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Differentiation of Oligodendrocyte Precursors is Impaired in the Prefrontal Cortex in Schizophrenia

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Abstract

The pathophysiology of schizophrenia involves disturbances of information processing across brain regions, possibly reflecting, at least in part, a disruption in the underlying axonal connectivity. This disruption is thought to be a consequence of the pathology of myelin ensheathment, the integrity of which is tightly regulated by oligodendrocytes. In order to gain insight into the possible neurobiological mechanisms of myelin deficit, we determined the messenger RNA (mRNA) expression profile of laser captured cells that were immunoreactive for 2', 3'-cyclic nucleotide 3'-phosphodiesterase (CNPase), a marker for oligodendrocyte progenitor cells (OPCs) in addition to differentiating and myelinating oligodendrocytes, in the white matter of the prefrontal cortex in schizophrenia subjects. Our findings pointed to the hypothesis that OPC differentiation might be impaired in schizophrenia. To address this hypothesis, we quantified cells that were immunoreactive for neural/glial antigen 2 (NG2), a selective marker for OPCs, and those that were immunoreactive for oligodendrocyte transcription factor 2 (OLIG2), an oligodendrocyte lineage marker that is expressed by OPCs and maturing oligodendrocytes. We found that the density of NG2-immunoreactive cells was unaltered, but the density of OLIG2-immunoreactive cells was significantly decreased in subjects with schizophrenia, consistent with the notion that OPC differentiation impairment may contribute to oligodendrocyte disturbances and thereby myelin deficits in schizophrenia.

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AUTHORS' CONTRIBUTIONS

SAM and CYP carried out LCM and RNA processing for microarray experiments. SAM performed qRT-PCR validation and immunohistochemistry, participated in the microarray data analysis and drafted the manuscript. KCS participated in data analysis and manuscript preparation. TUWW conceived of the study, participated in its design and data interpretation, and prepared the manuscript.

CONFLICTS OF INTEREST AND FINANCIAL DISCLOSURE

None.

INTRODUCTION

Schizophrenia can be conceptualized as the clinical manifestation of the dysfunction of information processing and integration of neural networks across brain regions, including the different areas of the cerebral cortex and subcortical domains, such as the thalamus and medial temporal structures. It is well established that neuronal circuits within these regions are functionally disturbed in schizophrenia. Furthermore, impaired myelin ensheathment of the axons that connect these neuronal circuits via the white matter can further exacerbate network dysfunction by disturbing the timing of the arrival of action potential, compromising spike-timing dependent potentiation and hence disrupting the precise temporal coherence of neuronal circuit output that is necessary for synchronized network activation (Fields, 2005; Fields et al., 2014; Pajevic et al., 2013) which, in turn, may underlie many of the cognitive and perceptual deficits that are characteristic of schizophrenia (Cho et al., 2006; Lisman and Buzsaki, 2008; Spencer et al., 2004; Uhlhaas et al., 2008; Uhlhaas and Singer, 2010).

Structural magnetic resonance imaging (MRI) investigations have converged upon the conclusion that the volume of the white matter is decreased in patients with schizophrenia (Bora et al., 2011; Breier et al., 1992; Buchanan et al., 1998; Sigmundsson et al., 2001), consistent with the notion that the integrity of myelin is compromised. Furthermore, diffusion tensor imaging (DTI) has revealed that fractional anisotropy, which is a measure of a combination of factors, including myelin sheath integrity, is also decreased in schizophrenia patients (Holleran et al., 2014). More direct evidence of myelin deficit in this illness has come from postmortem studies. Specifically, the density of oligodendrocytes has been found to be decreased by 25-31% in both the gray and white matter of the prefrontal cortex in schizophrenia subjects (Hof et al., 2003; Uranova et al., 2004; Uranova et al., 2007; Vostrikov and Uranova, 2011). Furthermore, ultrastructural examination of oligodendrocytes has revealed morphological changes associated with cell degeneration and death (Uranova et al., 2001; Uranova et al., 2007), in addition to disturbances in the integrity of myelinated axonal fibers (Uranova et al., 2011). Finally, gene expression profiling of RNA extracted from homogenized gray and white matter of the cerebral cortex have revealed that many oligodendrocyte- and myelin-associated genes appear to be differentially expressed in subjects with schizophrenia (Aston et al., 2004; Hakak et al., 2001; Haroutunian et al., 2007; Hof et al., 2002; Katsel et al., 2005; Mitkus et al., 2008; Sequeira et al., 2012; Sugai et al., 2004; Tkachev et al., 2003).

