Transcriptome alterations of prefrontal cortical parvalbumin neurons in schizophrenia

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Abstract

Schizophrenia is associated with dysfunction of the dorsolateral prefrontal cortex (DLPFC). This dysfunction is manifest as cognitive deficits that appear to arise from disturbances in gamma frequency oscillations. These oscillations are generated in DLPFC layer 3 via reciprocal connections between pyramidal cells and parvalbumin (PV)-containing interneurons. The density of cortical PV neurons is not altered in schizophrenia, but expression levels of several transcripts involved in PV cell function, including PV, are lower in the disease. However, the transcriptome of PV cells has not been comprehensively assessed in a large cohort of subjects with schizophrenia. In this study, we combined an immunohistochemical approach, laser microdissection, and microarray profiling to analyze the transcriptome of DLPFC layer 3 PV cells in 36 matched pairs of schizophrenia and unaffected comparison subjects. Over 800 transcripts in PV neurons were identified as differentially-expressed in schizophrenia subjects; most of these alterations have not previously been reported. The altered transcripts were enriched for pathways involved in mitochondrial function and tight junction signaling. Comparison with the transcriptome of layer 3 pyramidal cells from the same subjects revealed both shared and distinct disease-related effects on gene expression between cell types. Furthermore, network structures of gene pathways differed across cell types and subject groups. These findings provide new insights into cell type-specific molecular alterations in schizophrenia which may point toward novel strategies for identifying therapeutic targets.

CONFLICTS OF INTEREST

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INTRODUCTION

In schizophrenia, alterations in the dorsolateral prefrontal cortex (DLPFC) are thought to underlie certain cognitive deficits, including working memory impairments, associated with the disease. Multiple postmortem studies have detected gene expression alterations in the DLPFC of schizophrenia (SZ) subjects, including several transcriptome-wide studies (reviewed by Horvath and Mirnics, 2015). However, almost all transcriptome-wide studies have been conducted in total grey matter homogenates, likely masking cell-type specific alterations due to cellular heterogeneity. For example, alterations in certain mitochondria-related transcripts found in DLPFC layer 3 (L3) pyramidal cells (PCs) were not detected in DLPFC total grey matter from the same subjects. In L3 of the DLPFC, PCs and the subclass of inhibitory GABAergic neurons that express the calcium-binding protein parvalbumin (PV) are reciprocally connected to form microcircuits that generate gamma frequency oscillations associated with working memory. Thus, working memory impairments in SZ could be the result of PC and/or PV cell alterations.

Alterations in cortical PV neurons have been widely reported in SZ. Although the number of PV neurons does not appear to be altered in the disease, they contain lower mRNA and protein levels for the GABA-synthesizing enzyme GAD1, and the potassium channel KCNS3. Moreover, tissue levels of mRNA for the activity-dependent, PV neuron-enriched, transcriptional regulator EGR1 are also lower in SZ. Given the activity-dependent nature of EGR1, GAD1 and PV expression, these findings could reflect lower excitatory drive to PV cells in SZ.

However, a full interrogation of PV transcriptome alterations in the DLPFC in SZ has not been reported. Unlike PCs, PV cells cannot be identified for capture by laser microdissection (LMD) using only morphology; therefore, a specific cellular marker is required. Although PV immunoreactivity would seem to be an ideal marker for PV neurons, its expression is consistently reduced in SZ which could lead to a failure to identify the PV cells with the lowest PV levels and perhaps the most pronounced transcriptome alterations. However, most PV neurons are surrounded by perineuronal nets (PNNs), a condensed form of extracellular matrix. We recently reported that, in contrast to findings with other PNN markers, the density of PNNs labeled for the PNN component aggrecan is unaltered in the DLPFC in SZ.

In this study, PNNs were labeled with an aggrecan antibody and an immunohistochemical approach was used that is compatible with LMD. We first profiled aggrecan-positive PV cells and Nissl-stained PCs from the same unaffected comparison subjects (C) to confirm enrichment for gene products specific to each cell type, followed by transcriptome profiling of aggrecan-positive PV neurons in DLPFC L3 from 36 matched pairs of SZ and C subjects. Differentially-expressed genes (DEGs) in schizophrenia were then used for pathway and network analyses to identify biochemical pathway alterations in PV cells. Finally, we directly compared DEGs from PV cells and L3 PCs from the same 36 subject pairs.
METHODS (see Supplementary Methods for details)

Human subjects

Brain specimens (n=72) were obtained during autopsies conducted at the Allegheny County Office of the Medical Examiner (Pittsburgh, PA) after consent was obtained from the next-of-kin as previously described. Each subject with SZ (n=36) was matched to one C subject for sex and as closely as possible for age. Subject groups did not differ in mean age, post-mortem interval (PMI), RNA integrity number (RIN), brain pH, tissue storage time or race (Table 1, Supplementary Table 1).

