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Expression of Transcripts for Myelination-Related Genes in the Anterior Cingulate Cortex in Schizophrenia

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Abstract

Several recent studies have found changes in the expression of genes functionally related to myelination and oligodendrocyte homeostasis in schizophrenia. These studies utilized microarrays and quantitative PCR (QPCR), methodologies which do not permit direct, unamplified examination of mRNA expression. In addition, these studies generally only examined transcript expression in homogenates of gray matter. In the present study, we examined the expression of myelination-related genes previously implicated in schizophrenia by microarray or QPCR. Using in situ hybridization, we measured transcript expression of 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP), myelin-associated glycoprotein (MAG), transferrin (TF), quaking (QKI), gelsolin, myelin oligodendrocyte glycoprotein, v-erb-b2 erythroblastic leukemia viral oncogene homolog 3, erbb2 interacting protein, motility-related protein-1, SRY-box containing gene 10, oligodendrocyte transcription factor 2, peripheral myelin protein 22, and claudin-11 in both gray and white matter of the anterior cingulate cortex (ACC) in subjects with schizophrenia (n = 41) and a comparison group (n = 34). We found decreased expression of MAG, QKI, TF, and CNP transcripts in white matter. We did not find any differences in expression of these transcripts between medicated (n = 31) and unmedicated (n = 10) schizophrenics, suggesting that these changes are not secondary to treatment with antipsychotics. Finally, we found significant positive correlations between QKI and MAG or CNP mRNA expression, suggesting that the transcription factor QKI regulates MAG and CNP expression. Our results support the hypothesis that myelination and oligodendrocyte function are impaired in schizophrenia.

Introduction

While early hypotheses viewed schizophrenia as a dysfunction of discrete brain regions, recent models often conceptualize this illness as a disorder of the functional integrity of widely

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distributed circuitry. Connectivity within such circuits is dependent on myelination by oligodendroglia, a tightly regulated process that includes the initial myelination of axons during brain development and the coordination of myelin synthesis and turnover in the adult CNS. Converging lines of evidence suggest that the processes governing myelination and/or the regulation of oligodendroglia are abnormal in schizophrenia (Reviewed in: (Davis et al., 2003)). Studies using magnetic resonance, magnetization transfer, and diffusion tensor imaging support the hypothesis that there are white matter abnormalities in this illness, and pathological examination by electron microscopy suggests a role for alterations of the myelin sheath (Buchsbaum et al., 1998; Lim et al., 1998; Lim et al., 1999; Pfefferbaum et al., 1999; Foong et al., 2000; Agartz et al., 2001; Foong et al., 2001; Sigmundsson et al., 2001; Steel et al., 2001; Uranova et al., 2001; Bagary et al., 2003; Sun et al., 2003; Hubl et al., 2004; Uranova et al., 2004; Wang et al., 2004; Szeszko et al., 2005). A subset of myelin-related genes has been consistently implicated in schizophrenia by genetic studies (Reviewed in: (Davis et al., 2003)), and subsequent microarray and QPCR analyses have found decreases in mRNA expression of myelin-related genes in prefrontal and temporal cortices (Hakak et al., 2001; Tkachev et al., 2003; Aston et al., 2004; Dracheva et al., 2005a; Haroutunian et al., 2005).

One region in schizophrenia where myelination-dependent connectivity may be abnormal is the anterior cingulate cortex (ACC). Comparison of region-specific gene expression by microarray analyses found the second highest number of abnormally expressed transcripts in the ACC, after the superior temporal cortex, and well ahead of the DLPFC (Kastel et al., 2005). The ACC integrates cognitive function, motor control, and drive states; coordination of these modalities is often abnormal in schizophrenia (Paus, 2001; Heckers et al., 2004). Other studies have found abnormal activation of the ACC with tasks related to social functioning and behavior, a reduction in ACC glial cell number, and changes in GABAergic and glutamatergic gene expression in the ACC in this illness (Quintana et al., 2004; Stark et al., 2004; Woo et al., 2004). In addition, reductions in white matter volumes were detected in the ACC in schizophrenia using magnetic resonance imaging (Riffkin et al., 2005). Taken together with the aforementioned microarray data, these findings support a hypothesis of abnormal connectivity secondary to alterations in myelination and oligodendrocyte function in the ACC in schizophrenia.

