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Shared co-expression networks in frontal cortex of the normal aged brain and schizophrenia

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ABSTRACT

Previous studies on the brain of people with schizophrenia have identified structural changes and gene expression changes, suggesting that brain aging maybe accelerated in people with schizophrenia. To better characterize gene expression profiles in schizophrenia and in the aged population we constructed co-expression networks using RNA-Seq data from frontal cortex. The first data set analysed was from 62 subjects with schizophrenia and 51 unaffected controls ranging in age from 19 to 63 years. The second separate data set was from normal control individuals ranging in age from 29 to 106 years. In the first data set, we found two co-expression modules significantly associated with schizophrenia. One was a downregulated co-expression module enriched for neuron function related genes and the other was an upregulated immune/inflammation-related module. In the second data set of normal individuals, we found seven co-expression modules significantly correlated with age. A comparison of the co-expression modules from the two data sets revealed a significant consensus in nodes associated with schizophrenia and those associated with normal aging. The results indicate that a co-expression module related to neuronal function is downregulated and an immune/inflammation related co-expression module is upregulated, and associated with cells of the blood vessels, in both schizophrenia and in normal aging. This finding adds further support to the hypothesis that there may be accelerated brain aging in schizophrenia.

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1. Introduction

Schizophrenia is a common (Mueser and McGurk, 2004) but highly heterogeneous disorder (Arango et al., 2000). It is believed to be caused by the interplay between multiple genetic and environment factors, however the pathophysiology of the disorder is not well understood. While post-mortem studies have reported a number of neuropathological alterations in the brain of individuals with schizophrenia (Knable et al., 2002; Knable et al., 2004; Knable et al., 2001), high-throughput gene expression studies may identify the molecular mechanisms that underlie the pathophysiology of the disorder (Bray, 2008). Indeed, genome-wide expression profiling using RNA from post-mortem brain has identified changes in expression of genes in several biological processes including downregulation of genes related to synaptic and mitochondrial function (Iwamoto et al., 2005; Middleton et al., 2002; Mirnics et al., 2000; Prabakaran et al., 2004), and upregulation of genes associated with immune/inflammation response in schizophrenia cases (Arion et al., 2010; Arion et al., 2007; Fillman et al., 2013; Hess et al., 2016; Saetre et al., 2007; Schmitt et al., 2011; Shao and Vawter, 2008).

While aging is a risk factor for several neurodegenerative diseases including Alzheimer's disease (Lindsay et al., 2002) and Parkinson's disease (Collier et al., 2011), the relationship between normal aging and schizophrenia is largely unknown. Many biological functions, including brain function, decline during the aging process (Peters, 2006). Changes in gene expression in the brain during aging may contribute to the decline in brain function that occurs in older people and several previous studies show that genes related to synaptic or neuronal function are down-regulated (Erraji-Benchekroun et al., 2005; Loerch et al., 2008; Lu et al., 2004; Soreq et al., 2017) and those related to immune/inflammation response are up-regulated in the cortex during aging (Berchtold et al., 2008; Erraji-Benchekroun et al., 2005; Rhinn and Abeliovich, 2017; Soreq et al., 2017). Moreover, several lines of evidence suggest there are common features of normal aging and schizophrenia or there may be accelerated aging in patients with schizophrenia. Individuals with schizophrenia have a decreased life expectancy, cognitive decline and decline in white matter integrity (WM) (Bowie and Harvey, 2006; Cropley et al., 2017; Hennekens et al., 2005; Kirkpatrick et al., 2008; Kochunov et al., 2013). The frontal cortex of people with early-stage schizophrenia has been shown to share gene expression profiles with those with normal aging (Tang et al., 2009), however the sample

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size of this study was relatively small and a comparison of gene expression profiles from the subjects with schizophrenia and those with normal aging was not thoroughly investigated at a systems biology level. Coordinated gene expression plays a key role in brain functions (Oldham et al., 2008) and alterations in coordinated gene expression are implicated in psychiatric disorders (Gandal et al., 2018; Hwang et al., 2013; Torkamani et al., 2010). Thus, rigorous co-expression network analyses with a larger sample size is needed to validate the results and may provide further insights into the shared gene expression profiles between schizophrenia and normal aging. Consequently, we performed gene co-expression analyses using a large set of RNA-Seq data from the frontal cortex of individuals with schizophrenia and matched unaffected controls that ranged in age from 19 to 63 years of age. We then compared the results to RNA-seq data from the same brain region of normal unaffected controls ranging in age from 29 to 106 years of age with the overall aim of identifying gene expression networks that may be common to both schizophrenia and normal aging.

2. Materials and methods

2.1. RNA-Seq data from schizophrenia and matched controls

The RNA-Seq data was generated from the frontal cortex of 68 individuals with schizophrenia and 68 unaffected controls. The samples are from the Stanley Medical Research Institute (SMRI) tissue collections; the Neuropathology Consortium (NPC), the Array Collection (AC) and the New Stanley Collection (NSC). Two senior psychiatrists established a diagnosis of schizophrenia (DSM-IV) as described in Torrey et al. (2000). All normal controls included in these SMRI collections were considered psychiatrically and neurologically normal (Torrey et al., 2000). SMRI did not collect normal individuals over the age of 65 years because of the increased likelihood of comorbid neurologic and neurodegenerative disorders. In addition, a neuropathologist examined each brain to rule out any neurodegenerative changes or other cerebral pathology. A structured telephone interview with a first-degree family member was also conducted for all cases. The RNA-Seq data from the AC and the NSC were generated by Dr. D. van der Kooy (University of Toronto) and Dr. C. Lui (University of Illinois) respectively, and was deposited to the Stanley neuropathology consortium integrative database (SNCID) http://sncid.stanleyresearch.org. SMRI generated the RNA-seq data for the NPC and for several AC samples that were not sequenced in the original study. Library preparation and mRNA sequencing were performed as previously described (Kim et al., 2016a). Two individuals with schizophrenia and two unaffected controls were excluded from downstream analysis because they had autoimmune related conditions. It was then necessary to remove an additional 19 outlier samples in order to match the schizophrenia group and the normal control group for all the descriptive variables such as age of death, brain pH and PMI. For example, two individuals with schizophrenia were excluded because PMI was longer than 121 h and a clustering analysis detected two individuals with schizophrenia as outliers. Fifteen unaffected controls were also excluded because of older age at death, high brain pH or long PMI. The final network analysis included 113 RNA-seq data sets from the frontal cortex of 62 subjects with schizophrenia and 51 unaffected controls ranging in age from 19 to 63 years (Supplementary Tables 1 and 2). This data set was used to identify the co-expression networks significantly associated with schizophrenia.

