats, however. First, all three SNPs showing an effect on mRNA expression do not affect amino acid sequence and are not localized to known promoter domains (CNP rs2070106 was a synonymous exonic SNP and two OLIG2 SNPs were intronic), and thus it is unclear how they might affect gene expression levels. Synonymous SNPs can, however, alter expression by modulating mRNA secondary structure and thus its stability (Nackely et al. 2006), and polymorphisms in introns can affect gene function by affecting regulatory motifs within introns or splicing. Nevertheless, we have no evidence yet that any of the SNPs showing association with mRNA levels act through these mechanisms. It is also possible that these SNPs may monitor other undiscovered SNPs involved with regulation of expression or splicing (i.e., they may tag nearby functional SNPs). Second, these SNPs had similar effects in both diagnostic groups (as there was no main effect of diagnosis and no overall reduction of expression in patients). We could not confirm association of these SNPs with schizophrenia, but as we lacked power to conduct such a study in the postmortem collection, this is not surprising. It is possible, however, that if the genetic contribution to the expression of these genes is small and cohort-specific, it may not be readily detectable, especially in the face of multiple antemortem and postmortem confounding factors, which are difficult to avoid in human molecular studies. Finally, it is unclear why CNP expression was associated with a risk SNP in the DLPFC grey matter whereas the effects of SNPs on OLIG2 expression were detected in white matter.

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## References

Aberg K, Saetre P, Jareborg N, Jazin E. Human QKI, a potential regulator of mRNA expression of human oligodendrocyte-related genes involved in schizophrenia. Proc Natl Acad Sci U S A 2006;103:7482–7. [PubMed: 16641098]

Albertson DN, Pruetz B, Schmidt CJ, Kuhn DM, Kapatos G, Bannon MJ. Gene expression profile of the nucleus accumbens of human cocaine abusers: evidence for dysregulation of myelin. J Neurochem 2004;88:1211–9. [PubMed: 15009677]

Aston C, Jiang L, Sokolov BP. Microarray analysis of postmortem temporal cortex from patients with schizophrenia. J Neurosci Res 2004;77:858–66. [PubMed: 15334603]

Covault J, Lee J, Jensen K, Kranzler H. Nogo 3'-untranslated region CAA insertion: failure to replicate association with schizophrenia and demonstration of marked population difference in frequency of the insertion. Brain Res Mol Brain Res 2004;120:197–200. [PubMed: 14741411]

Davis KL, Haroutunian V. Global expression-profiling studies and oligodendrocyte dysfunction in schizophrenia and bipolar disorder. Lancet 2003;362:758. [PubMed: 13678867]

Davis KL, Stewart DG, Friedman JI, et al. White matter changes in schizophrenia: evidence for myelin-related dysfunction. Arch Gen Psychiatry 2003;60:443–56. [PubMed: 12742865]

Dracheva S, Davis KL, Chin B, Woo DA, Schmeidler J, Haroutunian V. Myelin-associated mRNA and protein expression deficits in the anterior cingulate cortex and hippocampus in elderly schizophrenia patients. Neurobiol Dis 2006;21:531–40. [PubMed: 16213148]

Dwork AJ, Mancevski B, Rosoklija G. White matter and cognitive function in schizophrenia. Int J Neuropsychopharmacol 2007:1–24.

Flynn SW, Lang DJ, Mackay AL, et al. Abnormalities of myelination in schizophrenia detected in vivo with MRI, and post-mortem with analysis of oligodendrocyte proteins. Mol Psychiatry 2003;8:811–20. [PubMed: 12931208]

Georgieva L, Moskvina V, Peirce T, et al. Convergent evidence that oligodendrocyte lineage transcription factor 2 (OLIG2) and interacting genes influence susceptibility to schizophrenia. Proc Natl Acad Sci U S A 2006;103:12469–74. [PubMed: 16891421]