Aggrecan immunolabeling and laser microdissection

The right hemisphere of each brain was blocked coronally, immediately frozen, and stored at −80°C. Samples from each subject pair were always processed together to control for experimental variance. Tissue sections (12 μm) were immunolabeled using an antibody raised against aggrecan. For each subject, two samples of 150 aggrecan-positive cells were individually dissected from L3 of adjacent tissue sections.

Microarray profiling

For each sample of PV cells, RNA was processed as previously described and loaded on an Affymetrix GeneChip® U219 (Affymetrix, Santa Clara, CA). For each subject, the two replicates were processed independently and replicate samples were averaged for data analysis. Three samples failed quality control analysis checks; thus, only a single sample was available for those three subjects (2 SZ and 1 C subject).

Analyses of cell type-specific transcript enrichment

Cell-type specific transcript enrichment was examined in 7 C subjects. Pyramidal cells (n=100 cells/sample), identified by a triangular shape in Nissl-stained sections, and aggrecan-positive cells (n=150 cells/sample) in L3 were collected from separate slides. Samples were profiled using the Affymetrix GeneChip® U219 and the data from those 14 samples were normalized together.

Statistical analysis

Detection of differentially-expressed transcripts, covariate and pathway analysis—After data filtering (Supplementary Figure 1) we followed a previously reported procedure to fit a random intercept model (RIM) for each subject group. This approach accounts for the matched design and adjusts for the effects of confounding covariates in a gene-specific manner.

We first tested whether continuous covariates present in both subject groups (pH, PMI, age, RIN) were potential mediators. The covariates that survived mediator analysis were then used along with additional categorical covariates in the confounding covariate selection process. The three covariates that appeared most often (age, RIN and suicide, Supplementary Table 2) were used in the final RIM. Differentially-expressed probes (DEPs) identified using the Storey procedure and a 5% false discovery rate (FDR) were used to...
perform pathway enrichment analysis using INGENUITY® pathway analysis (IPA) software (Qiagen).

**Network topology analysis**—We first tested if the DEGs detected in this study showed co-expression by using an unweighted network analysis\(^{26}\), and compared those networks to randomly generated gene networks. Unweighted gene co-expression network and hub gene analysis was performed independently for C and SZ subjects using all genes and based upon Pearson correlations\(^{27}\). Weighted gene coexpression network analysis (WGCNA) was then performed on all genes to determine if specific subsets of genes showed coexpression. WGCNA modules were then analyzed using IPA to determine if any modules were enriched for specific pathways.

**Reanalysis of pyramidal cell data**

In our previous study of L3 PCs from these same 36 subject pairs\(^4\), data were obtained using a different microarray platform, analyzed with different filtering methods and statistical models, and DEPs were determined using a meta-analysis combining data from L3 and L5 PCs. Furthermore, pathway analysis of DEGs was performed using publicly available databases\(^4\). Therefore, to compare gene expression findings across cell types, we reanalyzed the previously published L3 PC data and conducted the pathway analyses using exactly the same methods described above for PV cells.

**RESULTS**

**Aggrecan-positive cells are highly enriched for PV cell markers**

Gene expression profiles of aggrecan-positive cells demonstrated high levels of enrichment for gene products specific to PV cells or GABA neurons relative to PCs. Furthermore, there were comparably low levels of expression of PC and glial cell specific transcripts such as NEUROD6 and GFAP, respectively. These data demonstrate that aggrecan labeling, coupled with LMD, provides a robust means to selectively capture samples of cells highly enriched for PV neurons (Supplementary Results and Supplementary Figure 2).

**Differential gene expression from aggrecan-positive cells in schizophrenia**

In PV cells, 1,044 differentially-expressed probe sets (DEPs) were identified in SZ subjects, representing 872 unique differentially-expressed genes (DEGs) (see Tables 2 and 3 for DEGs with >40% change; see Supplementary Table 3 for all DEGs). Of these DEGs, 373 (42.8%) had lower expression and 499 (57.2%) had elevated expression in SZ.