To investigate the hypothesis that myelination and oligodendrocyte function are abnormal in schizophrenia, we examined expression of myelination-related genes previously implicated in this illness by microarray or QPCR analysis (Hakak et al., 2001; Tkachev et al., 2003; Aston et al., 2004; Dracheva et al., 2005a; Katsel et al., 2005). Since these techniques do not preserve the anatomy of the tissue and they rely on amplification of mRNA, we used in situ hybridization to directly measure transcript expression of 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP), myelin-associated glycoprotein (MAG), transferrin (TF), quaking (QKI), gelsolin (GEL), myelin oligodendrocyte glycoprotein (MOG), v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (ErbB3), erbB2 interacting protein (ErbB2IP), motility-related protein-1 (CD9), SRY-box containing gene 10 (SOX10), oligodendrocyte transcription factor 2 (OLIG2), peripheral myelin protein 22 (PMP22), and Claudin-11 (CLDN11) in both gray and underlying white matter of the ACC in schizophrenia.

Material and Methods

Subjects

A total of 75 subjects from the Mount Sinai Medical Center / Bronx VA Medical Center Department of Psychiatry Brain Bank were studied, 41 individuals diagnosed with schizophrenia and 34 comparison subjects with no history of psychiatric illness (Table 1)

(Davidson et al., 1995). Upon neuropathological examination, no evidence for neurodegenerative changes or Alzheimer disease was found in any of the subjects (Purohit et al., 1998). Brains were obtained after autopsy and one hemisphere was cut coronally into 10 mm slabs and frozen. Brain pH was measured using an aliquot of homogenized brain tissue and a pH meter. pH measurements were validated using correlation analysis by determining lactate levels in the same tissue homogenates from randomly selected cases. Anterior cingulate cortex was dissected at the level of the genu of the corpus callosum. Sections included the adjacent cingulum bundle. Tissue blocks were dissected from the dorsal surface of the corpus callosum extending 12–15 mm dorsally and extending 12–15 mm laterally from the midline. 15 µm sections were thawed onto slides previously treated with poly-L-lysine, dried, and stored at –80°C. Two slides were studied per subject.

In situ hybridization

The sensitivity and reproducibility of in situ hybridization has previously been well characterized (Williams, 1982; Downs and Williams, 1984; Stolz et al., 1989). mRNA expression was measured by in situ hybridization using subclones that were generated by amplifying unique segments of 2',3'-cyclic nucleotide 3'-phosphodiesterase (Abbreviation: CNP, Genebank accession number: NM_03313, region used for probe: 523–1066), myelin-associated glycoprotein (MAG, XM_01287, 305–835), transferrin (TF, XM_00279, 1172–1940), quaking (QKI, AF142421, 508–715), gelsolin (GSN, XM_01654, 1249–1649), myelin oligodendrocyte glycoprotein (MOG, U18800, 109–263), v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (ErbB3, NM_00198, 3081–3325), erbb2 interacting protein (ErbB2IP, NM_01869, 3531–3858), motility-related protein-1 (CD9, M38690, 330–666), SRY-box containing gene 10 (SOX10, BL007595, 1337–1560), oligodendrocyte transcription factor 2 (OLIG2, NM_00580, 423–628), peripheral myelin protein 22 (PMP22, BC019040, 293–637), and Claudin-11 (CLDN11, NM_00560, 610–821) from a human cDNA brain library (Human Adult Brain Unamplified cDNA Library, Edge Biosystems; Gaithersburg, MD) and Polymerase Chain Reaction (PCR). PCR-amplified cDNA segments were extracted (QIAquick Gel Extraction Kit, Qiagen, Valencia, CA), subcloned (Zero Blunt TOPO PCR cloning kit; Invitrogen, Carlsbad, CA), and confirmed by nucleotide sequencing. Sense and antisense probes for in situ hybridization were synthesized using 100 µCi of dried [³⁵S]UTP, 2.0 µl 5x transcription buffer (40 mM Tris-base, 6 mM Mg Cl₂, 2 mM spermidine, 10 mM NaCl, pH 7.9); 1.0 µl each of 10 mM ATP, CTP, and GTP; 1.0 µg linearized plasmid DNA; 0.5 µl RNase inhibitor; and 1.5 µl SP6 or T7 RNA polymerase. After 2 hours incubation at 37°C, 1.0 µl DNase (RNase-free) was added and incubated for 15 min at room temperature (RT). [³⁵S] Labeled cRNA was purified with a spin column (Micro Bio-Spin P-30 Tris Spin Columns, Bio-Rad Laboratories; Hercules, CA), diluted to 100 µl final volume, and 1.0 µl of 1 M dithiothreitol (DTT) was added to a final concentration of 0.01 M.

