Variations in oligodendrocyte-related gene expression across multiple cortical regions: implications for the pathophysiology of schizophrenia

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Abstract
The disconnectivity syndrome hypothesis of schizophrenia suggests that communication between multiple brain circuits and regions may be disrupted. Microarray studies analysed gene expression in 15 different brain regions derived from 13 persons with schizophrenia and controls. The superior temporal gyrus, cingulate gyrus and hippocampus evidence the greatest numbers of abnormally expressed genes. Gene ontology categorization suggested that gene classes associated with oligodendrocytes and myelin function were among the most profoundly affected. qPCR and additional microarray studies have validated these oligodendrocyte- and myelin-associated findings in independent cohorts. At least some of the affected genes are associated with the regulation of axoglial contacts, axon calibre and the integrity of functional elements involved in signal propagation. The confluence of emerging evidence shows that myelination abnormalities are major components of the neurobiology of schizophrenia and suggest that re-evaluation of some long-held hypotheses and beliefs regarding the biological substrates of schizophrenia may be warranted.

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Introduction
Abnormalities in neural networks – dis- or mis-connectivity and aberrant communication among different brain regions have been postulated to contribute to the central pathophysiology of schizophrenia (Arnold and Trojanowski, 1996; Davis et al., 2003; Haroutunian and Davis, 2000; Raedler et al., 1998; Stewart and Davis, 2004; Weinberger, 1995; Weinberger and Lipska, 1995). The neurobiological substrates of these connectivity and communication abnormalities remain unknown, but recent evidence, some of which is summarized here and in the Special Section of this issue, suggests that abnormalities in myelination and oligodendrocytes may be prominent features of schizophrenia (SZ).

Myelin-related abnormalities represent plausible candidates for influencing disease expression. It is possible that inadequate myelination, whether due to abnormal development, maintenance or oligodendrocyte death, contributes to some of the deficits necessary to impair or alter signal flow and brings about at least some of the symptoms of SZ. In addition to affecting signal conductance and propagation (Baumann and Pham-Dinh, 2001; Waxman and Bangalore, 2004), abnormalities in myelin and oligodendrocytes are well-positioned to account for many of the neurobiological disturbances associated with SZ: oligodendrocytes are involved in regulating glutamate function and are in turn affected by glutamate (Gallo and Ghiani, 2000; Matute et al., 2006; Park et al., 2004); oligodendrocyte precursors express dopamine D_2 and D_3 receptors (Bongarzone et al., 1998; Nikulina et al., 1995; Rosin et al., 2005); down-regulation of
oligodendrocyte-associated genes can affect the levels and metabolism of dopamine (Nikulina et al., 1995); as described by Segal et al. (2007), abnormalities in oligodendrocyte-associated gene expression in rodent models can influence neuronal morphology with features similar to those described in SZ (Benes et al., 1986; Rajkowska et al., 1998; Selemon et al., 1998); oligodendrocytes provide neurotrophic support (Deng et al., 2004; Du and Dreyfus, 2002); finally, there is an apparent temporal relationship between myelination and the onset of SZ (Benes, 1989; Benes et al., 1994; Giedd et al., 1999; Goldman-Rakic and Selemon, 1997; Huttenlocher, 1979; Terry et al., 1987). Thus, myelin gene expression abnormalities could have consequences for cytoarchitectural and chemoarchitectural organization that are consistent with some of the most robust neurobiological findings in SZ and with the disconnectivity hypothesis.

Direct evidence for the involvement of oligodendrocytes and myelin in SZ has come from analysis of post-mortem tissue from persons with SZ using microarray gene expression (Aston et al., 2004; Bahn and Jones, 2003; Hakak et al., 2001; Iwamoto et al., 2005; Katsel et al., 2004a, 2005a,b; Sugai et al., 2005; Tkachev et al., 2003), protein expression (Dracheva et al., 2005; Flynn et al., 2003), light and electron microscopic studies (Hof et al., 2003; Miyakawa et al., 1972; Uranova et al., 2001, 2004), and in-vivo neuroimaging (Buchsbaum et al., 1998; Foong et al., 2000, 2001; Lim et al., 1999). The majority of the post-mortem studies have focused on a single brain region – the prefrontal cortex. Neurobiological studies of SZ suggests, however, that multiple other brain regions, including portions of the limbic system and its associated circuits (prefrontal cortex, cingulate gyrus and hippocampus) are also profoundly affected (Benes et al., 1998, 2001; Benes, 1999; Goldman-Rakic, 1999; Haroutunian and Davis, 2000; Meyer-Lindenberg et al., 2001; Pulver et al., 1994; Rajkowska et al., 1998; Selemon et al., 1998; Selemon and Goldman-Rakic, 1999; Selemon and Rajkowska, 2003; Tamminga et al., 2000; Todtenkopf et al., 2005).