2.2. RNA-Seq data for normal brain aging study

To increase the sample for the normal aging study, the publicly available RNA-Seq raw data (FASTQ) files with accession number SRP051844 (Labadorf et al., 2015) were downloaded from the NCBI short read archive database (https://www.ncbi.nlm.nih.gov/sra). The data set consists of RNA-Seq reads from the frontal cortex of 20 cases with Huntington's disease and 49 neurologically normal controls (Labadorf et al., 2015), however only the data from the 49 normal controls was included in the analysis. We combined the 49 data sets from NCBI with the 66 data sets from SMRI resulting in 115 normal controls that range in age from 29 to 106 years. We conducted a clustering analysis on the adjusted transcript data and detected two outliers that were excluded from the network analysis. The final network analysis included RNA-seq data from the frontal cortex of 113 normal controls ranging in age from 29 to 106 years (Supplementary Table 3).

2.3. Microarray data for validation study of normal brain aging

Microarray data from the frontal cortex of a previous human brain aging study (Soreq et al., 2017) was downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) and used for a validation study. The data set (GSE36192) consists of normalized gene expression values from 455 frontal cortex samples and 456 cerebellum samples of unaffected controls ranging in age from 0.42 to 102 years. Since our normal aging study included RNA-seq data from the frontal cortex of 113 normal controls ranging in age from 29 to 106 years we used a comparable microarray dataset that contained 421 normal controls ranging in age from 16 to 102 years. Thus, we excluded data from individuals younger than 16 years of age. We also excluded data without microarray batch information. A clustering analysis was conducted on the adjusted transcript data and identified one outlier that was excluded from the network analysis. The final network analysis included microarray data from the frontal cortex of 420 normal controls ranging in age from 16 to 102 years.

2.4. Read mapping and read counting

Quality control of the raw sequence data, mapping the RNA-seq reads and quantifying the mapped reads were performed as previously described (Kim et al., 2016a). The raw data from all samples passed the initial quality control using FASTQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). All reads were then mapped to UCSC *H. sapiens* reference genome (build hg19) using TopHat v2.0.9 with UCSC refFlat gene model annotation file on the –G parameter (Trapnell et al., 2009). TopHat calls Bowtie v2.1.0 to perform the alignment with no >2 mismatches. We used the pre-built index files of UCSC *H. sapiens* hg19, which were downloaded from the TopHat homepage (http://tophat.cbcb.umd.edu/index.html). Counting of the mapped reads of the genes were performed by htseq-count (subprogram of HTseq) (Anders et al., 2015) with UCSC refFlat gene model annotation file, no strand specific option, and intersection-nonempty option.

2.5. Adjusting for confounding variables

Surrogate variable analysis (SVA) (Leek and Storey, 2007) was used to identify the potential confounding effects in the RNA-Seq data and the microarray data and then the confounding effects were adjusted using the resulting surrogate variables from the SVA. To identify the confounding variables in the RNA-Seq data for the normal aging study, we first divided the data into three age groups; young: ≤44, middle: 45–74 and old: ≥75, as previously described (Soreq et al., 2017). The age groups were used as the variable of interest then the surrogate variables were obtained using the SVA package (Leek and Storey, 2007). The standardized residuals from the linear regression including the surrogate variables were used to generate gene co-expression networks using WGCNA (Langfelder and Horvath, 2008). To control for confounding effects on the adjusted data, we performed a *post hoc* correlation analysis between the variables and the co-expression modules. Since PMI significantly correlated with most co-expression modules, we included PMI in the linear model. The standardized residuals from the linear regression including the surrogate variables and PMI were used to generate the final gene co-expression networks using WGCNA. Because

we combined the RNA-Seq data from multiple independent studies, a principal component analysis (PCA) was performed to visualize the existence of batch effects on the combined data before and after adjusting for such effects.

For adjusting the confounding variables in the microarray data, we first filtered out expression data without gene symbols and further filtered the genes with low variabilities using the biometric research branch (BRB)-array tools (http://linus.nci.nih.gov/BRB-ArrayTools.html). A gene was filtered out if <20% of expression values had at least 1.3-fold change from the median expression value for the gene. A final set of expression data for 10,431 genes was used for subsequent analyses. We then controlled for the known batch effect using the ComBat function in the SVA package (Leek and Storey, 2007). The surrogate variables were identified using the same methods as described above. Since PMI also significantly correlated with most co-expression modules, we included PMI in the linear model. The standardized residuals from the linear regression including the surrogate variables and PMI were used to generate gene co-expression networks using WGCNA (Langfelder and Horvath, 2008).