Sections were fixed with 4% (weight:volume) formaldehyde for 1 hour at room temperature (RT). Next, sections were rinsed three times in 2X SSC (300 mM NaCl/30 mM sodium citrate, pH 7.2) and incubated on a stir plate in 0.1 M triethanolamine (TAE), pH 8.0 / acetic anhydride, 1:400 (volume/volume) for 10 min at RT. Sections were then washed in 2X SSC for 10 min at RT, the tissue was dehydrated through graded alcohols and air-dried. [³⁵S] labeled riboprobe (1 x 10⁶ cpm), was applied in 100 µl of 50% formamide buffer (50% formamide, 10% dextran sulfate, 3X SSC, 50 mM Na₂HPO₄, pH 7.4, 1X Denhardt's solution, 100 µg/ml yeast tRNA) with 0.1% of 1M DTT per each slide. The slides were then covered with glass cover slips and stored in a humidified chamber saturated with 50% formamide overnight at 55°C for 18 hours. Next, the cover slips were removed and the sections were washed in 2X SSC for 2 min at RT, 2X SSC for 10 min at RT, and then incubated with RNase A (200 mg/ml in 10 mM Tris-HCl, pH 8.0/0.5 M NaCl) at 37°C for 30 min. Slides were then washed at RT two times for 15 min

in 2X SSC, 15 min in 1X SSC, 5 min in 0.5X SSC, two times for 60 min in 0.1X SSC at 55°C and 15 min in 0.1X SSC at RT. The sections were then dehydrated in graded alcohol solutions and air-dried. Finally the slides were placed in X-ray cassettes, apposed to film (Kodak BIOMAX MR Film, New England, Nuclear, Boston, MA) and developed after 10–31 days.

Data analysis

Images were digitized from films with a CCD camera with NIH Image software v16.1 and analyzed with Scion Image Beta 3b. Gray and white matter margins were identified based upon cellular patterns as defined by cresyl violet staining of sections from each subject. The pattern of expression for each of the probes was relatively uniform within the gray and underlying white matter, and no lamination was observed for any of the transcripts. The region analysed for each transcript included the entire profile of either the gray or the white matter of each section. Film background values were subtracted from gray scale values of either gray or underlying white matter regions of each section and converted to optical density. Values for two sections per subject were averaged and used for statistical analysis.

Statistical analyses

The phrase “Main effect for diagnosis” refers to the statistical result from the factorial ANOVA or ANCOVA analysis for the independent variable, diagnosis. The phrase “diagnosis by region interaction” refers the statistical result from the factorial ANOVA or ANCOVA analysis for the combined effects of the independent variables for diagnosis and isodense band. Correlation analysis for each transcript was performed to investigate possible associations between transcript expression and age, postmortem interval (PMI), and tissue pH. When significant associations with age, pH or PMI were found, analysis of covariance was utilized, otherwise analysis of variance was utilized, with diagnosis and region (gray matter or white matter) as the independent variables and optical density as the dependent variable. Post-hoc analysis was performed by Tukey’s HSD. For all tests, $\alpha = 0.05$. Correlation analyses were performed to probe for associations in gene expression for transcript that were significantly altered in schizophrenia and were previously demonstrated to be functionally linked or coordinately regulated.

Results

Sense and antisense probes were tested in sections of human cortex; specific labeling was only observed for sections incubated with antisense riboprobe (data not shown). We detected transcript expression in both white and gray matter of the ACC for all of the genes studied (Figures 1 and 2), although the gray matter labeling was considerably less than white matter for each gene studied, as expected for oligodendrocyte-associated genes.

We did not detect any associations between QKI, MAG, SOX10, OLIG2, CD9, CLDN11, MOG, ErbB2IP, or ErbB3 mRNA expression and age, PMI or pH. We did detect associations between CNP, GSN, and TF mRNA expression and pH (CNP: $r = 0.27$, $p < 0.03$, GSN: $r = 0.16$, $p < 0.05$, TF $r = 0.17$, $p < 0.03$) but not age or PMI. We also detected associations between PMP22 and age ($r = 0.20$, $p < 0.02$) and pH ($r = 0.17$, $p < 0.04$), but not PMI.