Gregorio SP, Mury FB, Ojopi EB, et al. Nogo CAA 3'UTR Insertion polymorphism is not associated with Schizophrenia nor with bipolar disorder. Schizophr Res 2005;75:5–9. [PubMed: 15820318]

Hakak Y, Walker JR, Li C, et al. Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. Proc Natl Acad Sci U S A 2001;98:4746–51. [PubMed: 11296301]

Hof PR, Haroutunian V, Friedrich VL Jr, et al. Loss and altered spatial distribution of oligodendrocytes in the superior frontal gyrus in schizophrenia. Biol Psychiatry 2003;53:1075–85. [PubMed: 12814859]

Kanazawa T, Glatt SJ, Tsutsumi A, et al. Schizophrenia is not associated with the functional candidate gene ERBB3: results from a case-control study. Am J Med Genet B Neuropsychiatr Genet 2007;144:113–6. [PubMed: 16958035]

Katsel P, Davis KL, Haroutunian V. Variations in myelin and oligodendrocyte-related gene expression across multiple brain regions in schizophrenia: a gene ontology study. Schizophr Res 2005;79:157–73. [PubMed: 16139990]

Kubicki M, McCarley RW, Shenton ME. Evidence for white matter abnormalities in schizophrenia. Curr Opin Psychiatry 2005;18:121–34. [PubMed: 16639164]

Lipska BK, Deep-Soboslay A, Weickert CS, et al. Critical factors in gene expression in postmortem human brain: Focus on studies in schizophrenia. Biol Psychiatry 2006;60:650–8. [PubMed: 16997002]

Liu J, Lewohl JM, Dodd PR, Randall PK, Harris RA, Mayfield RD. Gene expression profiling of individual cases reveals consistent transcriptional changes in alcoholic human brain. J Neurochem 2004;90:1050–8. [PubMed: 15312160]

Liu X, Qin W, He G, et al. A family-based association study of the MOG gene with schizophrenia in the Chinese population. Schizophr Res 2005;73:275–80. [PubMed: 15653272]

McInnes LA, Lauriat TL. RNA metabolism and dysmyelination in schizophrenia. Neurosci Biobehav Rev 2006;30:551–61. [PubMed: 16445981]

McCullumsmith RE, Gupta D, Beneyto M, et al. Expression of transcripts for myelination-related genes in the anterior cingulate cortex in schizophrenia. Schizophr Res 2007;90:15–27. [PubMed: 17223013]

Nackley AG, Shabalina SA, Tchivileva IE, et al. Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. Science 2006;314:1930–3. [PubMed: 17185601]

Narayan S, Kass KE, Thomas EA. Chronic haloperidol treatment results in a decrease in the expression of myelin/oligodendrocyte-related genes in the mouse brain. J Neurosci Res 2007;85:757–65. [PubMed: 17177202]

Novak G, Tallerico T. Nogo A, B and C expression in schizophrenia, depression and bipolar frontal cortex, and correlation of Nogo expression with CAA/TATC polymorphism in 3' -UTR. Brain Res 2006;1120:161–71. [PubMed: 17022955]

Peirce TR, Bray NJ, Williams NM, et al. Convergent evidence for 2',3'-cyclic nucleotide 3'-phosphodiesterase as a possible susceptibility gene for schizophrenia. Arch Gen Psychiatry 2006;63:18–24. [PubMed: 16389193]

Prabakaran S, Swatton JE, Ryan MM, et al. Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. Mol Psychiatry 2004;9:684–97. 643. [PubMed: 15098003]

Qin W, Gao J, Xing Q, et al. A family-based association study of PLP1 and schizophrenia. Neurosci Lett 2005;375:207–10. [PubMed: 15694262]

Rajkowska G, Goldman-Rakic PS. Cytoarchitectonic definition of prefrontal areas in the normal human cortex: II. Variability in locations of areas 9 and 46 and relationship to the Talairach Coordinate System. Cereb Cortex 1995;5:323–37. [PubMed: 7580125]