Myelin is actively maintained by a complex series of events that tightly regulate the generation, differentiation, survival and death of oligodendrocytes. In the adult rat brain, roughly 5-10% of cells are oligodendrocyte progenitors (OPCs) (Dawson, 2003; Polito and Reynolds, 2005), whereas approximately 20% of the cells in the white matter in humans may be OPCs (Lojewski et al., 2014). It is further estimated that up to 80% of OPCs are actively replicating or differentiating (Trotter et al., 2010; Young et al., 2013). Disturbances of any of the events that underlie the differentiation and mediate the integrity of oligodendrocytes could lead to myelin and myelination deficits.

In an attempt to gain insight into the possible molecular mechanisms specifically associated with the dysfunction of cells that belong to the oligodendrocyte lineage and thereby myelin and myelination deficits in the white matter, we profiled the expression of mRNA in these cells obtained by laser-capture microdissection (LCM). CNPase (2',3'-cyclic nucleotide 3'-phosphodiesterase) was chosen as a marker, because it is expressed in OPCs in addition to differentiating and myelinating oligodendrocytes (Belachew et al., 2001; Dawson, 2003; Levine et al., 1993; Polito and Reynolds, 2005; Reynolds et al., 2002; Scherer et al., 1994; Sprinkle, 1989; Tomassy and Fossati, 2014). Together with immunohistochemical validation of findings of our gene expression profiling experiment using oligodendrocyte lineage markers including neural/glial antigen 2 (NG2), a marker for OPCs, and oligodendrocyte transcription factor 2 (OLIG2), which is present in maturing and mature, myelinating oligodendrocytes, the preponderance of evidence derived from the present study appears to favor the interpretation that impairment in the differentiation of OPCs may contribute to the pathophysiology of white matter deficits of schizophrenia.

MATERIALS AND METHODS

Postmortem human brain tissue

Fresh-frozen tissue blocks containing the prefrontal cortex (Brodmann's area 9) from 9 schizophrenia and 9 normal control subjects, matched for age, sex and postmortem interval (PMI), were obtained from the Harvard Brain Tissue Resource Center (**Table 1**). A detailed methodology for tissue preparation, LCM and RNA processing has been described in detail elsewhere (Pietersen et al., 2011; Pietersen et al., 2009; Pietersen et al., 2014a; Pietersen et al., 2014b; Simunovic et al., 2009). Postmortem human brain collection procedures have been approved by the Partners Human Research Committee. Written informed consent for use of each of the brains for research has been obtained by the legal next-of-kin. The diagnosis of schizophrenia was made by two Board-certified psychiatrists by reviewing medical records and an extensive family questionnaire that included medical, psychiatric and social history. All of the brains included in this study were also examined by a Board-certified neuropathologist to rule out any neurological conditions. In addition, none of the subjects had any history of active substance abuse or dependence, as confirmed by toxicological analysis.

Laser-Capture Microdissection

Sections of 8 μm were cut on a cryostat, mounted on slides and stored at -80°C until use. Cells immunoreactive for CNPase, a myelin-associated enzyme that recognizes OPCs and both mature and immature oligodendrocytes, from the white matter (within 50 μm from the gray matter border) in the PFC were isolated using the Arcturus XT™ system (Life Technologies, Grand Island, CA). Approximately 400 cells per subject were captured onto a CapSure HS™ LCM cap (Life Technologies, Grand Island, CA). To avoid systematic biases, samples from schizophrenia and normal control subjects were processed for LCM in a random order.