2.6. Gene co-expression network analysis

There are two strategies in co-expression network analysis. The first one is to combine gene expression data from cases and controls and then generate co-expression networks using the combined data. The purpose of this strategy is to identify differentially regulated co-expression modules between cases and controls. The second strategy is to generate co-expression networks for cases and controls separately and then compare the co-expression modules for cases to those for controls, or *vice versa*. The purpose of this strategy is to identify differences in network connections between cases and controls. The goal of our study is to identify differentially regulated co-expression modules between schizophrenia as compared to controls and identify co-expression modules correlated with normal aging and then determine if there are consensus co-expression modules between these two sets of data. Thus we first generated co-expression modules using combined data from schizophrenia and matched controls to identify the differentially regulated co-expression modules and then generated co-expression modules using only data from controls to identify modules correlated with normal aging. To construct a weighted co-expression network we selected the power for which scale-free topology fitting index (R^2) is ≥0.9 (Zhang and Horvath, 2005). Correlation analyses were performed between co-expression modules and traits such as diagnosis, age and descriptive variables to identify modules that were associated with schizophrenia disease status, age and/or confounding factors. To perform correlation analysis between co-expression modules and normal aging, the actual age of death for each individual was used. To perform correlation analysis between co-expression modules and total lifetime intake of antipsychotics the normalized value for fluphenazine gram equivalent was used. To adjust for multiple testing when we performed the correlation analyses, we used the MPTCorr.r package (http://www.psych.umn.edu/faculty/waller/downloads/mpt/mptcorr.r) (Yoder et al., 2004) as previously described (Kim et al., 2016a). Briefly, correlations between the variables and the eigenvalues of multiple co-expression modules were subjected to 10,000 permutations to generate adjusted P-values using the package (Yoder et al., 2004). Adjusted P-values < 0.05 were considered significant. The network connections were visualized using Cytoscape (Shannon et al., 2003).

2.7. Consensus module analysis

The consensus co-expression modules from two independent networks were identified as previously described (Kim et al., 2016a). P-values of significance for each of the pairwise overlaps were obtained by permutation tests in R. Since we performed 1 million permutations, the lowest possible P-value we can obtain from our analysis is 1e-06. The consensus network connections were identified and visualized using Cytoscape (Shannon et al., 2003).

2.8. Cell type enrichment analysis

To identify the cell types associated with the genes enriched in each co-expression module, we downloaded a list of marker genes specific for 11 different brain cells isolated from mouse cortex (https://github.com/oganm/neuroExpressoAnalysis) (Mancarci et al., 2017). The cell marker gene sets were identified using microarray datasets from the major cell types of brain tissues and further validated using single cell RNA-Seq datasets from mouse cortex and human brain tissues (Mancarci et al., 2017). We then compared the nodes (genes) in each of our modules to the list of specific marker genes for each cell-type and evaluated the significance of overlap between the gene lists using Fisher's exact test. Adjusted P values were computed using the Bonferroni method to correct the error rate of multiple testing.

2.9. Functional annotation

The Database for Annotation, Visualization and Integrated Discovery database (DAVID) was used to identify the biological processes that were significantly over-represented by genes included in the co-expression modules (Dennis Jr. et al., 2003). Gorilla (http://cbl-gorilla.cs.technion.ac.il/) was used to annotate significantly enriched biological processes in genes of large co-expression modules whose node size was >3000 genes because DAVID does not allow >3000 genes or probes as input. False discovery rate (FDR) was computed using the Benjamini and Hochberg method to correct the error rate of multiple testing. FDR <0.05 was considered significant.

3. Results

3.1. Gene co-expression networks significantly associated with schizophrenia

We first compared the descriptive variables between schizophrenia cases and controls and found no significant differences in age, sex, PMI, RIN or brain pH (Supplementary Table 1). We used RNA-Seq data from three Stanley tissue collections, and therefore performed PCA for visual inspection of the batch effects on the data. Before adjusting for confounding factors there was a cluster of samples from the NSC. However, after adjusting for confounding factors the samples from the three collections were well mixed (Supplementary Fig. 1). This indicated that the SVA method efficiently removed possible confounding factors, including batch effect.

We then generated 18 co-expression networks using the RNA-Seq data from frontal cortex of the schizophrenia cases and matched controls. Of the 18 co-expression modules, two modules (Sch_M2 and Sch_M14) were significantly associated with schizophrenia and had adjusted P-values value < 0.05 after corrections of multiple testing using the multivariate permutation testing of correlations (Supplementary Table 4). While Sch_M14 module was not significantly correlated with any confounding variables tested, Sch_M2 was significantly correlated with brain pH (Supplementary Table 4). The Sch_M2 module was negatively associated with schizophrenia ($r = -0.27$, adjusted P-value = 0.04), indicating that the genes in the module were significantly down-regulated in the frontal cortex of people with schizophrenia as compared to unaffected controls. Vesicle transport, gamma-aminobutyric acid (GABA) signaling pathways, synaptic transmission and neuron development were significantly enriched in this module (Fig. 1A, Supplementary Table 5). *YWHAG*, *SYT1*, *ATP6V1C1*, *GUCY1B* and *SNAP91* are the top 5 genes with high connectivity in the module (Fig. 1C). The Sch_M14 module was positively associated with schizophrenia ($r = 0.28$, adjusted P-value = 0.03), indicating that the genes in the module were significantly up-regulated in schizophrenia.