Tan EC, Chong SA, Wang H, Chew-Ping Lim E, Teo YY. Gender-specific association of insertion/deletion polymorphisms in the nogo gene and chronic schizophrenia. Brain Res Mol Brain Res 2005;139:212–6. [PubMed: 15953657]

Tang F, Qu M, Wang L, et al. Case-control association study of the 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) gene and schizophrenia in the Han Chinese population. Neurosci Lett. 2007

Thompson PM, Hayashi KM, Simon SL, et al. Structural abnormalities in the brains of human subjects who use methamphetamine. J Neurosci 2004;24:6028–36. [PubMed: 15229250]

Tkachev D, Mimmack ML, Ryan MM, et al. Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. Lancet 2003;362:798–805. [PubMed: 13678875]

Usui H, Takahashi N, Saito S, et al. The 2',3'-cyclic nucleotide 3'-phosphodiesterase and oligodendrocyte lineage transcription factor 2 genes do not appear to be associated with schizophrenia in the Japanese population. Schizophr Res 2006;88:245–50. [PubMed: 17010574]

Wan C, Yang Y, Feng G, et al. Polymorphisms of myelin-associated glycoprotein gene are associated with schizophrenia in the Chinese Han population. Neurosci Lett 2005;388:126–31. [PubMed: 16039057]

Watanabe Y, Fukui N, Nunokawa A, et al. No association between the ERBB3 gene and schizophrenia in a Japanese population. Neurosci Res 2007;57:574–8. [PubMed: 17275115]

Xiong L, Rouleau GA, Delisi LE, et al. CAA insertion polymorphism in the 3'UTR of Nogo gene on 2p14 is not associated with schizophrenia. Brain Res Mol Brain Res 2005;133:153–6. [PubMed: 15661375]

Yang YF, Qin W, Shugart YY, et al. Possible association of the MAG locus with schizophrenia in a Chinese Han cohort of family trios. Schizophr Res 2005;75:11–9. [PubMed: 15820319]

Zai G, King N, Wigg K, et al. Genetic study of the myelin oligodendrocyte glycoprotein (MOG) gene in schizophrenia. Genes Brain Behav 2005;4:2–9. [PubMed: 15660663]

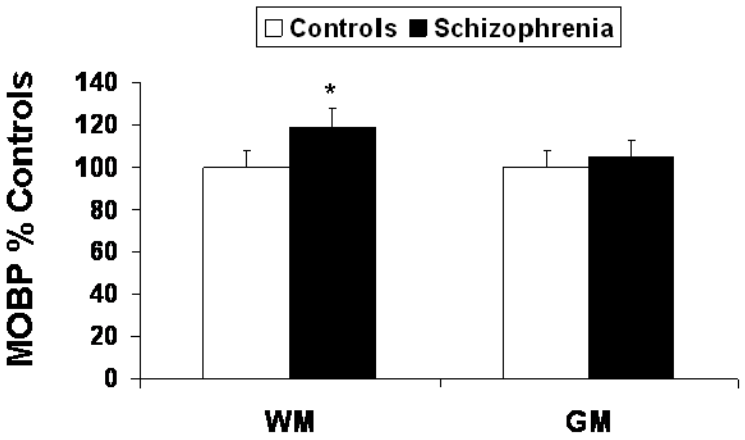


**Figure 1.**

Expression of normalized MOBP mRNA levels in schizophrenic patients and controls in the dorsolateral prefrontal cortex (DLPFC) white matter (WM) in 70 controls and 32 patients with schizophrenia and in the DLPFC grey matter (GM) in 64 controls and 30 patients with schizophrenia. Values are expressed as percentage of controls (MOBP % Controls, mean ± SE). *statistically significant from controls, $p<0.05$