Affymetrix platform-based microarray gene expression profiling

RNA processing—RNA isolation was performed using the Picopure™ RNA Isolation kit (Life Technologies, Grand Island, NY), with a DNase step (Qiagen, Valencia, CA). This typically resulted in approximately 1-25 ng of total RNA (Pietersen et al., 2011; Pietersen et al., 2009). The extracted RNA underwent two rounds of linear amplification using the RiboAmp® kit (Life Technologies, Grand Island, NY). A dilution of the resulting products (approximately 250 pg/μl) was used to determine the distribution of transcript lengths with the Experion StdSens Labchip (Bio-Rad Laboratories, Hercules, CA; **Supplementary Figure S1**). The concentration and purity of these samples were determined by absorbance measurements at the optical density of A260 and A280, using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA).

Data analysis—Each array was scanned twice and the Affymetrix Microarray Suite 5.1 software averaged the two images to compute an intensity value for each probe cell within each probe set. For the quality control step, we employed the Partek® software's built-in function (Partek, St. Louis, MO). We then normalized the data with Partek's standard normalization method (i.e. data has a mean of zero and a variance of one, and each column for each sample was divided by the average of all control samples). Differentially expressed genes were visualized by performing unsupervised hierarchical clustering as stringency of the filtering criteria (i.e. fold-change and FDR-adjusted p-value) was varied to determine a representative gene list for pathway analyses.

Pathway analyses were performed with two web-based algorithms, *Ingenuity Pathway Analysis* (Ingenuity Systems, Redwood City, CA) and *MetaCore* (GeneGo, New York, NY), to map the differentially expressed genes onto biological functions and canonical pathways (Pietersen et al., 2014a; Pietersen et al., 2014b). With *Ingenuity*, the significance for each of the identified pathways was determined via a Fisher's exact test, whereas GeneGo *Metacore* makes use of their algorithm for hypergeometric distribution, identifying pathways overrepresented with significant genes. Literature mining was then performed to elucidate the pathways or gene families that were particularly pertinent for oligodendrocyte functions.

Microarray and qRT-PCR validation—The TURBO Biotin labeling™ kit (end-labeling; Life Technologies, Grand Island, NY) was used to label the aRNA obtained from amplified samples. Gene expression profiling was performed using the Affymetrix Human X3P GeneChip®, which possesses an extreme 3' bias in its probe design and hence is particularly suitable for samples that are prone to RNA degradation, such as postmortem human brain tissue. This chip has also been shown to be superior to the more commonly used Affymetrix human U133 plus 2.0 chip in terms of data reproducibility (Caretti et al., 2008). The hybridization and scanning procedures were performed at the Partners HealthCare Center for Personalized Genetic Medicine, Cambridge, MA.

For validation of microarray data, cDNA was reverse transcribed from aRNA (200 ng input) using the High Capacity RNA-to-cDNA kit (Applied Biosystems, Carlsbad, CA). TaqMan®-based qRT-PCR (Applied Biosystems, Carlsbad, CA) was subsequently performed on selected differentially expressed genes within signaling pathways identified by

pathway analysis as dysregulated in schizophrenia and randomly chosen genes (**Supplementary Table S1**). Due to the small amount of RNA available from laser-captured samples, only a relatively small number of genes were included. Samples were normalized with respect to the housekeeping gene, hypoxanthine guanine phosphoribosyltransferase (HPRT), which has been shown to produce reliable results in human brain tissue as its expression has been shown not to be altered in disease states (Radonic et al., 2004). Normalization was performed against only one gene because of the limited quantity of RNA available from laser-captured materials. Negative controls (negative reverse transcription and no template controls) were performed to detect any contamination of the samples, such as genomic (g)DNA. Where necessary, samples were treated with Turbo DNA-free™ kit (Applied Biosystems, Carlsbad, CA) and the negative controls repeated to confirm gDNA removal before qRT-PCR was performed. Samples were run in duplicate and the average of the duplicates was taken as input for quantification using the $2^{-\Delta C_t}$ method (Livak and Schmittgen, 2001) or the relative expression software tool (REST) for group-wise comparisons of expression ratios (Pfaffl et al., 2002). A Spearman Rho's correlation analysis was then performed on the fold-changes determined by microarray and qRT-PCR. In accordance with Morey et al. (Morey et al., 2006), a correlation of >0.8 with a significance of $p < 0.05$ between qRT-PCR and microarray expression changes was considered validation of the microarray result.