It is essential therefore to determine the neuro-anatomical distribution of myelin-associated deficits in SZ and to ascertain which brain regions are most vulnerable. The studies summarized below have approached investigation of oligodendrocyte- and myelin-related (OMR) abnormalities in SZ from a brain ‘regions of vulnerability’ perspective. First, using large-scale microarray technology we ‘mapped’ the brain regions that evidence the greatest abnormalities in gene expression. Then, we determined whether the regions with the greatest numbers of abnormally expressed genes overlap with the regions that evidence the most pronounced abnormalities in the expression of OMR genes. Finally, we confirmed the gene expression deficits revealed by microarray using quantitative RT–PCR (qPCR) in multiple post-mortem brain regions derived from persons with ante-mortem assessed and diagnosed SZ and controls.

Microarray studies

Post-mortem tissue specimens from the superior frontal gyrus, frontal pole, insular cortex, dorsolateral prefrontal cortex, anterior and posterior cingulate gyrus, inferior parietal lobe, inferior, middle and superior temporal gyrus, parahippocampal gyrus/entorhinal cortex, primary visual cortex, hippocampus, caudate nucleus, and putamen of persons with SZ were compared to control subjects. The studies described were approved by the Mount Sinai Institutional Review Board and consent for autopsy was obtained from the legal next of kin of all subjects. All subjects were residents of nursing homes and chronic care facilities. The demographic characteristics and diagnostic procedures used for subject selection have been described extensively (Katsel et al., 2004b, 2005a). Microarray analysis was performed by Gene Logic Inc. (Gaithersburg, MD, USA) using Affymetrix (Santa Clara, CA, USA) HG-U133 A&B Human genome GeneChip® sets (containing probes for over 33,000 human genes) (Katsel et al., 2004b, 2005a). All of the brain specimens were derived from the left hemisphere and each dissected region was comprised of primarily grey matter. An average of 13 persons with SZ (range 9–21 cases per region) and 13 control subjects (range 8–18) contributed to each individual brain region analysed. Subjects did not differ significantly with respect to the demographic parameters of age, post-mortem interval (PMI) and brain tissue pH (age 74 yr and 80 yr, PMI 8.4 h and 11.7 h, brain pH 6.5 and 6.5 for control and SZ groups, respectively). All subjects died of natural causes and none had a history of licit or illicit drug abuse (tobacco excepted).

Schizophrenia-related alterations in regional gene expression

The total number of genes expressed in the different brain regions examined was relatively constant and ranged from 12045 in the primary visual cortex (BA 17) to 15594 in the putamen. Consistent with other studies (Evans et al., 2003; Harrison et al., 1997) the total number of expressed transcripts in controls
and persons with SZ were not significantly different from each other. The total number of genes with altered expression in SZ varied from brain region to brain region as summarized in Figure 1 (Katsel et al., 2005a). The superior temporal cortex (STC, BA 22) of the SZ group showed the largest number of altered transcripts. The anterior (BA 24/32) and posterior (BA 23/31) cingulate cortices followed the superior temporal gyrus with \( \sim 50\% \) fewer altered transcripts (\( \sim 170 \) altered genes), which was followed by the hippocampus which evidenced \( \sim 50 \) dysregulated genes. The rank ordering of brain regions with respect to the total number of altered transcripts remained nearly identical (Spearman’s \( r = 0.94, p = 0.0000003 \)), irrespective of whether high (fold change \( \geq 1.7 \), present calls \( \geq 80\% \)) or low (fold change \( \geq 1.4 \), present calls \( \geq 80\% \)) stringency criteria or \( p < 0.05 \) or \( p < 0.001 \) were used for detection suggesting that differential vulnerability of different brain regions to gene expression abnormality in SZ is independent of the criteria used to detect abnormal gene expression. These findings are consistent with post-mortem and neuroimaging studies that have repeatedly shown the temporal, medial temporal/hippocampal, cingulate and frontal cortical regions of the limbic pathway to be susceptible in SZ (Baumann and Bogerts, 1999; Benes, 2000; Bogerts, 1997; Buchsbaum, 1990; Copland et al., 2004; Harrison and Eastwood, 2001; Todtenkopf et al., 2005).