Consistent with prior studies performed on grey matter\(^9,12,13,15,20,28\), PV expression was 22.2% lower (q=0.03), KCNS3 was 31.5% lower (q=0.02) and EGR1 was 33.8% lower (q=0.08) in SZ subjects. Furthermore, two transcripts highly enriched in GABA neurons (SLC32A1, vesicular GABA transporter 1, 32.4% lower, q=0.01; GAD1, 17.8% lower, q=0.11), also had altered expression in PV cells in SZ. Moreover, other transcripts previously reported to be altered in SZ (e.g. HINT1\(^{29}\), BAG1\(^{30}\), RGS4\(^{31}\), GRIN2A\(^{32}\), and ATP1A3\(^{33}\)) were abnormally expressed in PV neurons from SZ subjects, indicating that alterations previously found in grey matter can, at least partially, be attributed to altered...
expression in PV cells. However, most transcript alterations appeared to be novel findings. Indeed, of the top 20 up- or down-regulated genes (ranked by largest percent change and q<0.05; Tables 2 and 3), a PubMed search using the term “schizophrenia” and the gene name identified only 10/40 DEGs as previously associated with SZ. Among the novel findings for PV cells were lower expression of both RTN4 and LYNX1, two “brakes” on molecular plasticity, several vacuolar ATPases involved in loading synaptic vesicles, and transcripts involved in scavenging free radicals. None of these findings appeared to be attributable to the effects of antipsychotics or other potentially confounding covariates (Supplementary Results).

Analyses of the DEGs using IPA identified 3 pathways that were significantly affected in PV neurons from SZ subjects: mitochondrial dysfunction, oxidative phosphorylation, and tight junction signaling (Table 4). Specifically, 27/141 genes in the mitochondrial dysfunction pathway were altered in SZ with 88.9% of the altered genes showing lower expression. In the oxidative phosphorylation pathway, 22/91 genes were altered; all 22 had lower expression and were part of the mitochondrial dysfunction pathway. Together these data suggest that PV cells likely have lower ATP production in SZ. Furthermore, 20% of the genes in the tight junction signaling pathway were altered in SZ subjects, with 12/21 altered genes showing lower expression.

Comparison to layer 3 pyramidal neurons

Given that PV cells and PCs in L3 form a local circuit that is critical for generation of gamma oscillations and working memory performance, the findings above were compared with those from L3 PCs obtained from the same subjects. Re-analysis of previously published data revealed 1,557 DEPs (5% FDR) in L3 PCs, representing 1,355 unique transcripts. All of the top 40 DEGs reported in the published analysis of L3 PCs were differentially-expressed in the new analysis. Furthermore, analysis of all 1,355 L3 PC DEGs identified 8 significantly altered pathways (Table 4), findings markedly similar to our published findings using different pathway analysis approaches.

Of the 872 PV cell DEGs, only 153 (17.5%) were also differentially-expressed in L3 PCs, with 137 showing a concordant direction of change. Therefore, over 80% of the significant gene expression alterations in DLPFC L3 PV cells in schizophrenia were not found in L3 PCs. Of the 719 PV cell DEGs that were not differentially-expressed in PCs, 43 were involved in extracellular matrix interactions. These cell type-specific, disease-related differences in gene expression cannot be fully explained by differences between the microarray platforms (only 77 PV cell DEGs were not present on the PC array) or by normal cell type differences in gene expression (281 DEGs in PV cells were filtered out as low-expressing genes in PCs). In summary, these comparisons demonstrate that SZ is associated with multiple transcriptome alterations in L3 that differ between PV cells and PCs.

As in PV cells, the mitochondrial dysfunction and oxidative phosphorylation pathways were strongly affected in PCs; however, the proportion of genes altered in those pathways was greater in PCs than PV neurons (Fisher’s Exact Test, p<0.05). Several pathways (including protein ubiquitination and EIF2 signaling) that were altered in PCs were unaffected in PV cells and the tight junction, endocytosis signaling and Rho pathways were equally affected.
in both cell types (Table 4). Interestingly, the upstream regulator analysis using IPA identified both shared and unique potential modulators of the gene expression alterations including a number of PV DEGs regulated by immune signaling pathways (Supplementary Results, Supplementary Table 4).