NG2 and OLIG2 Immunohistochemistry

Tissue blocks were sectioned at 20 μm , mounted on gelatin-subbed slides, and post-fixed in 4% paraformaldehyde for 20 minutes at room temperature. Sections were then incubated in endogenous enzyme block (1% H_2O_2 , 10% MeOH) for 15 minutes and, additionally, blocked using 2% bovine serum albumin (BSA) in 10% goat serum (Life Technologies, 16210-064, Grand Island, NY) at room temperature for 1 hour, followed by incubation in an anti-NG2 (1:200, Sigma-Aldrich, St. Louis, MO) or anti-OLIG2 (1:1,000, Phosphosolutions, Aurora, CO) antibody produced in rabbit (Sigma-Aldrich, St. Louis, MO) at 4°C overnight. Sections were then incubated at room temperature in biotinylated anti-rabbit IgG antibody produced in goat (1:500, Vector Labs, Burlingame, CA), followed by a 2-hour incubation in horseradish peroxidase-conjugated streptavidin (1:5,000, Zymed, San Francisco, CA) made in 1 Mol/L of phosphate buffer (PB) at room temperature, and finally in nickel-enhanced diaminobenzidine/peroxidase reaction (0.02% diaminobenzidine, 0.08% nickel-sulphate, 0.006% hydrogen peroxide in 1 Mol/L PB). Sections were finally dehydrated and coverslipped.

The number of NG2- or OLIG2-immunoreactive cells within three 100 $\mu\text{m} \times 100 \mu\text{m}$ squares that were 500 μm apart from one another were quantified by the same investigator (SAM) in a blind fashion using a StereoInvestigator system (MBF Bioscience, Williston, VT). Cell densities were compared between subjects with schizophrenia and normal control subjects using unpaired t-test. We also used correlation analysis to evaluate any potential confounding effect of each of the numerical covariates (i.e. age, PMI, antipsychotic exposure in terms of chlorpromazine equivalent) on the cell density measures. The possible confounding effect of sex was evaluated by comparing cell densities between the two sexes within each of the two subject groups.

RESULTS

Gene expression profile of CNPase-immunoreactive cells in schizophrenia

We identified 497 genes that were differentially expressed (i.e. fold-change >1.5 and $p < 0.05$) in the CNPase-immunoreactive cells in subjects with schizophrenia (**Supplementary Table S2**), the majority of which (56%) were downregulated. Pathway analysis revealed that many of the signaling cascades related to oligodendrocyte proliferation and differentiation appeared to be differentially regulated in schizophrenia subjects (**Table 2**). Validation of microarray findings were performed by statistically comparing gene expression changes detected by microarray and qRT-PCR (Morey et al., 2006), which were found to be highly significantly correlated ($R^2=0.85$, $p=0.005$; **Figure 1**).

Immunohistochemical quantification of OPC differentiation

In order to address the hypothesis that OPC differentiation might be altered in schizophrenia, we quantified cells that were immunoreactive for NG2, a marker for OPCs, and those that were immunoreactive for OLIG2, an oligodendrocyte lineage marker for OPCs, maturing and possibly also mature oligodendrocytes (Ligon et al., 2004; Ligon et al., 2006). We found that the density of NG2-immunoreactive cells was unaltered in schizophrenia ($1,973.3 \pm 447.6$ cells/mm²) compared to normal control ($2,124.0 \pm 462.6$ cells/mm²) subjects, but the density of OLIG2-immunoreactive cells was significantly ($p=0.032$) decreased by 21.3% in schizophrenia ($2,140.5 \pm 517.6$ cells/mm²) compared to normal control ($2,721.9 \pm 440.0$ cells/mm²) subjects (**Figure 2**). Our statistical analyses indicate that these findings were not affected by any of the potential confounding variables.