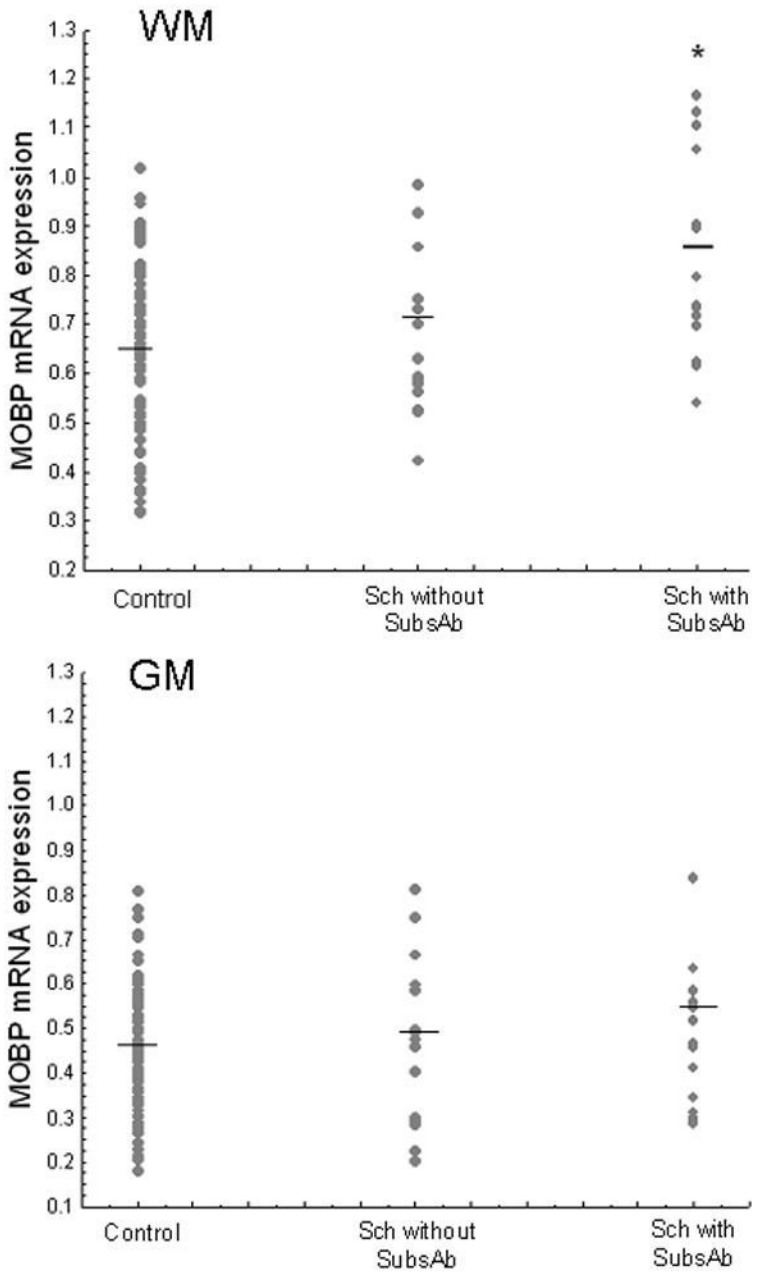


**Figure 2.**

Effect of a history of substance abuse on normalized MOBP mRNA expression in the dorsolateral prefrontal cortex **A**. white matter (WM) in 70 controls (Controls), 17 patients with schizophrenia without history of substance abuse (Sch without SubAb), and 15 patients with schizophrenia with history of substance abuse (Sch with SubAb) and in **B.** grey matter (GM), controls n = 64, schizophrenia without substance abuse n = 16, schizophrenia with substance abuse n = 14. None of the controls had a history of substance abuse. * statistically significant from schizophrenia patients without substance abuse, $p<0.05$.

