Expression profiles of schizophrenia susceptibility genes during human prefrontal cortical development

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Background: Disruption in normal development of the human prefrontal cortex (PFC) may lead to cognitive dysfunction that manifests in individuals with schizophrenia. We sought to identify genes associated with age that are implicated in schizophrenia. **Methods:** We generated genome-wide expression profiles for the PFCs of humans ranging in age from 1 month to 49 years using the Affymetrix HG-U133 plus 2.0 microarrays (54 675 transcripts). Based on the criteria of significance (false discovery rate [FDR]-adjusted $q < 0.001$ and $r^2 > 0.6$), we identified the genes associated with age in the PFC. We then performed functional annotation analyses of age-associated genes using the Gene Ontology and the Genetic Association Database (GAD). **Results:** We found robust age-dependent changes in gene expression in the PFCs of humans (2281 transcripts). The GAD analysis revealed that schizophrenia was an over-represented disease class, with 42 susceptibility genes included ($p < 0.001$, fold enrichment = 1.66, FDR = 1.5%). Among the 42 genes, glutamate receptor genes (*GRIA1*, *GRIK1*, *GRIK2*, *GRIN2D*, *GRIP1*, *GRM5*, *GRM7* and *SLC1A6*) were consistently downregulated across age. We confirmed microarray gene expression changes by the quantitative polymerase chain reaction experiment. **Limitations:** Although numerous genes undergo robust changes in expression during the PFC development, some of the changes may be confounded by known and unknown factors that are intrinsic to the postmortem brain studies. **Conclusion:** Multiple schizophrenia susceptibility genes undergo age-dependent expression changes in the human PFC, and any disruption in those genes during the critical period of development may predispose the individuals to schizophrenia.

Introduction

The human prefrontal cortex (PFC) continues to develop into adolescence and early adulthood and is one of the last cortical regions to mature structurally and functionally.[1] It is considered one of the most functionally advanced regions of the brain,[2] mediating working memory, attention, response inhibition and management of autonomic control.[3] Thus, any disruption in the development of the PFC may result in abnormalities in function that could manifest as the symptoms of schizophrenia that appear in adolescence and young adulthood.[2,4–7] For example, there is a peak in dopamine (DA) receptor 1 (DAR1) expression in the human PFC during adolescence and young adulthood,[8] a time that coincides with the attainment of adult-level competency in working memory tasks.[9,10] Since DAR1 plays an important role in memory function,[11–13] any disruption in the maturation of DAR1 in the PFC could manifest as abnormalities in cognitive function such as those that occur in schizophrenia. Moreover, increases in hormone and growth factor receptors also occur during adolescence.[14,15]

Although previous studies that have examined the expression of a limited number of genes during postnatal development can be informative, recent microarray studies that have examined the genome-wide expression patterns across development can be more exhaustive and may be better suited to identifying a group of genes that are associated with specific biological pathways. A recent microarray study examined the expression patterns of 31 schizophrenia susceptibility genes in the PFCs of humans ranging in age from 18 to 67 years.[16] Another microarray study of the developing mouse PFC showed that most changes in gene expression occurred between 2 and 4 weeks postnatal, which would correspond to time points earlier than 18 years in humans.[17] This has been corroborated by recent microarray analyses of gene expression in the human PFC showing that the most dramatic changes in expression occur between birth and 10 years of age.[18–20] Thus, the

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earlier study involving older participants may have missed the window of the most dramatic expression changes in schizophrenia susceptibility genes. Using a genome-wide expression microarray and the Genetic Association Database (GAD),[21] we sought to identify genes in the PFC that are significantly associated with age and implicated in schizophrenia.