To further explore the differential gene expression changes in PCs and PV cells, we used an unweighted network analysis to determine if the DEGs detected in each cell type showed correlated expression (co-expression or connectedness)\(^\text{26}\). The DEGs from PV cells had a significantly higher connectedness than randomly generated networks in both C and SZ subjects (Figure 1A), suggesting that many of DEGs from PV cells in SZ show coordinated expression, regardless of disease status. Interestingly, the DEGs from PCs were highly connected only in SZ subjects, (Figure 1A), suggesting that the DEGs identified in each cell type are regulated by different mechanisms.

To examine more fully if the highly correlated expression of the DEGs represented the correlated expression of genes within particular biological pathways, a genome-wide weighted gene coexpression network analysis (WGCNA) was performed in the PV cells and PCs from SZ subjects. Fifty-four percent of the PV cell DEGs fell into one of eight modules of genes showing highly correlated expression in PV cells. Five of these modules were significantly enriched with DEGs (Figure 1B) and IPA analysis of these modules found that one was significantly enriched for the mitochondrial dysfunction/oxidative phosphorylation pathways. Interestingly, this module also contained other genes that were differentially-expressed in SZ in this study, including GAD2, GRIN2A, RGS4, LYNX1, PRDX 3, and GABRA1.

Genome-wide WGCNA of the PCs in SZ subjects identified 13 coexpression modules, which contained 56% of the DEGs. Five modules were enriched for PC DEGs. Interestingly, three of these modules were enriched for the IPA pathways mitochondrial dysfunction/oxidative phosphorylation and EIF2 signaling, while the other eight modules did not show any specific pathway enrichment (Figure 1B). Moreover, the PV module enriched for mitochondrial transcripts showed significant overlap with one of the three PC mitochondrial modules.

An analysis of potential network hubs in each cell type identified 16 genes that behaved as network hubs in both PV cells and PCs, with one of those genes, RTN4, also differentially-expressed in SZ in both cell types. However, the majority of genes identified as network hubs (645 in PV cells and 704 in PCs) were cell type-specific.

**DISCUSSION**

Using an immunohistochemical approach coupled with LMD to sample L3 PV cells from the DLPFC, we detected numerous transcriptome alterations in SZ subjects, including some transcripts previously reported to show altered expression in SZ\(^{12,13,20,29–33,35,36}\). However, most SZ-related transcriptome alterations in PV cells were novel discoveries, including lower levels of two well-recognized “plasticity brakes”, RTN4 and LYNX1, as well as reduced levels of several vacuolar ATPases and genes activated in response to oxidative phosphorylation. The DEGs identified in PV cells in SZ showed coordinated expression, suggesting that these genes are regulated by disease-related mechanisms. In contrast, the DEGs identified in PCs were highly connected only in SZ subjects, suggesting that these genes are regulated by different mechanisms. The overlap between the PV and PC modules enriched for mitochondrial dysfunction further supports the idea that these pathways are critical in SZ. The identification of potential network hubs in each cell type provides insights into the cellular mechanisms underlying SZ.

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stress. Covariate analyses suggested that these transcript alterations likely reflect the underlying disease process and not factors that are frequently co-morbid with schizophrenia or potential confounds of postmortem studies. In addition, our recent work suggests these findings are not likely to reflect factors that frequently accompany serious psychiatric illness as PFC L3 PCs from subjects with bipolar or major depressive disorder do not show the same transcriptome alterations. However, we cannot definitively exclude the possible contribution of factors other than the schizophrenia disease process to our findings. Differentially-expressed transcripts in PV cells were enriched in gene pathways involved in mitochondrial function. Furthermore, the cell type-specificity of these transcriptome alterations was supported by findings that 1) only 17.5% of the DEGs in PV cells were also detected in L3 PCs from the same subjects, 2) the gene co-expression network structure of the DEGs differed across cell types, and 3) pathway alterations shared between PV cells and PCs were more pronounced in PCs.