**Relationship of the myelin-related genes to brain regional changes in gene expression**

Gene ontology (GO) classification is one method for the grouping of genes into functional classes to facilitate interpretation of large-scale microarray studies. The gene expression results of the study cited above were subjected to GO categorization (Katsel et al., 2005b) by functional class scoring (FCS) (Pavlidis et al., 2002a,b, 2004) using the ErmineJ software (version 2.0b5, Columbia University, NY, USA). An average of \( \sim 15000 \) individual transcript \( p \) values per region were subjected to the FCS using 4123 GO terms from three independent GO areas: biological process; molecular function; and cellular component. GO classes categorized as nerve maturation/myelination, peripheral nervous system development, regulation of action potential and axonogenesis and heterophilic cell adhesion all include genes related to oligodendrocyte function and myelin formation and had FCS values that were the highest scores in the cingulate cortex, temporal cortex and in the hippocampus relative to all other classes. Thus, in the brain regions that showed the greatest number of aberrantly expressed genes in SZ, genes that relate to functional classes involving myelination and oligodendrocyte-neuron interactions were among the most affected genes.

Among the most affected genes within the GO classes that subsume OMR genes were myelin-associated glycoprotein (MAG), 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP), quaking homologue, KH domain RNA-binding protein (mouse) (QKI) and transferrin (TF). They were strongly down-regulated in multiple brain regions including the cingulate cortex and the hippocampus. Mice with natural or experimentally induced mutations or knockouts of some of these genes are characterized by severe dysmyelination of the central nervous system (Ebersole et al., 1996; Nave, 1994; Sidman et al., 1964; Weiss et al., 2000) including reduced brain size, enlarged ventricles and corpus callosum atrophy, all of which are features consistent with the neuropathology observed in SZ (Lappe-Siefke et al., 2003). The expression of many of these genes (e.g. TF and QKI) coincides with the development of oligodendrocytes and the onset of myelination (Arvidsson...
et al., 1991; Connor et al., 1987, 1993; Larocque et al., 2002, 2005; Li et al., 2000).

TF and QKI, for example, have been implicated in supporting metabolic demands associated with the production and maintenance of myelin and in oligodendrocyte differentiation (Ryder and Williamson, 2004; Wu et al., 2002), respectively and their reduced expression in SZ may therefore influence/hinder the generation of mature oligodendrocytes and the production of myelin by surviving oligodendroglia. Recent independent studies have validated gene expression decreases of QKI isoforms in SZ and have provided genetic evidence for the association of QKI to SZ in a large pedigree from northern Sweden (Aberg et al., 2006a).

Similarly, the reduced expression of CNP mRNA and protein has been confirmed in SZ (Dracheva et al., 2005; Flynn et al., 2003) and CNP has been implicated in SZ by genetic linkage studies (Peirce et al., 2006).

Structural and functional domains of axons, such as nodes of Ranvier, the paranodal, juxtaparanodes, and internodal regions, are regulated by oligodendrocytes and mediated by a complex of adhesion and adherens junction proteins on both sides of axoglial contacts (Boyle et al., 2001; Davis et al., 1996; Lambert et al., 1997; Poliak and Peles, 2003; Coetzee et al., 1996). Genes encoding for proteins involved in these junctional and nodal loci [e.g. CD9, MAG, myelin-associated lipoprotein (MAL), tight and intercellular junction and peripheral myelin protein (PMP22), claudin (CLDNs-11 and -5), ankyrin 3, tight junction protein (TJP2/ZO2)], are abundantly expressed in both neurons (Bauer et al., 1999) and in myelinating Schwann cells (Dyer, 2002; Poliak et al., 2002). These genes were also associated with the OMR GO terms and were strongly down-regulated (2.0- to 2.4-fold) in the cingulate cortex and hippocampus of persons with SZ. Disrupting the integrity of at least some of these genes and proteins (e.g. PMP22, PLP or MAG) affects signal propagation (Auld et al., 1995; Baumgartner et al., 1996; Bhat et al., 2001; Boyle et al., 2001; Coetzee et al., 1996) and voltage-dependent potassium channels (Kv) (Poliak and Peles, 2003; Salzer, 2003; Schaeren-Wiemers et al., 2004) and may contribute to the disconnectivity syndrome described in SZ.