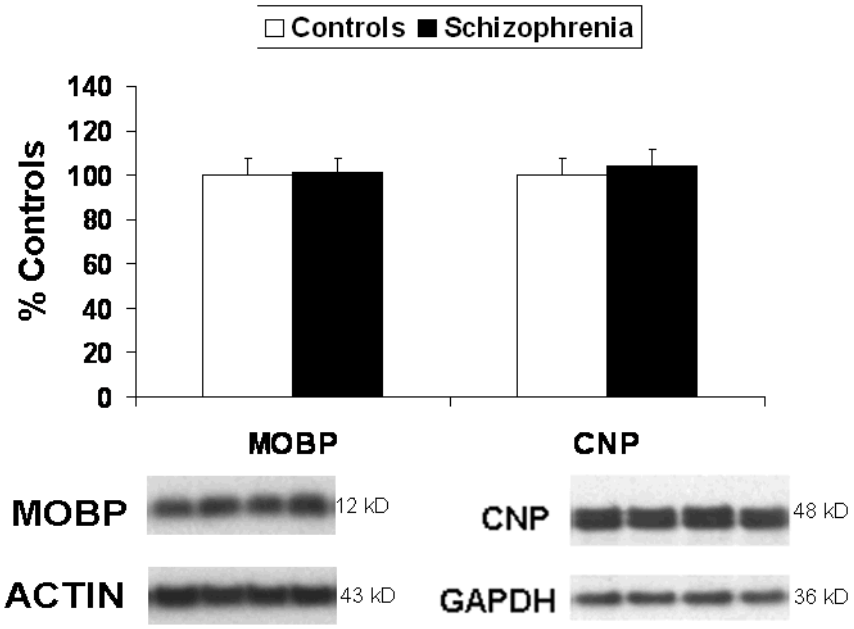


**Figure 3.**

MOBP and CNP protein immunoreactivity in DLPFC white matter in controls (n=47) and in patients with schizophrenia (n=26). Immunobloting was performed using antibodies against MOBP and a loading control Actin as well as CNP and a loading control GAPDH. MOBP and CNP immunoreactivity in controls and patients with schizophrenia were normalized to control proteins (Actin and GAPDH, respectively) and expressed as a percentage of controls (mean ± SE). There were no differences in immunoreactivity between the diagnostic groups.

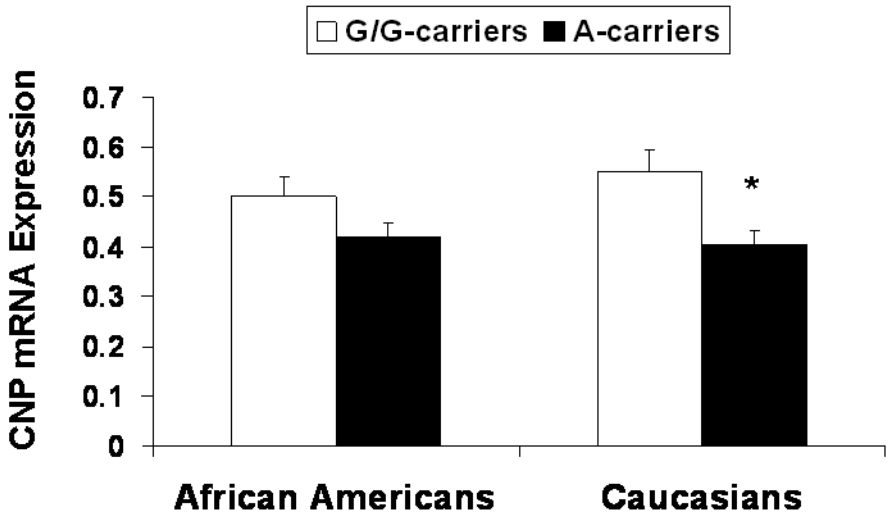


**Figure 4.**

Effect of race and CNP genotype (rs2070106) on normalized CNP mRNA expression in the dorsolateral prefrontal cortex grey matter ("African American G/G" n = 39, "African American A-carriers" n = 11, "Caucasian G/G" n = 15, "Caucasian A-carriers" n = 13). Caucasian individuals carrying a high risk A allele showed significantly lower levels of CNP expression compared to Caucasian subjects homozygous for the G allele. (mean ± SE) * statistically significant from Caucasian G/G, $p < 0.05$.