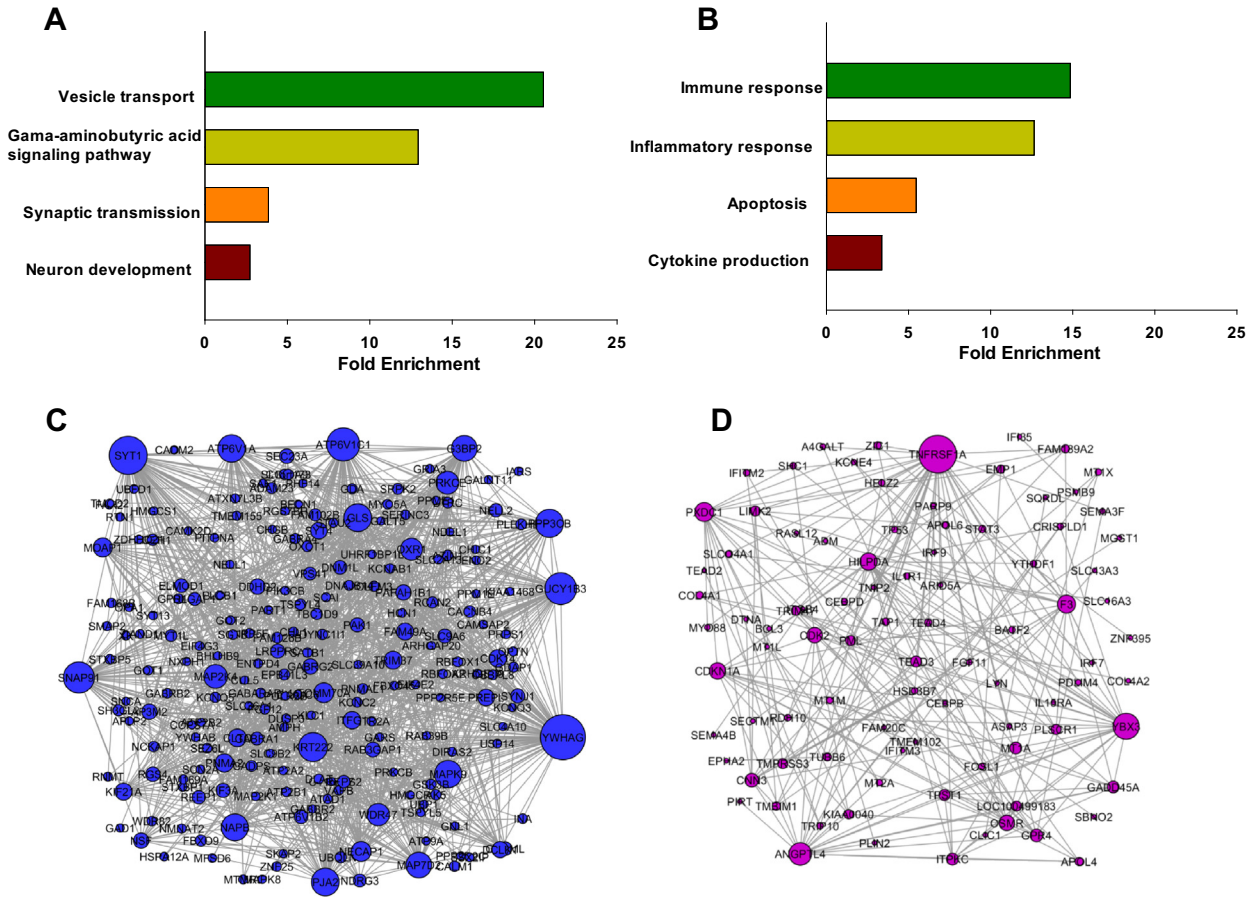

Fig. 1. Differentially expressed co-expression modules in the frontal cortex between schizophrenia and matched controls. The co-expression modules were constructed using combined RNA-Seq data from the frontal cortex of 62 subjects with schizophrenia and 51 unaffected normal controls from the SMRI collection (see Methods). The Sch_M2 module (A, C) is negatively associated with schizophrenia while the Sch_M14 module (B, D) is positively associated with schizophrenia. Major biological processes (Gene ontology) significantly enriched in the genes in the Sch_M2 module (A) and the Sch_M14 module (B). The x-axis represents the fold enrichment score. The network connections of the Sch_M2 module genes (C) and the Sch_M14 module genes (D) with topological overlap above the threshold of 0.05 were visualized using Cytoscape (Shannon et al., 2003). The hub genes are larger circles in the network.

Immune and inflammation response, apoptosis and cytokine production were significantly enriched in this module (Fig. 1B, Supplementary Table 6). *TNFRSF1A*, *YBX3*, *ANGPTL4*, *PXDC1* and *F3* are the top 5 hub genes in this module (Fig. 1D).The eigengene values in the two modules were significantly negatively correlated (Supplementary Fig. 2).

3.2. Gene co-expression networks significantly correlated with normal aging

Sixteen co-expression modules were generated from RNA-Seq data from the frontal cortex of unaffected controls that ranged in age from 29 to 106 years, and seven modules were significantly correlated with age (all adjusted P-values < 0.05, Fig. 2, Supplementary Table 7). PCA indicated that the samples from the different studies were well mixed after adjusting for potential confounding variables (Supplementary Fig. 3). Three of the modules positively correlated with age, indicating that expression levels increased with age in the frontal cortex. Immune response, inflammation response, defense response, apoptosis, and regulation of angiogenesis were significantly enriched in these modules (Supplementary Tables 8–10). In contrast, four modules negatively correlated with age. Modules related to biological processes such as synaptic transmission, ion transport, GABA signaling pathway and ATP synthesis coupled proton transport decreased with age in the frontal cortex (Supplementary Tables 11–14). The Age_M11 neuron related module was negatively correlated with the Age_M4 immune/inflammation response module (Supplementary Fig. 4).

In order to replicate the co-expression modules that were significantly correlated with normal aging in the frontal cortex, we used gene expression data derived from a different technology. We used publicly available microarray data from 420 frontal cortex samples from 16 to 102 years of age. PMI was included in the linear model because PMI was strongly associated with gene expression levels even after adjusting for surrogate variables. The standardized residuals from the linear regression were used to generate gene co-expression networks. Thirteen co-expression modules were built using the microarray data and seven of them were significantly correlated with age (all adjusted P-values < 0.05, Supplementary Table 15). Two modules were negatively correlated with age and were significantly enriched for biological processes such as mitochondrial ATP synthesis coupled electron transport, fatty acid metabolic process, proton transport (Supplementary Tables 16, 17). Five modules positively correlated with age and were significantly enriched for biological processes such as immune response, inflammation response, apoptosis and vasculogenesis (Supplementary Tables 18, 19). While six modules from the RNA-Seq data have consensus with the modules from the microarray data (Fig. 3), one module (Age_M3) did not have any consensus with the modules from the replication data.