DISCUSSION

Findings of the gene expression profiling experiment reported here suggest that dysregulation of the differentiation of OPCs may contribute to oligodendrocyte and myelin deficits of schizophrenia. To address this hypothesis, we immunohistochemically visualized cells that expressed NG2, a marker for OPCs, and OLIG2, an oligodendrocyte lineage marker for OPCs, maturing oligodendrocytes and some mature, myelinating oligodendrocytes. We found that the density of NG2-immunoreactive cells was unaltered, but the density of OLIG2-immunoreactive cells was significantly decreased in subjects with schizophrenia. Hence, even though the sample size of our gene expression profiling experiment was relatively small, a key hypothesis derived from findings of this experiment is in fact substantiated by our immunohistochemical findings. It should be noted, however, that these findings by themselves do not necessarily allow us to definitively conclude that oligodendrocyte differentiation is impaired, as the observed decrease in OLIG2-immunoreactive cells could reflect a deficit in the mature but not the maturing oligodendrocytes. Nevertheless, the interpretation of these findings in the context of the microarray observations does appear to favor the argument that impairment in the differentiation of OPCs contributes to the pathophysiology of schizophrenia.

Using LCM in combination with the Affymetrix platform of microarray, we identified signaling pathways that appear to be differentially regulated in cells of oligodendrocyte

lineage in the white matter in the prefrontal cortex in schizophrenia. One of the most noticeable observations of this study is the dysregulation of platelet-derived growth factor (PDGF) signaling in subjects with schizophrenia (**Table 2**). For instance, PDGF receptor alpha (PDGFRA), which is specifically expressed in OPCs (Armati and Mathey, 2010; Baracskey et al., 2007; Wilson et al., 2006), was found to be upregulated by 3.8-fold. Previous studies have found that in the absence of PDGF, all OPCs stop dividing and instead differentiate into oligodendrocytes (Durand and Raff, 2000), which leads to the downregulation of PDGFRA (Hart et al., 1989). In this context, the observation that PDGFRA is upregulated in schizophrenia is consistent with the interpretation of impaired differentiation of OPCs into mature oligodendrocytes.

The substrate of PDGFRA, PDGF, regulates the survival, proliferation and differentiation of OPCs in part via phosphoinositide 3-kinase (PI3K) signaling. In fact, PIK3CA was also found to be upregulated by 3.4-fold in subjects with schizophrenia. Furthermore, in light of the fact that sonic hedgehog (SHH) appears to promote the OPC fate (Armati and Mathey, 2010; Mitew et al., 2013; Tomassy and Fossati, 2014), we found that SHH signaling was differentially regulated in subjects with schizophrenia. Other signaling pathways found to be differentially regulated in this study (**Table 2**), such as NOTCH signaling, are known to inhibit the oligodendrocyte differentiation process (Wang et al., 1998), whereas pathways such as the thromboxane A2 signaling cascade have been shown to promote OPC proliferation and oligodendrocyte survival (Lin et al., 2005; Mir and Le Breton, 2008). WNT signaling, on the other hand, appears to either promote or inhibit oligodendrocyte differentiation in a context-dependent fashion (Emery, 2010; Guo et al., 2015; Mitew et al., 2013). Similarly, TGF-beta signaling has been shown to play an important role in regulating the balance between OPC proliferation and oligodendrocyte differentiation (Zhang et al., 2010). Finally, cell cycle G1-S growth factor regulation pathway also appears to be affected. Altogether, the preponderance of evidence derived from this study is consistent with the hypothesis that mechanisms that regulate the generation and differentiation of oligodendrocytes are involved in the pathophysiology of this illness.

To more directly address the hypothesis that oligodendrocyte differentiation may be dysregulated in schizophrenia, we compared the density of NG2- and OLIG2-immunoreactive cells between schizophrenia and normal control subjects. Because NG2 is a specific OPC marker, our finding that the density of NG2-immunoreactive neurons was unchanged in schizophrenia suggests that OPCs are numerically unaltered. In this context, because OLIG2 is expressed in both OPCs and maturing oligodendrocytes in addition to some mature oligodendrocytes, our observation of decreased density of OLIG2-immunoreactive cells in schizophrenia subjects, together with a previous study showing reduced immunoreactivity for CNPase (Flynn et al., 2003), a marker for the entire oligodendrocyte lineage, is consistent with the notion that oligodendrocyte differentiation is impaired in schizophrenia. Future studies should focus on further delineating the specific stage(s) during oligodendrocyte differentiation and maturation that are affected in schizophrenia by employing stage-specific markers.