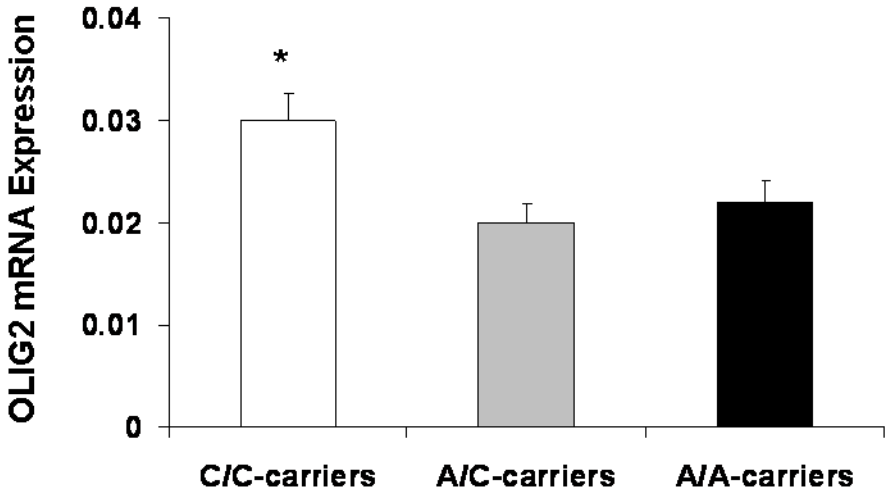


**Figure 5.**

Effect of OLIG2 genotype (SNP rs1059004) on OLIG2 mRNA expression in the dorsolateral prefrontal cortex white matter. Individuals homozygous and for an A allele and heterozygotes had significantly lower OLIG2 mRNA expression than subjects homozygous for the C allele, “C/C” n = 37, “A/C” n = 40, “A/A” n = 14. (mean ± SE), * $p<0.01$.

**Table 1**

Summary of postmortem findings of oligodendrocyte-related gene expression abnormalities in schizophrenia.

| Study | Brain Regions Studied | Brain Collection | Method | Positive Findings | Comments | Numbers S/C | Age |
|---|---|---|---|---|---|---|---|
| Hakak et al. (2001) | PFC | Mount Sinai | Microarray | CNP, MAG, ErbB3, MAL, TF, gelsolin reduced | MOBP, OLIG2 not reported | 12 / 12 | >70 |
| Hof et al. (2003) | SFG | Mount Sinai | IHC | Number of CNP positive cells reduced | MOBP, MAG, OLIG2, ErbB3 not tested | 7 / 7 | >75 |
| Flynn et al. (2003) | PFC | NYPI | ELISA | CNP reduced | No change for MAG; MOBP, CNP, OLIG2, ErbB3 not tested | 13 / 11 | ~46 |
| Tkachev et al. (2003) | PFC | Stanley | Microarray/ QPCR | OLIG2, MAG, ErbB3 reduced | No change for MOBP; CNP not reported | 15 / 15 | N/A |
| Prabakaran et al. (2004) | PFC | Stanley | Proteomics | CNP reduced in WM | MOBP, MAG, OLIG2, ErbB3 not reported | 10 / 10 | N/A |
| Aston et al. (2004) | TMPGy | Stanley | Microarray/ qPCR | MAG, ErbB3 reduced | MOBP, CNP, OLIG2 not reported | 12 / 14 | ~45 |
| Katsel et al. (2005) | Multiple | Mount Sinai | Microarray | CNP, MAG, OLIG2, ErbB3 reduced | MOBP not reported | ~13 / 13 | >70 |
| Dracheva et al (2006) | HIPP, CING, CAU, PUT | Mount Sinai | qPCR, Western | CNP, MAG reduced | No change for MOBP; OLIG2, ErbB3 not tested | ~25 / 20 | >75 |
| Aberg et al. (2006) | FC | Stanley+Maudsley +Harvard | qPCR | MAG reduced | MOBP, CNP, OLIG2 not tested | 55 / 55 | N/A |
| Novak G et al 2006) | FC | Stanley | qPCR | NOGO C increased | MOBP, CNP, MAG, ErbB3, OLIG2 not tested | 8/14 | ~45 |
| McCullumsmith et al. (2007) | CING | Mount Sinai | ISH | MAG, CNP, QKI, TF reduced in WM (not GM) | No change for OLIG2, ErbB3; MOBP not tested | 41 / 34 | >75 |