**PV cell transcriptome alterations**

In SZ over 800 unique transcripts were differentially expressed in DLPFC L3 PV cells. Several of these findings were validated by prior studies using qPCR measures of DLPFC grey matter homogenates and/or quantitative in situ hybridization at cellular level resolution. For example, we replicated previous reports of reductions in the PV cell-specific transcripts PVALB, KCNS3, and SLC32A1, as well as lower levels of GABA neuron transcripts including SLC32A1 (vGAT1), EGR1 (Zif268), and GAD1 in the DLPFC from schizophrenia subjects. A prior study using 8 subject pairs assessed PV cell transcriptome alterations in L3 of the superior temporal gyrus (STG). Of the DEGs detected in that study, 15 overlap with those found in the DLPFC, but alterations in PVALB, KCNS3 and SLC32A1 were not detected in the STG. These differences might be attributable to cross-study differences in brain regions analyzed, subject ages, methods for identifying PV cells, microarray platforms and/or statistical approaches.

Therefore, many of the transcriptome changes we detected, at both the individual transcript and pathway levels, have not been previously reported in PV neurons. RTN4 and LYNX1 have been suggested to serve as “plasticity brakes” as their expression levels increase with reduced neural plasticity. RTN4 was reported to be lower in cortical grey matter homogenates in SZ and we found lower RTN4 expression in both PV cells and PCs. Interestingly, RTN4 was identified as a gene network hub in both cell types, suggesting that lower expression of RTN4 correlates with many of the gene expression changes seen in both cell types. Components of another plasticity brake, perineuronal nets, are also lower in SZ, findings which suggest that in SZ cortical PV neurons and PCs are in a state of heightened plasticity, similar to that seen during developmental critical periods. This interpretation is further supported by reduced PV expression, as lower PV expression has been correlated with increased neural plasticity. Reduced levels of transcripts in PV neurons involved in scavenging free radicals would result in increased insults due to oxidative stress. These findings are especially interesting since heightened plasticity/cortical immaturity has been speculated to be present in SZ and related to elevated levels of oxidative stress. Finally, reduced expression of several vacuolar ATPases would likely
disrupt the pH gradient necessary for loading neurotransmitter into vesicles, and thus further altering GABA signaling from PV neurons\textsuperscript{46}.

Pathway analysis identified oxidative phosphorylation and mitochondrial function as severely compromised in PV cells. Indeed, >85\% of the DEGs detected in these pathways showed decreased expression, suggestive of reduced ATP generation and increased formation of reactive oxygen species. Interestingly, reductions in cellular redox state have recently been reported in SZ subjects\textsuperscript{47} and PV cell-specific disruption of mitochondrial function in mice alters gamma oscillations, sociability and sensory gating\textsuperscript{48}. Furthermore, the alterations in gene expression involved in mitochondrial function are strikingly similar to previous results from PCs in the DLPFC\textsuperscript{4}, total grey matter homogenates in other brain regions\textsuperscript{49,50} in SZ subjects and the murine 22q.11 microdeletion model\textsuperscript{51}.

**Comparison of PV cell and PC transcriptome alterations**

Of the DEGs from PV cells and PCs in DLPFC L3, >80\% were differentially expressed only in one cell type. A major pathway selectively altered in PV cells involved gene products that interact with the extracellular matrix (ECM). This finding is especially interestingly given recent evidence of schizophrenia-related alterations in PNNs\textsuperscript{42,43}. Since no major PNN components, including aggrecan, displayed differential expression in this study, post-transcriptional alterations of ECM components may explain reports of PNN deficits in SZ.

Other cell type-specific alterations in schizophrenia were also detected. First, the number of transcript alterations was greater in PCs than PV cells (7.5\% versus 4.6\% of probes, respectively, at 5\% FDR). Second, the effects on translation (EIF2 pathway) and protein ubiquitination were found only in PCs. This difference appears to be robust as alterations in these pathways were still not detected in PV cells when less stringent q values were used. Third, the upstream regulator analysis identified many potential cell type-specific regulators, including PV cell alterations in SZ that may be the result of increased immune signaling. However, the DEGs involved with mitochondrial function shared regulators across cell types. Fourth, the PC DEGs were only linked in SZ subjects. In contrast the DEGs in PV cells showed a high degree of coexpression in both C and SZ subjects. Therefore, the altered coexpression of the PV cell DEGs in SZ could reflect a disease-specific alteration in a specific upstream regulator, which also regulates the expression of these DEGs in C subjects. Since the PC DEGs only become linked in SZ, it is plausible that a SZ-specific set of alterations drives the coexpression of these genes only in the illness.