3.3. Comparison of modules associated with schizophrenia and those correlated with normal aging

To identify abnormalities in the frontal cortex that may be common to both schizophrenia and normal aging, we compared the modules that

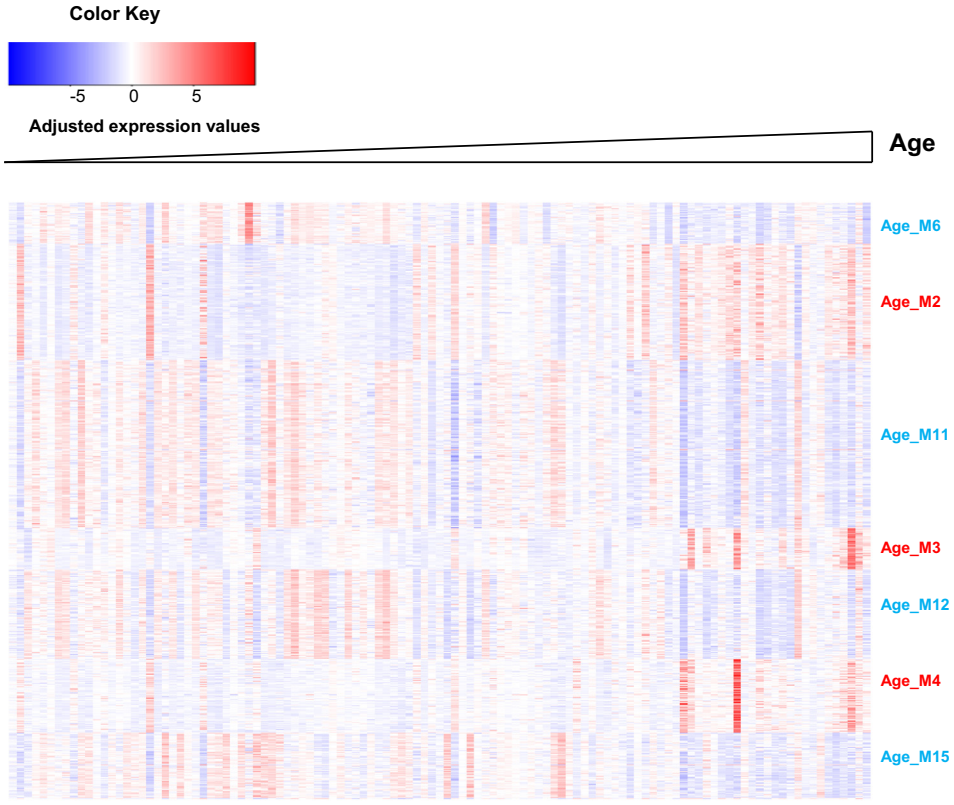

Fig. 2. Heatmap of 7 co-expression modules correlated with age in the frontal cortex. The co-expression modules were constructed using combined RNA-Seq data from the frontal cortex of 66 normal controls from the SMRI collection and 47 normal controls from SRP051844 (see Methods). The color code of the heatmap encodes adjusted expression values of genes from each module. Names of modules with expression positively correlated with age are written in red and negatively correlated with age are written in blue.

were associated with schizophrenia to the modules that were significantly correlated with age. A significant number of nodes (264) were common to both the Sch_M2 module and the Age_M11 module ($P < 1e{-}06$; Fig. 4A). These modules were negatively associated with schizophrenia and age, respectively. The Age_M11 module was reproduced in the replication study using microarray data. Genes related to synaptic transmission, transmembrane transport and GABA signaling pathways were significantly enriched in the 264 common genes (Supplementary Table 20), including *GABRG2*, *GABRA4*, *GABRA3*, *GABRB2*, *CACNB4* (Fig. 4C). To identify the cell types that may be associated with genes in these co-expression modules we compared the nodes (genes) of each module to the list of cell-type specific marker genes generated from 11 different brain cells isolated from mouse cortex. Sch_M2 module was significantly enriched with pyramidal neuron markers such as *CNKSR2*, *KCNV1*, *NPTX1* and *SV2B* (adjusted $P = 0.004$), while there was no cell specific marker significantly enriched in the Age_M11 module.

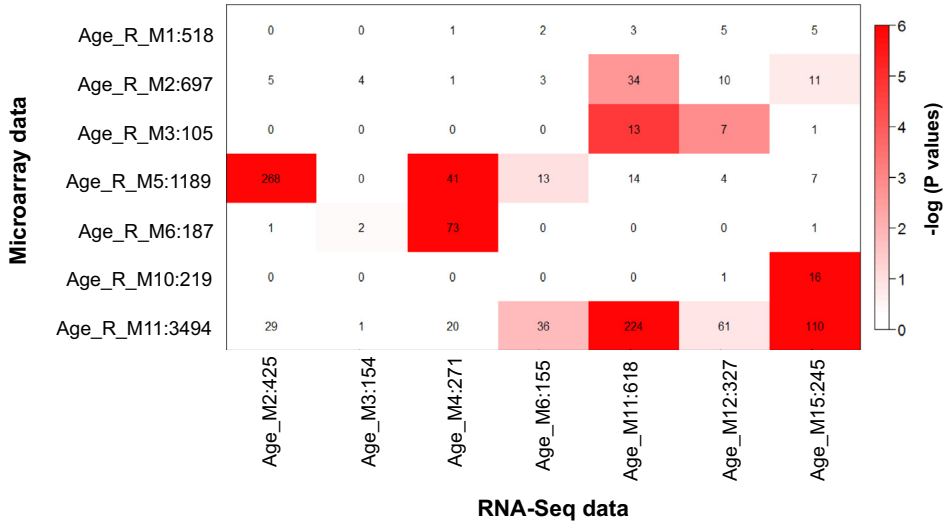

Fig. 3. Pairwise comparisons between modules correlated with age and generated from RNA-Seq data or from microarray data. The color code of the heatmap encodes –log (P-value). The P-values were calculated by permutation test for the overlap of the two modules. The numbers in the heatmap indicate gene counts in the intersection of two modules. The label to the left of the y-axis indicates modules from the microarray data and gene counts in each module. The label to the bottom of the x-axis indicates modules from the RNA-Seq data and gene counts in each module.