qPCR studies

Microarray approaches offer a means of escaping the confines of hypotheses driven by treatment and responses to pharmacological agents and allow for the generation of hypotheses anchored to a firm neurobiological foundation (Davis et al., 2003; Katsel et al., 2004a). More quantitative experimental methods are required, however, to confirm or refute the results of microarray analyses. qPCR gene expression studies represent one such approach.

Table 1 summarizes portions of the results of one confirmatory study (Dracheva et al., 2005) in which the expression of multiple oligodendrocyte-associated

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<th>Anterior cingulate gyrus</th>
<th>Putamen</th>
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<td>SZ: 30</td>
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<td>FC  p FC p</td>
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<tr>
<td>MAG x 1.83 0.0005</td>
<td>-1.83 0.0005 ( \frac{-2.05}{0.0450} )</td>
<td>-1.13 0.5363 1.11 0.7730</td>
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<tr>
<td>CNP x -1.70 0.0004</td>
<td>-1.70 0.0004 ( \frac{-1.89}{0.0033} )</td>
<td>-1.11 0.5137 1.09 0.7398</td>
</tr>
<tr>
<td>SOX10 x -1.93 0.0003</td>
<td>-1.93 0.0003 ( \frac{-1.30}{0.0953} )</td>
<td>-1.28 0.1270 -1.09 0.6859</td>
</tr>
<tr>
<td>CLD11 x -1.99 0.0015</td>
<td>-1.99 0.0015 ( \frac{-2.18}{0.0023} )</td>
<td>-1.27 0.1821 -1.31 0.2209</td>
</tr>
<tr>
<td>SGK1 x -2.13 0.0014</td>
<td>-2.13 0.0014 ( \frac{-2.71}{0.0002} )</td>
<td>-1.37 0.1129 -1.56 0.0760</td>
</tr>
<tr>
<td>PMP22 x -1.75 0.0005</td>
<td>-1.75 0.0005 ( \frac{-1.87}{0.0053} )</td>
<td>-1.23 0.1249 -1.09 0.7016</td>
</tr>
<tr>
<td>MBPEx1x -1.07 0.4555</td>
<td>-1.07 0.4555 ( \frac{1.06}{0.6797} )</td>
<td>1.41 0.0295 1.39 0.0596</td>
</tr>
<tr>
<td>MBPEx2x -0.6236</td>
<td>1.15 0.3996 ( \frac{1.01}{0.9848} )</td>
<td>1.32 0.0502 1.52 0.1272</td>
</tr>
<tr>
<td>MOBP x 1.07</td>
<td>-1.07 0.6236 ( \frac{-1.23}{0.4928} )</td>
<td>1.41 0.0610 1.69 0.1340</td>
</tr>
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Statistically significant values are shown in bold.

* MBPEx1 probe was used to measure total MBP.
* MBPEx2 probe was used to measure only 20.2 kDa and 21.5 kDa isoforms of MBP.
genes MAG, CNP, SRY (sex determining region Y) – box 10 (SOX10), claudin11 and other myelin-associated transcripts were explored in multiple post-mortem brain regions (anterior cingulate gyrus, hippocampus, caudate nucleus and putamen – data for the anterior cingulate gyrus and putamen only are shown in Table 1) derived from a large cohort of subjects with SZ and normal controls ($n = 19–30$). The subjects in this study were drawn from the same population as those participating in the microarray studies described above. There was extremely close agreement between the qPCR results and the microarray analysis, despite the use of brain tissue specimens derived from nearly independent cohorts.