Methods

Postmortem brain tissue

We obtained fresh frozen postmortem PFC tissue (Brodmann area 46) of 48 individuals ranging in age from 1 month to 49 years from the National Institute of Child Health and Human Development Brain and Tissue Bank for Developmental Disorders (UMBB; NICHHD contract# NO1-HD8–3283). The Institutional Review Board of the University of Maryland, Baltimore, reviewed and approved the collection protocol. All individuals from whom samples were obtained were free of neurologic and psychiatric symptoms at the time of death, and toxicological analyses showed them to be free of drug use. We included samples if the pH was above 6.35[22] and the RNA was of good quality, as determined by the Bioanalyzer 2100 electrophoresis system (Agilent Technologies).

Microarray experiment

We extracted total RNA from 300 mg of grey matter from the middle frontal gyrus (BA 46) using the Trizol method (Invitrogen)[23] and purified it through a Qiagen RNA miniKit column. Purified RNA was carried through the Affymetrix preparation protocol (www.affymetrix.com), and we hybridized each sample to the Affymetrix HG-U133 plus 2.0 GeneChip to assess genome-wide expression profiles of 54 675 transcripts.

Quality control of microarrays

We processed and analyzed raw microarray data using the R statistical language (R Foundation for Statistical Computing, www.r-project.org) and the Bioconductor packages.[24] A robust multiarray average algorithm allowed for normalization of expression values (log base 2) for each probeset.[25] We assessed microarray chip quality using a pair-wise sample correlation with hierarchical clustering analysis (Appendix 1, available at www.cma.ca/jpn). Based on the quality-control procedures, 2 samples were considered outliers and removed from further analysis. We included all probesets (54 675) in the present analysis.

Multiple regression analysis of age

First we analyzed individual variables to identify potential confounding variables affecting the expression of a significant number of genes in the data set. Then, with a threshold of $p < 0.001$, we calculated the number of transcripts significantly regulated by each variable. We considered the following variables for all individuals whose tissue samples we included in the study: age, sex, race, brain pH, postmortem interval (PMI) and mRNA quality (RIN). We used a linear model to analyze continuous variables such as age, brain pH, PMI and RIN, and discrete variables such as race and sex.

After the individual variable analysis, we performed multiple regression analyses to identify genes associated with age. Since brain pH was the second-most influential factor affecting the expression of about 3% of the probesets, we included brain pH as a covariate in this model. Thus, we analyzed gene expression across age in a series of linear regression models, one model for each gene, including age (log base 2) and brain pH as independent variables and gene expression (log base 2) as the dependent variable. To correct for multiple testing of genes, we adjusted the calculated p values corresponding to the age covariate for each gene to give an overall false discovery rate (FDR) of 5% using the q value package from BioConductor (www.bioconductor.org). The q value is an extension of the FDR. Whereas p value is a measure of significance in terms of false-positive rates, the q value is a measure in terms of the FDR. Thus, the q value FDR approach is a sensible measure of the balance between the number of true-positive and false-positive results that is automatically calibrated and easily interpreted in high-throughput studies, as described previously.[26] We set the criteria of genes associated with age at $q < 0.001$ and adjusted coefficient $r^2 > 0.6$.

Functional annotation of age-associated genes

We used the NCBI Database for Annotation, Visualization and Integrated Discovery (DAVID 2008, http://david.abcc.ncifcrf.gov) as a standard source for gene annotation information.[27] In the DAVID annotation system, a modified Fisher exact test measures the gene-set enrichment in the annotation terms. In addition the set of probesets associated with age is used in an annotation term × annotation term contingency test to identify the association between the significant genes of interest and each annotation term. Both a raw and adjusted p value (using Benjamini–Hochberg's method) for each contingency test is calculated and the criterion for a significant Gene Ontology (GO) term includes an FDR-adjusted $p < 0.05$.[28] The DAVID 2008 also provides a function to associate a set of genes to disease phenotypes using a similar modified Fisher exact test. Thus, we were able to map the age-associated genes to disease phenotypes using information from the Genetic Association Database (GAD) (http://geneticassociationdb.nih.gov/).[21] The GAD is an archive of published genetic association studies with more than 28 000 records and includes major disease classes such as immune, cardiovascular, metabolic, neurodegenerative and psychiatric disorders and cancer. We set a significance criterion for association between age-associated genes and disease phenotypes at an FDR-adjusted $p < 0.05$, as described above.