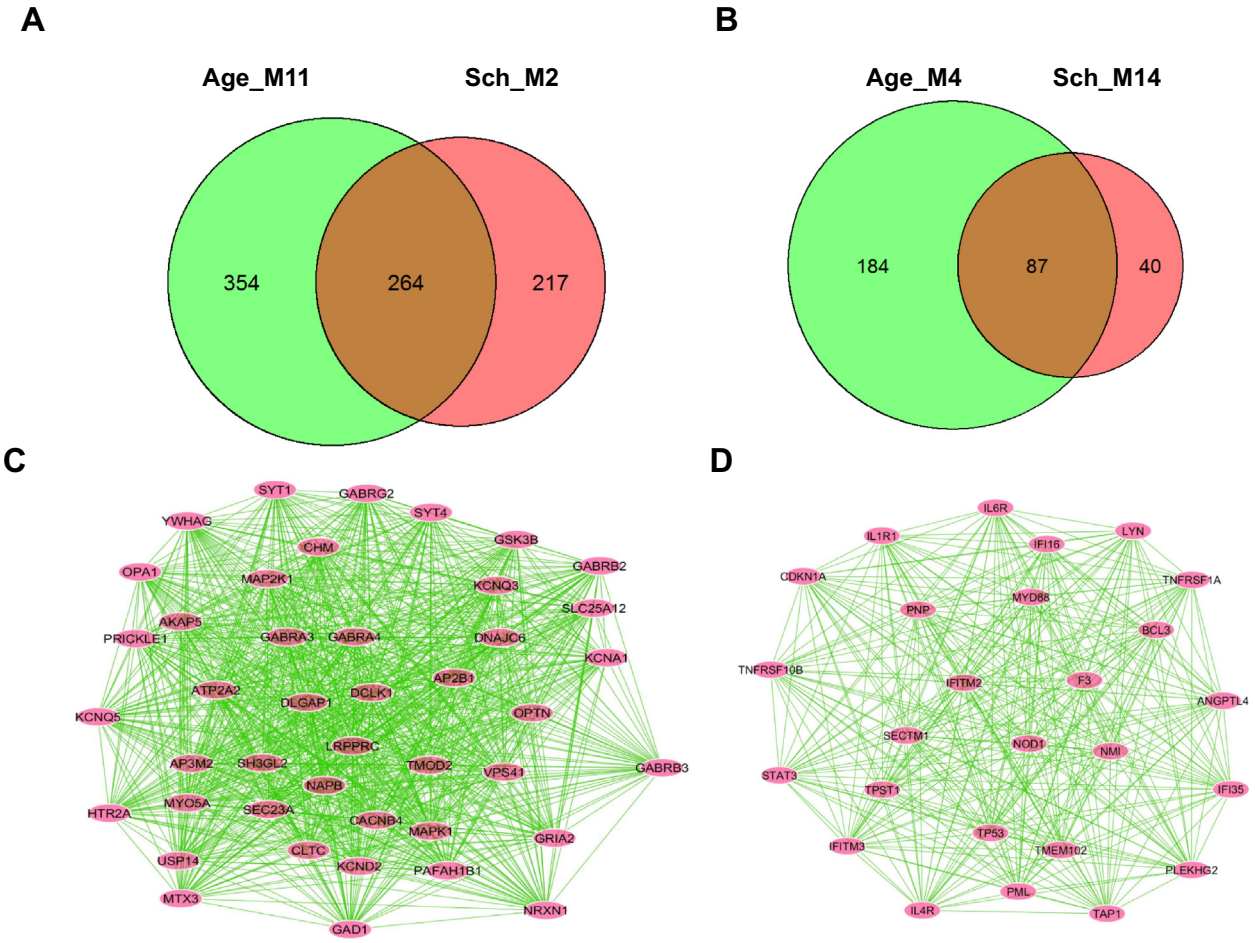

Fig. 4. Comparative network analysis between two modules associated with schizophrenia and the corresponding modules that correlated with age. Venn diagram shows the number of genes that were both common and unique to the module associated with schizophrenia, Sch_M2 and to the module correlated with age, Age_M11 (A) and to the Sch_M14 module and Age_4 module (B). Among 264 genes common to both the Sch_M2 and Age_M11 modules, the network connections of only 43 genes related to synaptic transmission, intracellular transport and gamma aminobutyric acid signaling pathway were visualized (C). Among 87 genes common to both the Sch_M14 module and Age_4 modules, the network connections of only 26 genes related to immune/inflammation responses and regulation of apoptosis were visualized (D). Networks were visualized using Cytoscape (Shannon et al., 2003).

In addition, a significant number of nodes (87) were common to both the Sch_M14 module and the Age_M4 module ($P < 1e{-}06$; Fig. 4B). The Age_M4 module was also reproduced in the replication study using the microarray data. These modules were positively associated with schizophrenia and age, respectively, and the common genes were significantly enriched for processes related to immune/inflammation response (Supplementary Table 21), including *TPST1*, *TNFRSF1A*, *NMI*, *TNFRSF1A*, *LTBR*, *MYD88*, *LYN*, *TNFRSF1A*, *IL1R1*, *IFITM2*, *IL4R*, *IFITM3* (Fig. 4D). The Sch_M14 module was highly enriched with genes for markers specific to endothelial cells (adjusted $P = 2.4e{-}05$) and for activated microglia (adjusted $P = 1.6{-}e05$). The Age_M4 module was also highly enriched with genes for markers of endothelial cells (adjusted $P = 7.8e{-}10$) and activated microglia (adjusted $P = 8.8e{-}15$). The genes *PECAM1*, *CD93* and *EMP1* mark endothelial cells and are common to both modules. The genes *MYD88*, *PPP1R18*, *RAB20* and *SLCO4A1* mark activated microglia and were common to both modules. TheAge_M3 that did not reproduce from microarray data did not have any consensus with the two modules (Sch_M2 and Sch_M14) associated with schizophrenia.