Abbreviations: CING – cingulate cortex, CAU – caudate, CNP - 2',3'-cyclic nucleotide 3'-phosphodiesterase, ErbB3 - v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian), ISH – *in situ* hybridization, IHC – immunohistochemistry, FC – frontal cortex, GM- grey matter, HIPP – hippocampus, MAG – myelin-associated glycoprotein, MOBP - myelin-associated basic protein, NYPI – New York Psychiatric Institute, OLIG2 - oligodendrocyte-lineage transcription factor 2, PFC- prefrontal cortex, PUT – putamen, SFG – superior frontal gyrus, TMPGy – temporal gyrus, WM- white matter, N/A – not available.

**Table 2**

Summary of association findings of oligodendrocyte-related genes and schizophrenia.

| Study | Population | n (S/C) | Findings |
|---|---|---|---|
| Wan C et al (2005) | Han Chinese | 470/470 | Association of SNPs in MAG with schizophrenia |
| Yang YF et al (2005) | Han Chinese | 413 patients and parents | Association of MAG haplotype with schizophrenia |
| Qin W et al (2005) | Han Chinese | 487 patients and parents | Association of SNP in PLP1 with male patients with schizophrenia |
| Zai G et al (2005) | Caucasians/Asian/ African American | 111 patients and families | No association of SNPs in MOG with schizophrenia |
| Liu X et al (2005) | Han Chinese | 532 patients and families | Association of MOG haplotype with schizophrenia |
| Covault J et al (2004) | Caucasian/ African American | 77/243 | No association of NOGO polymorphisms and schizophrenia |
| Tan E et al (2005) | Chinese | 363/253 | Association of polymorphisms in NOGO in female patients with schizophrenia |
| Gregorio SP et al (2005) | Brazil | 181/427 | No association of NOGO polymorphisms and schizophrenia |
| Xiong L et al (2005) | Caucasian European | 462/153 | No association of NOGO polymorphisms and schizophrenia |
| Peirce et al. (2006) | Caucasian/UK | 708/711 | Exonic SNP predicting lower CNP expression is associated with schizophrenia |
| Georgieva et al. (2006) | Caucasian/UK | 673/716 | Association of SNPs in OLIG2 with schizophrenia |
| Usui et al. (2006) | Japanese | 759/757 | No association of SNPs in OLIG2 and CNP |
| Kanazawa T et al (2007) | Japanese | 193/121 | No association of SNPs in ErbB3 and schizophrenia |
| Watanabe Y et al (2007) | Japanese | 399/438 | No association of SNPs in ErbB3 and schizophrenia |
| Tang et al. (2007) | Han Chinese | 426/439 | No association of SNPs in CNP |

Abbreviations: CNP - 2',3'-cyclic nucleotide 3'-phosphodiesterase, ErbB3 - v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian), MAG – myelin-associated glycoprotein, MOBP - myelin-associated basic protein, MOG – myelin oligodendrocyte glycoprotein, OLIG2 - oligodendrocyte-lineage transcription factor 2, NOGO (RTN4R) - reticulon 4 receptor, PLP1 - proteolipid protein 1

**Table 3**

Characteristics of subject cohorts used for mRNA expression and protein measurements. AA- African American; A – Asian; H – Hispanic; C – Caucasian; F –female; M – male; PMI – postmortem interval (hrs); RIN – Agilent RNA integrity number (on a scale 1-10). For age, PMI, pH and RIN the data re presented as Mean ± SD.