**Dysfunction of layer 3 PV neurons and PCs in schizophrenia**

The presence of multiple alterations in both DLPFC L3 PV neurons and PCs in SZ raises the question of whether the alterations in one cell type are a cause or consequence of alterations in the other\textsuperscript{52}, or related to a common upstream mechanism that directly affects both PV neurons and PCs. Although mitochondrial dysfunction is shared between PCs and PV neurons in SZ, these alterations are more robust in PCs. Furthermore, more pathways are significantly affected in PCs, suggesting that the alterations in PV neurons might be a consequence of upstream alterations in PCs. One factor driving the PC transcriptome changes could be reduced excitatory drive to these neurons. Findings of lower spine
density\textsuperscript{53,54} (the site of most excitatory synapses), lower levels of the excitatory NMDA receptor subunit GRIN2A\textsuperscript{32} (also found here), and lower expression in mitochondrial pathway transcripts\textsuperscript{4} (which reflect levels of neuronal activity) in DLPFC L3 PCs in SZ support this interpretation. Additionally, the alterations in layer 3 PCs representing an “upstream” component of the disease process is supported by recent findings that olfactory epithelial cells from SZ patients show robust alterations in EIF2/translation\textsuperscript{55}, similar to the PC-specific changes reported here. Because changes in olfactory epithelial cells (which are excitatory neurons) are unlikely to be driven by brain-specific circuit abnormalities, this finding may represent a primary molecular pathology in the disease. Furthermore, these olfactory epithelial neurons did not show alterations in mitochondrial function, suggesting that changes in mitochondrial function may be “downstream” consequences of changes in neural circuitry. The lower activity of PCs would result in additional downstream consequences in PV cells, which receive substantial excitatory input from neighboring PCs\textsuperscript{56}. If the level of excitatory drive to PV neurons is responsible for coordinating the expression of a subset of genes in these cells (specifically those involved in mitochondrial function and GABA synthesis), alterations in this drive would alter the expression of these genes without disrupting their coordinated expression, as found in the present study. This idea is supported by the WGCNA analysis of the PV transcriptome, since most mitochondrial gene expression alterations are found in the same coexpression network as GAD1 and GAD2. However, some PV cell-specific alterations, such as lower levels of several molecules correlated with neural plasticity and PV cell type-specific altered splicing of ERBB4\textsuperscript{57}, suggests that primary alterations in PV cells might also be critical in DLPFC circuitry abnormalities in SZ.

In summary, these findings demonstrate the importance of studying the molecular alterations associated with SZ at the level of specific cell types. Our findings reveal distinct disease-related effects on gene expression in L3 PV cells as well as transcriptome alterations that are shared with L3 PCs. Comparison of the findings across cell types provides additional evidence suggesting that many of the alterations in PV cells could be secondary to events in the L3 PCs that innervate them. To what extent the transcriptome alterations present in L3 PV cells are conserved across other classes of cortical GABA neurons is an important area for future investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
A. Differential network structure and disruptions in layer 3 PV cell (left) and PC (right) networks. The mean degree of connectivity of the DEGs (x’s) were compared to randomly generated networks (ovals) across cell types and diagnoses (unaffected comparison-C and schizophrenia-SZ). Any network plotted above the whiskers in the box plots is significantly connected (p<0.001). B. WGCNA from L3 PV cells and PCs. Genome-wide coexpression modules are detected in L3 PV cells (top) and L3 PCs (bottom). Coexpression modules in each cell-type are identified by a randomly assigned color and the same color in different cell types does not represent the same coexpression module. Modules that are significantly enriched for DEGs are bolded and those that are enriched for mitochondrial transcripts are
shaded gray. The PV red and PC turquoise modules have significant overlap in gene expression, suggesting a shared regulatory network structure across cell types.
**Table 1**

Summary of subject characteristics.

|                  | Control | Schizophrenia | Statistics |
|------------------|---------|---------------|------------|
| Number           | 36      | 36            |            |
| Sex              | 27 M, 9 F | 27 M, 9 F    | X^2 = 0.0, p=1.0 |
| Race             | 30 W, 6 B | 24 W, 12 B    | X^2 = 2.67, p=0.10 |
| Age (years)      | 48.1 (13.0) | 46.9 (12.4)  | F=0.16, p=0.69 |
| PMI^* (hours)    | 17.6 (6.1) | 18.0 (8.8)   | F=0.04, p=0.84 |
| Brain pH         | 6.7 (0.2)  | 6.6 (0.4)    | F=3.36, p=0.07 |
| RIN**            | 8.3 (0.6)  | 8.2 (0.6)    | F=0.42, p=0.52 |
| Storage time (months at −80°C) | 151.1 (43.4) | 154.6 (45.9) | F=0.09, p=0.77 |