We detected a main effect for diagnosis for QKI ($F(1, 138) = 6.2$, $p < 0.02$) and MAG ($F(1, 135) = 4.3$, $p < 0.04$), but not SOX10, OLIG2, CD9, CLDN11, MOG, ErbB2, or ErbB3 transcript expression (Figures 3 and 4). We detected a main effect for region for QKI, MAG, SOX10, OLIG2, CD9, CLDN11, MOG, ErbB2IP, and ErbB3 mRNA expression. We did not detect any diagnosis by region interactions for these transcripts. Post-hoc analysis revealed decreased QKI ($p < 0.03$) and MAG ($p < 0.04$) mRNA expression in the white matter in

schizophrenia, and higher levels of mRNA expression for QKI, MAG, SOX10, OLIG2, CD9, CLDN11, MOG, ErbB2IP, and ErbB3 in the white matter compared to the gray matter (Figures 3 and 4, $p < 0.05$ for all transcripts). Using ANCOVA with pH (and age for PMP22) as a covariate, we detected a main effect for diagnosis for CNP ($F(1, 135) = 7.6$, $p < 0.01$) and TF ($F(1, 147) = 6.7$, $p < 0.01$), but not GSN or PMP22 mRNA expression (Figures 3 and 4). We detected a main effect for region for CNP, TF, PMP22 and GSN mRNA expression. We did not detect any diagnosis by region interactions for these transcripts. Post-hoc analysis revealed decreased CNP ($p < 0.04$) and TF ($p < 0.04$) mRNA expression in the white matter in schizophrenia, and higher levels of mRNA expression for CNP, TF, PMP22 and GSN in the white matter compared to the gray matter (Figures 3 and 4, $p < 0.05$ for all transcripts).

To assess the role of antipsychotic treatment on the expression of CNP, TF, QKI, and MAG we compared medicated ($n = 31$) and unmedicated (within six weeks of death) subjects with schizophrenia (Figure 5). We did not find any differences in transcript expression between unmedicated and medicated schizophrenics for these 4 genes in either the white or gray matter. To assess the role of gender on the expression of CNP, TF, QKI, and MAG we analysed transcript expression in male versus female subjects (data not shown). We did not find any differences in transcript expression between males and females for these 4 genes in either the white or gray matter.

We also found significant positive correlations between QKI transcript expression and MAG or CNP mRNA expression (MAG: $r = 0.529$, $p < 0.01$; CNP: $r = 0.647$, $p < 0.01$) (Figure 6).

Discussion

Using in situ hybridization, we found decreased mRNA expression in the white matter of the ACC of four functionally related genes that have well-characterized roles in myelination and the regulation of oligodendrocytes. These results confirm a subset of previous findings of altered expression of myelination-related genes in schizophrenia, supporting the hypothesis that abnormalities of white matter axonal tracks contribute to schizophrenic pathophysiology (Hakak et al., 2001; Tkachev et al., 2003; Aston et al., 2004; Dracheva et al., 2005a; Haroutunian et al., 2005). Since we only found changes in expression of myelination-related genes in the white matter, and high levels of these genes (CNP, QKI, TF and MAG) are reportedly expressed by oligodendrocytes (Bloch et al., 1985; Barbarese et al., 1988; Sprinkle, 1989; Hardy, 1998), decreased gene expression is likely secondary to either a decrease in the number of oligodendrocytes, a normal number of oligodendrocytes but with diminished gene expression, or both. Decreases in oligodendrocyte density and altered morphology have both been reported in schizophrenia (Uranova et al., 2001; Hof et al., 2002; Davis et al., 2003; Hof et al., 2003; Uranova et al., 2004), suggesting that a combination of cell loss and oligodendrocyte dysfunction could be contributing to the gene expression abnormalities we and others have detected. However, we only found changes in some but not all oligodendrocyte-associated transcripts, suggesting that our findings are more likely due to perturbed gene expression rather than changes in oligodendrocyte density.

We found decreased expression of TF mRNA in the white matter in the ACC. This result is consistent with microarray and QPCR findings of decreased TF mRNA expression in DLPFC samples containing both gray and white matter in schizophrenia, and is further supported by six positive linkage studies for the TF gene in schizophrenia (Hakak et al., 2001; Davis et al., 2003; Tkachev et al., 2003). Several preclinical studies have demonstrated a critical role for TF in myelination and oligodendrocyte maturation (Espinosa de los Monteros et al., 1999; Saleh et al., 2003). TF is an iron transport glycoprotein synthesized in the liver by hepatocytes, by distinct types of well-differentiated epithelial cells, and by oligodendrocytes and the choroid

plexus in the CNS (Skinner and Griswold, 1980; Bloch et al., 1985; Bloch et al., 1987; Lee et al., 1987; Tu et al., 1991). TF made by oligodendrocytes is functionally distinct from TF synthesized by other tissues, likely secondary to alternative splicing (de Arriba Zerpa et al., 2000; Duchange et al., 2002). TF expression correlates with the synthesis of myelin and the normal development of oligodendrocytes in rodent models, and the replacement of TF with exogenous apo-transferrin restores myelination in P5 rat pups (Bartlett et al., 1991; Connor et al., 1993; Saleh et al., 2003). Thus, a decrease in TF expression is consistent with the hypothesis that synthesis and maintenance of myelin is disrupted in schizophrenia.