| | n | race | sex | age | PMI | pH | RIN |
|---|---|---|---|---|---|---|---|
| **White matter- mRNA** | | | | | | | |
| Controls | 73 | 43AA/24C/3A/3H | 23F/50M | 41.4±15.1 | 32.1±14.4 | 6.6±0.28 | 6.7±1 |
| Schizophrenia patients | 33 | 20AA/11C/2H | 13F/20M | 48.8±15.9* | 38.5±18.5 | 6.4±0.32* | 6.2±1.1* |
| **Grey Matter- mRNA** | | | | | | | |
| Controls | 68 | 40AA/21C/3A/4H | 21F/47M | 41.2±14.7 | 32.3±14.1 | 6.6±0.25 | 6.8±1.19 |
| Schizophrenia patients | 31 | 17AA/12C/2H | 14F/17M | 46.8±15.3 | 37.4±17.3 | 6.4±0.28* | 6.8±1.13 |
| **White Matter- protein** | | | | | | | |
| Controls | 47 | 31AA/13C/2A/1H | 13F/34M | 41.5±13.5 | 32.4±14.1 | 6.58±0.25 | 6.6±1 |
| Schizophrenia patients | 26 | 13AA/11C/2H | 9F/17M | 44.7±16.3 | 36.3±13.4 | 6.51±0.28 | 6.2±1.3 |

* $p<0.05$

**Table 4**

Contribution of demographic and tissue related variables to non-normalized and normalized gene expression levels in schizophrenics and controls determined using forward stepwise multiple regression analysis

| | adj $R^2$ | DX β | Age β | Sex β | pH β | PMI β | RIN β | Sm β | AS β |
|---|---|---|---|---|---|---|---|---|---|
| **White Matter** | | | | | | | | | |
| MOBP | 0.229 | n.s. | -0.25** | | n.s. | n.s. | 0.432**** | n.s. | n.s. |
| CNP | 0.256 | n.s. | -0.29** | | n.s. | n.s. | 0.405**** | n.s. | n.s. |
| MAG | 0.197 | n.s. | -0.26** | | n.s. | n.s. | 0.379**** | n.s. | n.s. |
| OLIG2 | 0.116 | n.s. | -0.23* | | n.s. | n.s. | 0.279** | n.s. | n.s. |
| NF | 0.234 | n.s. | n.s. | n.s. | n.s. | n.s. | 0.445**** | n.s. | n.s. |
| MOBP/NF | 0.127 | 0.225* | n.s. | n.s. | n.s. | 0.224* | n.s. | n.s. | n.s. |
| CNP/NF | 0.044 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| MAG/NF | 0.029 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| OLIG2/NF | 0.001 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| **Grey Matter** | | | | | | | | | |
| MOBP | 0.038 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| CNP | 0.058 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| MAG | 0.061 | n.s. | n.s. | n.s. | n.s. | -0.21* | n.s. | n.s. | n.s. |
| OLIG2 | 0.235 | n.s. | n.s. | n.s. | 0.287** | -0.186* | n.s. | n.s. | n.s. |
| NF | 0.145 | n.s. | n.s. | n.s. | n.s. | -0.227* | 0.273** | n.s. | n.s. |
| MOBP/NF | 0.009 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| CNP/NF | 0.007 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| MAG/NF | 0.04 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| OLIG2/NF | | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |

* p<0.05,

** p<0.01,

*** p<0.001,

**** p<0.0001;

n.s.- not significant;

Abberviations: adj $R^2$ – adjusted coefficient of determination; β – standardized regression coefficient; AS – agonal state (1=<10 min; 2=>1hr) Dx – diagnosis (1-controls, 2-schizophrenia patients); Sex – 1-female, 2-male; PMI – postmortem interval; RIN – RNA integrity number; Sm – smoking history (1-No, 2-Yes); NF – normalizing factor Other abbreviations as in Tables 1 and 2.