Values are mean (SD)

* Postmortem interval

** RNA integrity number
Table 2

Top differentially-expressed genes in PV cells with > 40% increase (q<0.05) in expression in schizophrenia subjects relative to unaffected comparison subjects.

| Gene Symbol | Gene Name                                                                 | % increase |
|-------------|---------------------------------------------------------------------------|------------|
| MT2A*       | metallothionein 2A                                                        | 112.0      |
| MGAT4A      | mannosyl (alpha-1,3-)glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isozyme A | 96.7       |
| GPR56       | G protein-coupled receptor 56                                             | 80.0       |
| TPCN2       | two pore segment channel 2                                                | 69.3       |
| CRIP1       | cysteine-rich PDZ-binding protein                                         | 66.7       |
| C9orf3      | chromosome 9 open reading frame 3                                         | 62.9       |
| AGT         | angiotensinogen (serpin peptidase inhibitor, clade A, member 8)           | 60.5       |
| ATM*        | ATM serine/threonine kinase                                               | 59.2       |
| KLKB1       | kallikrein B, plasma (Fletcher factor) 1                                   | 59.0       |
| MAP3K2      | mitogen-activated protein kinase kinase kinase 2                          | 53.8       |
| ANKRD20A5P  | ankyrin repeat domain 20 family, member A5, pseudogene                    | 53.8       |
| HNRNPLL     | heterogeneous nuclear ribonucleoprotein L-like                            | 49.7       |
| GOLGA2P10   | golgin A2 pseudogene                                                      | 49.3       |
| RAB13*      | RAB13, member RAS oncogene family                                         | 44.7       |
| LOC100131826| TSSP3028 (hypothetical protein)                                            | 44.5       |
| FAM107A     | family with sequence similarity 107, member A                             | 44.2       |
| PTPRG*      | protein tyrosine phosphatase, receptor type. G                            | 42.5       |
| UBE2L3      | ubiquitin-conjugating enzyme E2L 3                                        | 42.2       |
| IGKC        | immunoglobulin kappa constant /// immunoglobulin kappa variable 1–39 (gene/pseudogene) /// --- /// immunoglobulin kappa variable 1D-39 /// --- /// | 41.3       |
| BGN         | biglycan                                                                  | 41.1       |
| NOV         | nephroblastoma overexpressed                                              | 40.9       |
| SOX9        | SRY (sex determining region Y)-box 9                                      | 40.7       |
| P2RY14      | purinergic receptor P2Y, G-protein coupled, 14                             | 40.6       |
| SMAD3       | SMAD family member 3                                                      | 40.4       |

* Denotes gene previously implicated with schizophrenia
Table 3
Top differentially-expressed genes in PV cells with > 40% decrease (q<0.05) in expression in schizophrenia subjects relative to unaffected comparison subjects.