Our data do not appear to implicate genes directly associated with myelination and myelin synthesis as differentially regulated in schizophrenia. Perhaps myelin deficit in the white

matter in schizophrenia may be more of a consequence of an “inadequate” supply of myelinating oligodendrocyte instead of a deficit in the synthesis of myelin by oligodendrocytes, although these two possibilities need not be mutually exclusive. In fact, previous studies have found that some of the myelin-associated genes appear to be differentially expressed in schizophrenia, although findings from these studies have not always been consistent (Aston et al., 2004; Hakak et al., 2001; Katsel et al., 2005; Mitkus et al., 2008; Sequeira et al., 2012; Sugai et al., 2004; Tkachev et al., 2003). Our findings are in line with previous studies showing that the density of oligodendrocytes is decreased in both the gray and white matter of the prefrontal cortex in schizophrenia subjects (Hof et al., 2003; Uranova et al., 2007; Vostrikov and Uranova, 2011), although a lack of significant change in the density of these cells has also been reported (Hercher et al., 2014; Uranova et al., 2004). Insofar as the number of oligodendrocytes is in fact decreased in schizophrenia, this reduction has been speculated to represent a deficit in the normal age-dependent increase in the number of oligodendrocytes (Vostrikov and Uranova, 2011), perhaps reflecting an intrinsic deficit in cell lineage programming and differentiation. The decrease in the number of oligodendrocytes together with the resultant myelin deficit may together contribute to the observed reduction in white matter volume in various brain regions, including the prefrontal cortex, in patients with schizophrenia (Bora et al., 2011; Breier et al., 1992; Buchanan et al., 1998; Sigmundsson et al., 2001).

Oxidative stress is increasingly recognized as an important mechanism that may lead to the cellular insults characteristic of the pathophysiology of schizophrenia (Bitanhirwe and Woo, 2011; Do et al., 2009; Do et al., 2015; Fournier et al., 2014; Kulak et al., 2013). For instance, animal studies have shown that the inhibitory neurons that contain the calcium-binding protein parvalbumin, which are strongly implicated in contributing to the functional deficits of the cerebral cortex in schizophrenia, are particularly vulnerable to oxidative insult (Cabungcal et al., 2013a; Cabungcal et al., 2013b; Morishita et al., 2015). Likewise, oligodendrocytes are also especially susceptible to oxidative injury (Monin et al., 2014), which may in part contribute to the apparent decrease in the number of oligodendrocytes in schizophrenia (Hof et al., 2003; Uranova et al., 2004). The fact that there is an apparent failure in OPC differentiation to replenish the oligodendrocyte pool is likely to magnify the pathophysiological consequence of oxidative insult to oligodendrocytes. In this context, it is of interest to point out that haloperidol has previously been shown to potentially promote oligodendrocyte differentiation (Fang et al., 2013; Wang et al., 2010) and quetiapine has been found to directly facilitate myelin regeneration by replenishing mature oligodendrocytes in animals (Zhang et al., 2012). Hence, the efficacy of antipsychotics in the treatment of schizophrenia may, at least in part, be mediated by regulating oligodendrocyte lineage.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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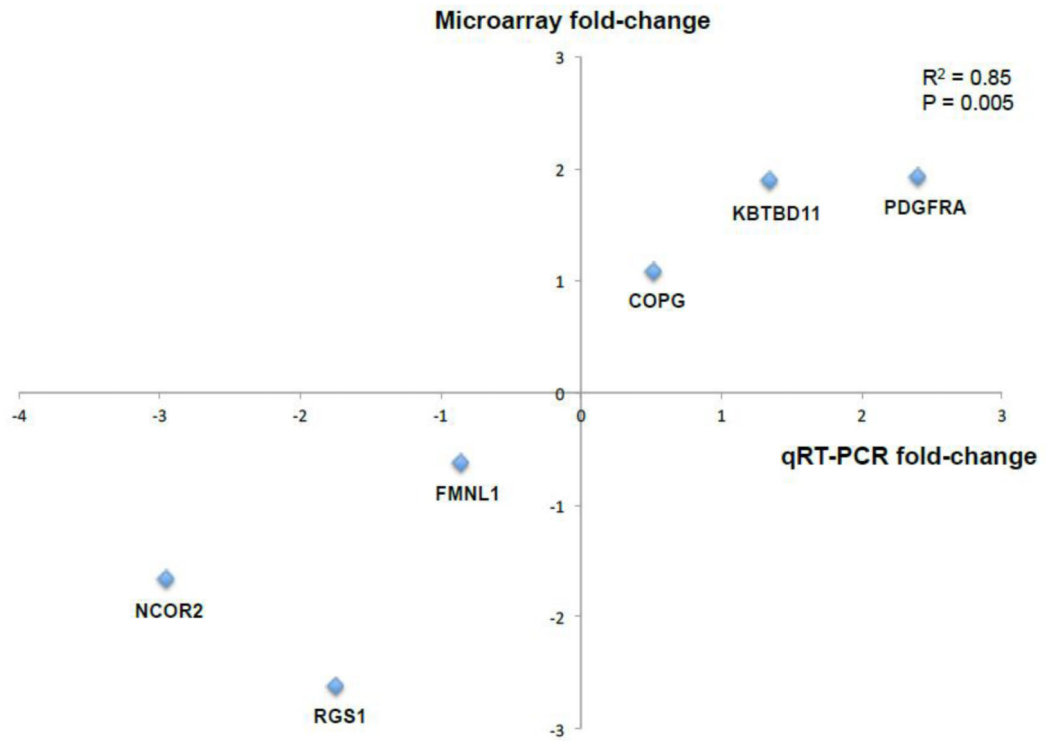