We found a decrease in the expression of MAG in white matter in schizophrenia using a probe that will detect mRNAs for both the L-MAG and S-MAG splice variants. This result is consistent with microarray findings of decreased MAG mRNA expression in the DLPFC and the middle temporal gyrus, and QPCR findings of decreased MAG mRNA in the DLPFC, the ACC and the hippocampus (HPC) in schizophrenia (Hakak et al., 2001; Tkachev et al., 2003; Aston et al., 2004; Dracheva et al., 2005a). The involvement of MAG in schizophrenia is further supported by three positive linkage studies for the MAG gene in this illness (reviewed in: (Davis et al., 2003)). However, a different study did not find statistically significant changes in MAG protein expression in gray matter from the anterior PFC using ELISA (Flynn et al., 2003), although MAG protein levels were reduced by 27%. MAG is a glycoprotein component of the myelin sheath that is characterized as an inhibitor of axon outgrowth, an effect restricted to adult neurons (Filbin, 2003; Grados-Munro and Fournier, 2003; Yiu and He, 2003; Cui, 2006). Interestingly, iron deficient rats, used as an analogous model of iron deficiency in humans that is linked to hypomyelination, have decreased MAG and TF protein expression in the white matter, suggesting that these gene products are high yield markers of the fidelity of myelin synthesis and maintenance processes (Ortiz et al., 2004).

We also found a decreased expression of CNP in white matter in schizophrenia. This result is consistent with findings of decreased CNP mRNA expression in DLPFC by microarray analysis and in the ACC and HPC by QPCR (Hakak et al., 2001; Dracheva et al., 2005a). Two other studies have found decreases in CNP protein expression in the anterior PFC and the HPC by ELISA and Western blot analyses, respectively (Flynn et al., 2003; Dracheva et al., 2005a). The role of CNP in myelination is less well understood than for TF or MAG. CNP is expressed by both mature oligodendrocytes as well as oligodendrocyte precursors (Lappe-Siefke et al., 2003). One function attributed to CNP is facilitation of the assembly and linkage of microtubules to membranes, an effect blocked by phosphorylation of CNP (Bifulco et al., 2002). While the specific role(s) of CNP in myelination remains unclear, CNP expression is abnormal in schizophrenia in a region specific manner at multiple levels of gene expression using distinct but complimentary techniques.

Similar to TF, MAG and CNP, QKI mRNA expression has recently been examined in schizophrenia (Haroutunian et al., 2005). We found decreased QKI transcript expression using a probe that detects three isoforms of the QKI gene. The QKI gene codes for a family of alternatively spliced gene products (QKI-5, QKI-6 and QKI-7) that regulate myelination by Schwann cells and oligodendrocytes in the CNS, and that have been extensively studied using viable mutants (Hardy, 1998). For example, the 5' promoter region of the QKI gene is disrupted in the quakingviable (qk^v) mutant mouse, resulting in severe myelination defects in the CNS, including abnormal compaction of myelin and dysregulation of cytoplasmic loop formation (Hardy, 1998). Interestingly, these deficits are similar, albeit on a smaller scale, to ultrastructural defects of the myelin sheath found in the prefrontal cortex and caudate nucleus in schizophrenia (Uranova et al., 2001; Uranova et al., 2004). Taken together with the above findings of alterations in TF, MAG, and CNP expression, these data suggest that myelination

and oligodendrocyte function are impaired in schizophrenia, supporting the hypothesis of altered connectivity in this illness.