4. Discussion

Aging affects multiple brain functions including cognitive performance (Peters, 2006) and is a risk factor for several neurodegenerative diseases including Alzheimer's disease (Lindsay et al., 2002) and Parkinson's disease (Collier et al., 2011). However, the relationship between normal aging and schizophrenia has generally been speculative (DeLisi, 1997; Kirkpatrick et al., 2008). While we identified two co-expression modules significantly associated with schizophrenia, there were seven co-expression modules significantly correlated with age. Thus, there appear to be more global gene expression changes in the frontal cortex during normal aging than in schizophrenia. Moreover, while there was only one co-expression module related to immune/inflammation response positively associated with schizophrenia (Sch_M14), there were two immune/inflammation related modules (Age_M3 and Age_M4) positively correlated with age. However, within each age-related module, there was a different complement of cell type specific genes differentially enriched and thus each module represents a different immune/inflammation related process. The Age_M4 module was highly enriched with endothelial cell markers and activated microglia markers, and a significant number of the nodes (genes) were also common to the Sch_M14 module. Thus, the up-regulated immune/inflammation related process represented in these two modules involves multiple cell types, including the endothelial cells, and indicates that these modules are representing a common process in the pathophysiology of schizophrenia and normal aging and may indicate an accelerated aging process in schizophrenia.

The disorder we now know as schizophrenia was originally named dementia praecox` by (Kraepelin, 1971 (original 1919)) who described it as a chronic deteriorating brain disease, characterized by rapid cognitive disintegration. Several studies since then have suggested that there may be accelerated brain aging in schizophrenia. Recently, structural

MRI brain imaging studies combined with machine learning algorithms have identified significantly accelerated brain structural aging in schizophrenia (Cropley et al., 2017; Koutsouleris et al., 2014; Nenadic et al., 2017; Schnack et al., 2016), and a microarray study identified 311 probe sets that were differentially expressed between schizophrenia and matched controls and that were also correlated with normal aging (Tang et al., 2009). While these studies all provide evidence for an accelerated brain aging process in schizophrenia, recent epigenetic studies were unable to provide support for the accelerated aging hypothesis of schizophrenia (McKinney et al., 2017; Voisey et al., 2017). However, it is possible that there are non-epigenetic mechanisms driving the aging process in the schizophrenia brain and additional functional studies will be necessary to identify these mechanisms.

The co-expression module Sch_M2 was significantly negatively associated with schizophrenia and was associated with neuronal functions such as synaptic transmission. This is consistent with the results from previous gene expression profiling studies (Fromer et al., 2016; Torkamani et al., 2010) and from our previous network analysis that reported a similar neuron related co-expression module that was significantly downregulated in the hippocampus (Hwang et al., 2013) of individuals with schizophrenia as compared to unaffected controls. The Sch_M2 module included several genes related to the GABAergic system including *GAD1*, *GABRA1*, *GABBR2*, *GABRB2* and *GABRG2*, and thereby adds further support for GABAergic neuronal dysfunction hypothesis of schizophrenia pathophysiology (Curley et al., 2011; Gonzalez-Burgos et al., 2010; Hashimoto et al., 2003; Nakazawa et al., 2012). Moreover, the age associated module (Age_M11) included a significant number of nodes in common with the Sch_M2 module. These nodes were associated with the same biological process, the GABA signaling pathway. GABA is the main inhibitory neurotransmitter in the brain. The balance between excitatory and inhibitory signaling can modulate the gamma oscillations that are necessary for normal cognitive processing (Bartos et al., 2007; Gao and Penzes, 2015). Dysregulation of genes related to the GABAergic system may disrupt the excitatory/inhibitory balance in the brain and eventually contribute to the cognitive deficits found in schizophrenia. Because a significant number of the nodes were common to both the Age_M11 and the Sch_M2 module, it appears this same set of down-regulated genes related to neuronal function may be contributing to the cognitive deficits that occur in individuals with schizophrenia and in normal aged people. Unfortunately information on the cognitive performance of cases in the SMRI brain collection is not available. Future studies that collect prospective data on the cognitive performance of people with schizophrenia and unaffected controls are needed to determine if the co-expression modules related to neuronal function are associated with cognitive deficits in schizophrenia and normal aged people.

We also found the Sch_M2 neuron related co-expression module was significantly negatively correlated with the Sch_M14 immune/inflammation response module. Likewise, the Age_M11 neuron related module was negatively correlated with Age_M4 immune/inflammation response module. These correlations corroborate a study in prefrontal cortex that showed the immune related IFITM genes (that are expressed in the endothelial cells) are expressed at levels inversely correlated with markers of the GABAergic neurons in schizophrenia (Siegel et al., 2014). While the significant negative correlations between the two modules suggests there may be a common factor causing the upregulation of the immune related module and downregulation of the neuron related module, it may also be that a deficit in one of the modules is affecting or causing the other. However, the results are correlational and not causal and therefore, animal model, or cell culture, studies will need to identify if there is a shared upstream cause of both co-expression modules or if one of the modules has a role in causing the dysregulation of the other.

The results of this study corroborate previous RNA-Seq studies from several different brain collections that also identified immune/inflammation-related genes or co-expression modules upregulated in the cerebral cortex (Fillman et al., 2013), hippocampus (Hwang et al., 2013), choroid plexus (Kim et al., 2016a), amygdala (Chang et al., 2017) and peripheral blood (Xu et al., 2012) of individuals with schizophrenia as compared to unaffected controls. Moreover, a large scale genome wide association study has shown the most significant association between schizophrenia and common variants is on the major histocompatibility complex (MHC) region of chromosome 6 (Schizophrenia Working Group of the Psychiatric Genomics, 2014). Here we find an immune/inflammation related co-expression module significantly upregulated in the frontal cortex of schizophrenia cases as compared to controls, that is also consistent with a previous RNA-Seq study conducted on frontal cortex in another brain collection (Fillman et al., 2013).