| Gene Symbol | Gene Name | % decrease |
|-------------|-----------|------------|
| ANAPC13     | anaphase promoting complex subunit 13 | 58.4       |
| TOLLIP      | toll interacting protein | 57.7       |
| NDUFA13     | NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 13 | 54.8       |
| PITPNB      | phosphatidylinositol transfer protein, beta | 52.4       |
| EFTUD2      | elongation factor Tu GTP binding domain containing 2 | 51.8       |
| FEZ1 *      | fasciation and elongation protein zeta 1 (zygin I) | 50.9       |
| WDR41       | WD repeat domain 41 | 49.8       |
| RAC1 *      | ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1) | 49.4       |
| NBR1        | neighbor of BRCA1 gene 1 | 48.9       |
| RIOK1       | RIO kinase 1 | 48.4       |
| PPT1 *      | palmitoyl-protein thioesterase 1 | 47.8       |
| ACTR3       | ARP3 actin-related protein 3 homolog (yeast) | 47.5       |
| RGS4 *      | regulator of G-protein signaling 4 | 47.3       |
| UQCRNC1     | ubiquinol-cytochrome c reductase core protein I | 46.5       |
| ATP6V1G1    | ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G1 | 46.4       |
| RTN4 *      | reticulon 4 | 46.1       |
| TRIM37      | tripartite motif containing 37 | 45.7       |
| DNAJC15     | DnaJ (Hsp40) homolog, subfamily C, member 15 | 45.7       |
| EIF5        | eukaryotic translation initiation factor 5 | 45.5       |
| ESRRG       | estrogen-related receptor gamma | 45.3       |
| APBB1IP     | amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein | 45.3       |
| SRPRB       | signal recognition particle receptor, B subunit | 45.2       |
| NTM         | neurotrimin-like // neurotrimin | 45.1       |
| FAM174A     | family with sequence similarity 174, member A | 44.6       |
| SLC4A1AP    | solute carrier family 4 (anion exchanger), member 1, adaptor protein | 44.4       |
| CLCN4       | chloride channel, voltage-sensitive 4 | 44.2       |
| FAT1 *      | FAT atypical cadherin 1 | 43.6       |
| SUSD1       | sushi domain containing 1 | 43.6       |
| IDH3B       | isocitrate dehydrogenase 3 (NAD+) beta | 43.5       |
| GRIP1 *     | glutamate receptor interacting protein 1 | 43.1       |
| PPP3CB *    | protein phosphatase 3, catalytic subunit, beta isoyme | 43.1       |
| RAB11A      | RAB11A, member RAS oncogene family | 42.9       |
| NACA        | nascent polypeptide-associated complex alpha subunit | 42.7       |
| RHOQ        | ras homolog family member Q | 42.4       |
| Gene Symbol | Gene Name                                                                 | % decrease |
|------------|----------------------------------------------------------------------------|------------|
| MRPL33     | mitochondrial ribosomal protein L33                                       | 41.8       |
| RAD21      | RAD21 homolog (S. pombe)                                                  | 41.0       |
| NDUFS2     | NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa (NADH-coenzyme Q reductase) | 40.4       |
| NR1D2      | nuclear receptor subfamily 1, group D, member 2                           | 40.1       |

* Denotes gene previously implicated with schizophrenia.
Table 4
Comparison of altered gene pathways in DLPFC layer 3 PV cells and PCs in schizophrenia.

| Pathway                                | PV cell analysis | PC analysis | PC analysis |
|----------------------------------------|------------------|-------------|-------------|
|                                        | DEGs in pathway  | p-value     | % DEGs decreased | DEGs in pathway  | p-value | % DEGs decreased |
| Oxidative Phosphorylation               | 22 (24.2%)       | < 10<sup>−5</sup> | 90.9%         | 61 (67%)         | < 10<sup>−31</sup> | 100%       |
| Mitochondrial Dysfunction               | 27 (19.1%)       | < 10<sup>−4</sup> | 88.9%         | 73 (51.8%)       | < 10<sup>−30</sup> | 100%       |
| Tight Junction Signaling                | 21 (20%)         | < 10<sup>−3</sup> | 57.1 %        | 16 (14.8%)       | 0.21     | 81.3%         |
| ProteinUbiquitination Pathway           | 20 (9.9%)        | 0.26        | 55%           | 55 (24.4%)       | < 10<sup>−2</sup> | 92.7%       |
| EIF2 Signaling                          | 10 (6.2%)        | 1           | 60%           | 41 (24.4%)       | < 10<sup>−5</sup> | 90.2%       |
| Clathrin-mediated Endocytosis Signaling | 20 (15.5%)       | 0.006       | 55%           | 31 (25%)         | < 10<sup>−4</sup> | 87.1%       |
| Superpathway of Cholesterol Biosynthesis| 2 (9.1%)         | 0.57        | 100%          | 10 (43.5%)       | < 10<sup>−2</sup> | 100%       |
| Regulation of Actin-based Motility by Rho| 14 (21.9%)      | < 10<sup>−3</sup> | 78.6%        | 16 (28.6%)       | < 10<sup>−3</sup> | 87.5%       |
| Pyrimidine Deoxyribonucleotides De Novo Biosynthesis I | 0 (0%) | 1 | 0% | 7 (46.7%) | < 10<sup>−3</sup> | 85.7% |

* Number and % of differentially expressed genes (DEGs) in each pathway. Bolded values represent pathways significant at a 5% FDR.