Preclinical studies suggest a functional link between QKI and the myelination-related genes MAG and CNP (Hardy, 1998). MAG mRNA expression is abnormal in qk^V mice, and expression of protein for both the L-MAG and S-MAG splice variants is decreased in these animals (Hardy, 1998). This effect might be mediated by QKI modulation of MAG mRNA splicing, increased endocytosis of MAG from the plasma membrane, or differential activation of non-receptor tyrosine kinases such as Fyn (Hardy, 1998). The link between QKI and CNP is less well established. CNP protein expression is decreased in qk^V mice, while CNP mRNA is only minimally diminished, suggesting that abnormal CNP levels are due to a post-transcriptional alteration in CNP expression or degradation (Zhang and Feng, 2001). Since QKI apparently regulates expression of MAG and CNP, we performed correlation analysis and found an association between expression of QKI and MAG or CNP in schizophrenia (Figure 6), suggesting that in schizophrenia decreases in QKI mRNA expression may lead to decreases in MAG and CNP gene expression.

We detected PMP22 mRNA expression in the gray and white matter in the ACC. This result was somewhat surprising given that PMP22 was until recently only reported to be expressed in the peripheral nervous system. Our finding is consistent with a recent report describing PMP22 expression in several regions of the human CNS (Ohsawa et al., 2006).

Reverse power calculations demonstrated that $\beta < 0.05$ for each of our transcript studies, indicating that transcript expression for SOX10, OLIG2, CD9, CLDN11, MOG, GSN, PMP22, ErbB2IP, and ErbB3 was unchanged in the white and gray matter of the ACC in schizophrenia. The absence of changes in mRNA expression for these genes is divergent from previous studies that found decreases in transcript expression for SOX10, CLDN11, MAL, GEL, ErbB3, MOG, and OLIG2 (Hakak et al., 2001; Tkachev et al., 2003; Aston et al., 2004; Dracheva et al., 2005a). Differences in brain region and schizophrenic cohort could account for these disparate findings. In one study using tissue from the Stanley Foundation Consortium (SFC), expression of SOX10, MOG, CLDN11, OLIG2, and ErbB3 mRNA was performed via microarray analysis and QPCR in BA9 homogenates containing gray and white matter (Tkachev et al., 2003). In two other studies from the same brain bank as the tissue in the present study, changes in MAL, GEL, and ErbB3 were found by microarray analysis of mRNA from homogenates of gray matter from BA 46 (Hakak et al., 2001), and changes in SOX10, CLDN11, and PMP22 were detected by QPCR in samples of gray matter from the ACC and the HPC (Dracheva et al., 2005a). A fourth study using tissue from the SFC that found decreased ErbB3 mRNA by microarray in BA 21 homogenate did not comment on whether the samples included white matter (Aston et al., 2004).

In addition to differences in brain region and cohort, all of these studies relied on either QPCR or microarrays, techniques which require homogenization of the sample and amplification of the mRNA prior to quantification. We examined mRNA expression using in situ hybridization, a technique that does not involve amplification of the mRNA and that preserves the tissue anatomy permitting evaluation of mRNA expression in white and gray matter regions within the same section of brain tissue. Despite the anatomic advantages provided by in situ hybridization, the significantly lower expression of the studied genes in the gray matter may have hampered our ability to observe significant reductions in their expression in gray matter. Similar to the region effects observed in the current study, preliminary QPCR studies of gray vs. white matter have suggested that oligodendrocyte-related gene expression may be 3–6 fold lower in gray matter than in the underlying white matter of the frontal cortex (unpublished observations, C. Copland & V. Haroutunian, 2005).

There are several potential limitations of this study. We performed a secondary analysis of transcript expression in unmedicated (no antipsychotic medications within six weeks of death, $n = 10$) versus medicated ($n = 31$) subjects with schizophrenia, and did not detect any significant changes between these groups for CNP, MAG, TF, or QKI mRNA expression, suggesting that our findings may not be due to treatment with antipsychotics. However, despite being antipsychotic free within at least 6 weeks of death, all of the subjects in the unmedicated group had lifelong treatment with antipsychotics, leaving open the possibility that our findings are due to a long-lasting effect of chronic drug treatment on transcript expression. Another concern is that we studied aged individuals, a potential limitation of this collection as compared to those studied from other brain banks. However, recent studies of the ontogeny of myelin-associated gene expression across the human lifespan have indicated that the expression of many of these genes is relatively stable after age 30 in both gray and white matter (Copland et al., 2004). Finally, since we measured mRNA expression in brain sections, our findings do not indicate whether or not there was a change in protein expression, and do not specify the type of cell or synapse where gene expression is altered. However, studies conducted in this same cohort of subjects suggest that the protein expression of one of the studied genes, CNP, is significantly downregulated in the hippocampal formation (Dracheva et al., 2005b).