Figure 1. Gene expression changes detected by microarray and qRT-PCR are highly significantly correlated ($R^2=0.85$, $p=0.005$).

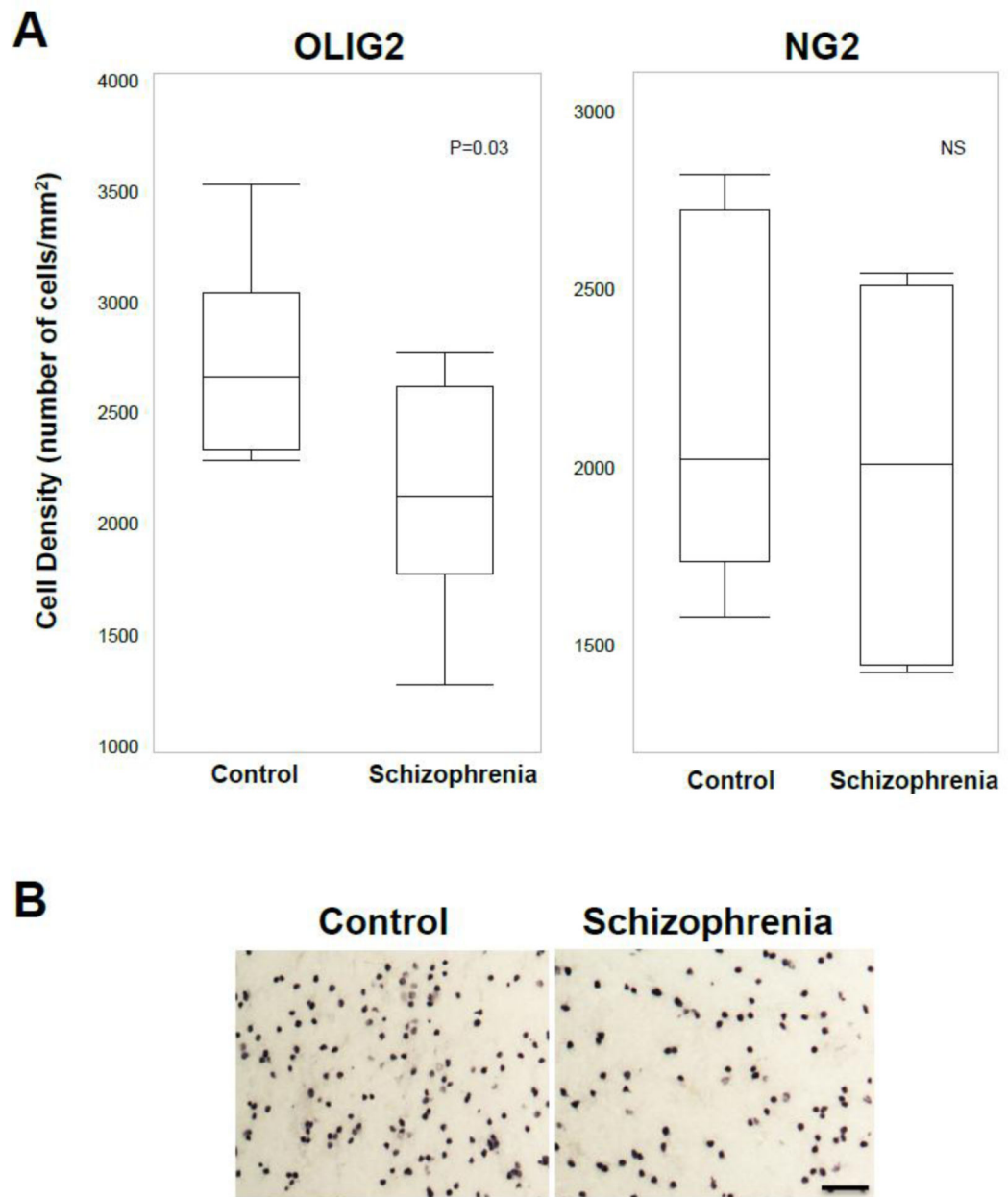


Figure 2.

A. The density of OLIG2-immunoreactive cells is significantly decreased whereas the density of NG2-immunoreactive cells is unaltered in subjects with schizophrenia. **B.** Photomicrographs showing OLIG2-immunoreactive cells in a schizophrenia (right) and a normal control (left) subjects. Scale bar=50 μ m.

Table 1

Cases used in the present study

Diagnosis	Age	Sex	PMI	aRNA concentration ng/ul
Schizophrenia	69	F	23.08	923.13
Schizophrenia	75	F	18.66	457.45
Schizophrenia	58	M	25.33	519.9
Schizophrenia	77	M	25.33	1159.7
Schizophrenia	66	M	21.75	845.9
Schizophrenia	63	M	26.16	450.07
Schizophrenia	60	F	17.13	706.1
Schizophrenia	60	M	22.17	1091.2
Schizophrenia	55	F	22	772.6
	64.8 ± 7.6		22.4 ± 3.0	769.6 ± 262.4
Control	69	F	18.65	1700
Control	78	F	18.7	295.16
Control	58	M	20.75	665.7
Control	76	M	23.92	684.6
Control	58	M	19.28	1437.88
Control	63	M	17.92	968.2
Control	60	F	21.67	645
Control	61	M	21	644.6
Control	63	F	23.5	800.4
	65.1 ± 8.4		20.1 ± 2.1	871.3 ± 437.9

Table 2

Differentially regulated signaling pathways associated with oligodendrocyte differentiation in schizophrenia

Pathway	FDR-adjusted p value
Normal and pathological TGF-beta-mediated regulation of cell proliferation	1.395e-6
Signal transduction_NOTCH signaling	2.674e-5
Development_PDGF signaling via MAPK cascades	1.611e-4
Development_Thromboxane A2 signaling pathway	8.202e-4
Development_Growth factors in regulation of oligodendrocyte precursor cell proliferation	8.515e-4
Cell cycle_G1-S Growth factor regulation	2.412e-4
Development_Hedgehog signaling	2.477e-3
Apoptosis_Anti-Apoptosis mediated by external signals via MAPK and JAK/STAT	5.738e-3
Signal transduction_WNT signaling	1.647e-2

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