Several markers of the inflammatory response have been proposed as potential biomarkers for symptom severity in schizophrenia patients (Lai et al., 2016; Liu et al., 2010; Perkovic et al., 2017), for example, IL-1β and TNF-α levels are significantly correlated with scores on the general psychopathology subscale of the Positive and Negative Syndrome Scale (Liu et al., 2010). We found genes related to cytokines, such as IL1R1, TNFRSF1A, TNFRSF10B and TNFRSF12A, that were included in the Sch_M14 module, indicating that this module may be associated with symptom severity in schizophrenia. However, symptom severity scores are not available for the individuals with schizophrenia in the SMRI brain collections. Future prospective studies that collect data on symptom severity over the lifetime of the subjects will be necessary to investigate the association between the immune-related modules and symptom severity in schizophrenia patients.

The immune/inflammation-related Sch_M14 module also included genes specifically associated with defense response to virus such as *IRF9*, *PLSCR1*, *IFITM2*, *IFITM3*, *PML*, and *IFI16*. The *IFITM2* and *IFITM3* genes were included in the upregulated immune/inflammation modules associated with schizophrenia in the hippocampus (Kim et al., 2016b) and choroid plexus (Kim et al., 2016a) in our previous studies. The *IFITM2* and *IFITM3* genes are expressed in endothelial cells in the brain (Siegel et al., 2014) and their primary function is to restrict infection by viruses (Diamond and Farzan, 2013). The Sch_M14 module was also highly enriched for other endothelial cell specific markers. Taken together, these studies suggest that there may be an upregulated immune/inflammation related co-expression network of genes that is expressed in endothelial cells of the cortical blood vessels as they respond to virus or virus like particles, such as human endogenous retrovirus. Interestingly, exposure to various infectious agents is associated with cognitive deterioration in older individuals (Nimgaonkar et al., 2016) and in those with schizophrenia (Prasad et al., 2012).

RNA-Seq studies that use postmortem brain tissue can only provide a snapshot of the gene expression profile at the time of death. Since it is not possible to determine if the individuals with schizophrenia in the SMRI tissue collections were actively psychotic at the time of death we cannot determine if the neuroinflammation is related to the exacerbation of psychosis or not. However, there is evidence for immune activation in the brain of individuals at ultra-high risk for developing schizophrenia but for who the symptoms are not yet manifest (Bloomfield et al., 2016). This indicates that the immune activation is occurring very early in the course of the disease and is not just coincident with psychotic episodes or as a result of living with a chronic disease. Previous gene expression profiling studies, including one using the SMRI Array collection data, showed that there were high and low inflammation sub-groups in individuals with schizophrenia (Fillman et al., 2013; Fillman et al., 2014). These results suggest that the upregulated gene expression network related to the immune/inflammation response may be specifically associated with a particular feature of schizophrenia that has so far eluded identification. Further gene expression profiling in brain tissue from schizophrenia cases that have been followed prospectively and that collect extensive symptom and epidemiological data and that also documents the presence of psychotic features at the time of death may more accurately answer these questions.

One of the main limitations of this study is controlling for the potential confounding effects that some of the descriptive and clinical variables may have on the gene expression data. These effects are generally more serious on data generated from post-mortem tissue as compared to animal tissue or cultured cells. Furthermore, it is very difficult to remove the confounding effects in gene expression data using a statistical analysis. We therefore performed a *post hoc* correlation analysis between the known variables and the co-expression modules built with the standardized residuals from the linear regression, including the surrogate variables, to evaluate the statistical model we initially used. We found that PMI was highly correlated with most co-expression modules that correlated with normal aging. Thus, we included PMI in the linear model for the normal aging study. Moreover, the Sch_M2 module that was significantly associated with schizophrenia also correlated with brain pH. While our control and schizophrenia groups were matched for average PMI and brain pH, further studies with pair-wise matching of individuals between groups for descriptive variables may be required to exclude all the potential confounding effects on gene expression profiling.

Antipsychotic medications may also affect gene expression levels in RNA-seq data from human post-mortem samples. However, in our study, there was no significant correlation between a total lifetime intake of antipsychotics and the two co-expression modules associated with schizophrenia (Sch_M2 and Sch_M14). Thus, medication unlikely contributed to the differential expression of the two modules. While numerous RNA-Seq studies on post-mortem tissue have consistently identified up-regulated immune/inflammation related genes in the brain of individuals with schizophrenia as compared to unaffected controls, one recent large-scale RNA-Seq study using the prefrontal cortex and hippocampus failed to identify differential expression of immune genes in individuals with schizophrenia as compared to controls (Birnbaum et al., 2018). In human post-mortem studies, inadequate correction for confounding factors will likely inflate the possibility for type I and type II statistical errors. Our results show that the expression profile of immune/inflammation related genes in the frontal cortex of younger individuals with schizophrenia overlapped to a high degree those of much older normal people. Thus, the broad age range of the cases and the inclusion of very old normal control individuals in the Birnbaum et al. (2018) study may inflate the type II error and thereby fail to detect an effect that is actually present. In contrast, the ages at death in our current case/control study are all relatively young.

In conclusion, we generated co-expression networks from frontal cortex of individuals with schizophrenia and matched unaffected controls and from a cohort of normal individuals that spanned a very broad age range. We found that both the schizophrenia cohort and the normal aging cohorts had an upregulated immune/inflammation related co-expression network and a downregulated neuronal function related network with many genes common to the overlapping networks. This data provides further evidence to support the hypothesis that there may be accelerated brain aging in schizophrenia that implicates the immune system.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2018.09.010.

Note

Gene symbols for each co-expression module are listed in Supplementary Table 22. The experimental design, including information regarding sample selection, is shown in Supplementary Fig. 5. RNA-Seq raw data (FASTQ) files from frontal cortex of the individuals from the three Stanley tissue collections are publicly available for download at http://sncid.stanleyresearch.org (SNCID).

Contributors

SK and DL designed the study. SK, MW and YJ conducted the data analysis. All authors contributed and have approved the manuscript.

Conflicts of interest

The authors declare that they have no conflict of interest.

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