In summary, this work is the first to systematically examine expression of myelination related genes in the white matter in schizophrenia using a technique that does not rely on mRNA amplification. We found decreased expression of transcripts for QKI, MAG, CNP, and TF in the white matter of the ACC in schizophrenia, supporting the hypothesis that there are deficits in myelin synthesis and maintenance as well as oligodendrocyte function contributing to altered connectivity in this illness.

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Figure 1.

In situ hybridization using [^{35}S] labeled antisense riboprobes of a representative subject for motility-related protein-1 (CD9), Claudin-11 (CLDN11), gelsolin (GEL), myelin-associated glycoprotein (MAG), myelin oligodendrocyte glycoprotein (MOG), and peripheral myelin protein 22 (PMP22). Labeling for each is seen in the white matter (WM) and gray matter (GM) of the anterior cingulate cortex. The dashed line indicates the boundary of the white and gray matter, the solid line indicates the pial surface. Scale bar: 1mm

Figure 2.

In situ hybridization using [^{35}S] labeled antisense riboprobes of a representative subject for 2', 3'-cyclic nucleotide 3'-phosphodiesterase (CNP), erbB2 interacting protein (ErbB2IP), v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (ErbB3), oligodendrocyte transcription factor 2 (OLIG2), quaking (QKI), SRY-box containing gene 10 (SOX10), and transferrin (TF). Labeling for each is seen in the white matter (WM) and gray matter (GM) of the anterior cingulate cortex. The dashed line indicates the boundary of the white and gray matter, the solid line indicates the pial surface. Scale bar: 1mm

Figure 3.

Motility-related protein-1 (CD9), Claudin-11 (CLDN11), gelsolin (GEL), myelin-associated glycoprotein (MAG), myelin oligodendrocyte glycoprotein (MOG), and peripheral myelin protein 22 (PMP22) transcript expression levels in the white matter (WM) and gray matter (GM) of the anterior cingulate cortex in schizophrenia and a comparison group. Decreased transcript expression was detected for MAG in the WM.

Figure 4.

2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP), erbB2 interacting protein (ErbB2IP), v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (ErbB3), oligodendrocyte transcription factor 2 (OLIG2), quaking (QKI), SRY-box containing gene 10 (SOX10), and transferrin (TF) transcript expression levels in the white matter (WM) and gray matter (GM) of the anterior cingulate cortex in schizophrenia and a comparison group. Decreased transcript expression was detected for CNP, QKI, and TF in the WM.

Figure 5. 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP), transferrin (TF), quaking (QKI), and myelin-associated glycoprotein (MAG) transcript expression levels in white matter (WM) and gray matter (GM) of the anterior cingulate cortex in unmedicated (no antipsychotics within 6 weeks of death) and medicated subjects with schizophrenia.

Figure 6.

Correlation analysis of quaking (QKI) and 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) or myelin-associated glycoprotein (MAG) transcript expression levels in the anterior cingulate cortex for all subjects studied ($n = 75$). We found significant positive associations between QKI and CNP transcript expression and QKI and MAG transcript expression.