In addition to providing confirmation of brain region-specific myelin-associated changes in gene expression, this study illustrates a second point of interest, namely the expression of isoforms of the same gene. In this instance, two sets of MBP isoforms were represented on the microarray and the expression of neither set was reliably down-regulated in SZ. However, this is not always true. For example, two alternatively spliced variants of MAG have been identified – L-MAG and S-MAG (Miescher et al., 1997). L-MAG is associated with oligodendrocytes myelinating small diameter axons and S-MAG is associated with myelination of large-diameter axons (Butt et al., 1998). Since MAG isoforms were not uniquely represented on the microarray the expression of L-MAG and S-MAG was studied by qPCR (Copland et al., 2004) and S-MAG was found to be more severely under-expressed in SZ than L-MAG. The preferential association of S-MAG with large-calibre axons suggests that signal propagation in large-calibre axons may be particularly affected in SZ (Rajkowska et al., 1998). It is also important to note that recent evidence from MAG knockout mice presented in this issue by Segal et al. (2007) shows that experimentally induced ablation of MAG leads to abnormal morphology of neurons in layer III of the prefrontal cortex, where large-calibre axons predominate, and is consistent with observations of layer-III-associated neuropil reductions in SZ (Rajkowska et al., 1998; Selemon et al., 1998).

Concluding remarks

The results of SZ-related changes in myelin-associated gene expression, including down-regulation of genes encoding for proteins critical to the function of nodal loci on nerve fibres and axogial interactions, provide provocative evidence for functional abnormalities in myelin and oligodendrocytes as well as for disturbances in nerve signal conductance and signal propagation in nerve signal conductance and signal propagation in SZ. It is easy to postulate that these abnormalities may contribute significantly to the disconnectivity syndrome that has been proposed to underlie some of the symptoms of SZ (Arnold and Trojanowski, 1996; Davis et al., 2003; Haroutunian and Davis, 2000; Raedler et al., 1998; Weinberger, 1995; Weinberger and Lipska, 1995). The results described here were based on studies of tissue homogenates of the cortex and they do not address layer-specific changes in gene expression or changes in gene expression that may exist in grey vs. white matter. Studies underway in our laboratories are attempting to address these questions using laser capture microscopy of specific cell types within specified cortical laminae.

The initial report of myelin-associated gene expression changes (Hakak et al., 2001) was soon followed by other studies (Aberg et al., 2006b; Aston et al., 2004; Bahn and Jones, 2003; Dracheva et al., 2005; Hakak et al., 2001; Iwamoto et al., 2005; Katsel et al., 2004a, 2005a,b; Sugai et al., 2005; Tkachev et al., 2003) with remarkably similar findings reported by independent investigators using independent cohorts and divergent methodological platforms. Neuro-anatomy and neuroimaging studies provide complementary and supportive data (Benes, 1989; Hof et al., 2003; Miyakawa et al., 1972; Uranova et al., 2001, 2004). The level of experimental data convergence demonstrated by these studies and those published in this issue is nearly unprecedented in neurobiological studies of SZ and suggests that it may be appropriate to revisit some of our firmly held conceptualizations of the neurobiology of the disease.

If myelin and myelination deficits represent a major abnormality in the disease, then we must begin to consider how this abnormality may affect our neurotransmitter system-based hypotheses of the disease, the volumes of data that support them, and the mechanisms by which therapeutic agents affect the disease and its symptoms. For example, we may ask whether the long-held hypotheses of abnormal dopamine system function cannot be re-conceptualized within a myelination framework. Is it possible that some of the abnormalities in dopamine function relate to how dopaminergic systems interact with oligodendrocytes or that dopamine system abnormalities are influenced by myelination deficits? Can dopamine system-based treatment strategies be viewed as treatments that at least in part overcome some of the consequences of nerve conductance and signal propagation defects caused by aberrant myelination? Can we view the known abnormalities of
the glutamate system in SZ as abnormalities that not only affect neurotransmission directly, but also as abnormalities that affect and are in turn affected by oligodendrocytes (Domercq et al., 2005; Matute et al., 2006). Recent technological advance in genomics, proteomics, metabolomics and laser capture microscopy provide the opportunity to begin to evaluate questions such as those posed above, as heretical as they may seem.

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Statement of Interest

None.

References