Table 1

Subject Characteristics						
Diagnosis	Age (Years)	Gender	Rx	PMI (Minutes)	pH	Cause of Death
CTL	96	F	N	195	6.7	Cardiopulmonary failure
CTL	90	F	N	250	6.0	Cardiopulmonary arrest
CTL	74	F	N	180	6.0	Cardiopulmonary failure
CTL	88	F	N	305	6.4	Cardiopulmonary failure
CTL	98	F	N	85	6.6	Cardiopulmonary failure
CTL	69	M	N	255	6.3	Lung cancer
CTL	82	F	N	340	6.1	Cardiopulmonary arrest
CTL	80	F	N	285	6.2	Cardiopulmonary failure
CTL	85	F	N	260	6.3	Acute myocardial infarction
CTL	102	F	N	423	6.5	Acute myocardial infarction
CTL	73	F	N	203	6.3	Acute myocardial infarction
CTL	84	M	N	1110	6.2	Myocardial infarction
CTL	101	F	N	280	6.8	Coronary artery disease
CTL	78	F	N	600	6.2	Cardiopulmonary arrest
CTL	96	F	N	270	6.3	Cardiac arrhythmia
CTL	95	M	N	245	6.5	Cardiopulmonary failure
CTL	65	M	N	230	6.8	Renal failure
CTL	89	F	N	1140	6.7	Cardiopulmonary arrest
CTL	83	F	N	370	6.8	Cardiopulmonary arrest
CTL	66	M	N	454	6.6	Cardiac arrest
CTL	82	F	N	1440	6.3	Cardiopulmonary failure
CTL	81	F	N	1128	6.1	Cardiopulmonary arrest
CTL	79	F	N	410	6.6	Acute coronary thrombosis
CTL	70	M	N	1230	5.9	Cardiopulmonary arrest
CTL	76	M	N	175	6.3	Cardiopulmonary arrest
CTL	93	M	N	250	6.4	Acute myocardial infarction
CTL	74	F	N	285	6.8	Cardiopulmonary arrest
CTL	80	F	N	230	6.3	Cardiopulmonary arrest
CTL	59	M	N	1225	6.6	Cardiopulmonary arrest
CTL	58	M	N	737	6.2	Coronary artery disease
CTL	54	M	N	1080	6.7	Sudden death
CTL	66	F	N	960	6.5	Accident
CTL	70	M	N	1080	6.2	Sudden death
CTL	72	M	N	720	6.6	Sudden death
	79 ± 12	13M/21F		542 ± 404	6.4 ± 0.3	
SCZ	80	F	N	415	5.8	Acute respiratory insufficiency
SCZ	58	M	Y	401	6.2	Cardiopulmonary arrest
SCZ	84	M	N	315	7.0	Cardiopulmonary arrest
SCZ	52	M	N	1770	5.9	Cardiopulmonary failure
SCZ	61	M	Y	212	6.5	Acute cardiac failure
SCZ	76	F	Y	510	6.1	Cardiopulmonary arrest, cancer
SCZ	58	M	Y	800	6.9	Cardiopulmonary failure
SCZ	57	M	Y	1220	6.1	Cardiopulmonary arrest
SCZ	63	M	Y	372	5.9	Cardiopulmonary arrest
SCZ	69	F	Y	820	6.2	Cardiopulmonary failure
SCZ	87	M	N	670	6.5	Cardiopulmonary failure
SCZ	68	M	Y	335	6.8	Cardiopulmonary failure
SCZ	86	M	Y	420	6.3	Cardiopulmonary arrest
SCZ	85	M	Y	424	6.8	Pneumonia
SCZ	83	F	N	1225	7.1	Cardiopulmonary arrest, cancer
SCZ	57	M	Y	940	6.4	Cardiopulmonary failure
SCZ	85	M	Y	320	6.3	Cardiopulmonary arrest
SCZ	73	M	Y	475	6.5	Cardiopulmonary failure
SCZ	66	M	Y	725	6.5	Acute cardiac failure
SCZ	86	M	N	320	6.2	Acute myocardial infarction
SCZ	76	F	Y	1270	6.1	Cardiogenic shock
SCZ	97	M	Y	555	6.5	Cardiopulmonary arrest
SCZ	86	M	N	845	6.7	Cardiopulmonary arrest
SCZ	66	M	Y	504	6.7	Cardiopulmonary arrest
SCZ	93	M	N	1060	6.6	Cardiopulmonary arrest
SCZ	82	M	N	1126	6.6	Cardiopulmonary arrest
SCZ	92	M	Y	1062	6.7	Cardiopulmonary arrest
SCZ	68	M	Y	1036	6.6	Pneumonia
SCZ	70	F	Y	794	6.5	Unknown
SCZ	69	M	Y	940	6.7	Respiratory failure
SCZ	76	M	Y	995	6.7	Cardiopulmonary arrest
SCZ	74	F	Y	417	6.3	Cardiopulmonary arrest
SCZ	86	F	Y	1092	5.8	Cardiopulmonary arrest
SCZ	59	F	Y	1315	5.7	Cardiopulmonary arrest
SCZ	84	M	N	1064	6.7	Cardiopulmonary arrest
SCZ	53	F	Y	377	5.9	Cancer
SCZ	82	M	Y	683	6.7	Respiratory failure
SCZ	73	M	Y	525	6.2	Cardiopulmonary arrest

Diagnosis	Age (Years)	Gender	Rx	PMI (Minutes)	pH	Cause of Death
SCZ	90	F	Y	465	6.0	Cardiopulmonary arrest
SCZ	77	F	Y	583	6.0	Cardiopulmonary arrest
SCZ	68	M	Y	534	6.3	Cardiopulmonary arrest
<hr/>						
	75 ± 12	29M/12F		730 ± 360	6.4 ± 0.4	

Abbreviations: control (CTL), schizophrenia (SCZ), postmortem interval (PMI), female (F), male (M), no antipsychotic treatment within 6 weeks of death (N), antipsychotic treatment within six weeks of death (Y). Summary data presented as means ± standard deviation.