Quantitative polymerase chain reaction (qPCR) validation

We extracted total RNA from the PFC tissue samples of the same individuals used in the microarray experiment and assessed the quality of RNA with the Bioanalyzer 2100

(Agilent). We further purified RNA with the PureLink Micro-to-Midi Total RNA Purification System (Invitrogen) synthesized complementary DNA (cDNA) with reverse transcription–PCR using oligo dT primers. We used predesigned and validated Qiagen QuantiTect SYBR primers for the qPCR: *GRIA1* (QT01001665, NM_000827), *GRIK1* (QT00080332, NM_000830), *GRIK2* (QT00016597, NM_021956), *GRIN2D* (QT00065324, NM_000836), *GRIN3A* (QT00043617, NM_133445), *GRM5* (QT00026502, NM_000842), *GRM7* (QT00069727, NM_000844) and *SLC1A6* (QT01008392, NM_005071). We selected 3 endogenous control genes, *B2M* (QT00088935, NM 004048), *GUSB* (QT00046046, NM_000181) and *ACTB* (QT00095431, NM_001101), for the experiment. Using a 384-well format with the ABI Prism7900HT real-time detector (Applied Biosystems), 1 μL aliquots of Qiagen QuantiTect SYBR primer, 10 μL qPCR PCR Master mix (Applied Biosystems) and 10 μL diluted cDNA were mixed together and pipetted into single wells of the qPCR plate. For no template controls for each gene tested, we added water instead of cDNA. Thermocycle conditions were 1 cycle for 2 minutes at 50°C, 1 cycle for 15 minutes at 95°C, and 40 cycles for 15 seconds at 95°C and 1 minute at 60°C. We measured fluorescence during the 60°C step for each cycle, as recommended by the manufacturer. Using SDS2.2 software (Applied Biosystems), we quantified reactions by the cycle threshold method. We calculated an average quantity value (Qty mean) for each sample from the triplicates of that sample for each gene. The data for each gene are expressed as Qty mean for the gene of interest or geometric mean of Qty mean for the 3 endogenous control genes. We performed linear regression analyses with age (log base2) and pH as independent variables and normalized expression values for each gene as a dependent variable.

Results

Demographic and postmortem variables

Table 1 provides a summary of the demographic information for the 46 individuals from whom we obtained postmortem brain tissue samples (age 1 month–49 years, 31 male and 15 female, 25 black and 21 white). For illustrative purposes, we divided individuals into 7 age groups: neonate, infant, toddler, school age, teenage, young adult and adult. The means and standard deviations (SD) for brain pH (6.7, SD 0.16), PMI (18.0, SD 7.54 h) and RNA integrity number (8.5, SD 0.66) indicate that the brain tissue samples were of good quality. Detailed demographic information on each individual is provided in Appendix 2, available at www.cma.ca/jpn.

We examined the effect of individual demographic variables on gene expression using a threshold of $p < 0.001$. This analysis revealed that 20% of the probesets are regulated by age, and 3.1% are regulated by brain pH. Other variables such as PMI (1%), RIN (0.5%), race (0.3%) and sex (0.2%) affected a relatively small number of probesets in this analysis. Principal component analysis confirmed that the first component in the data set was highly correlated with age, which accounts for about 20% of the total variance in the data (data not shown). Therefore, the predominant pattern of mRNA-level change was a gradual increase or decrease in expression as a function of age.

Genes associated with age

Individual variable analysis revealed that brain pH was the second-most influential factor, affecting the expression of 3.1% of the probesets ($p < 0.001$). The other 4 factors affected a much smaller percentage of probesets (< 1%), so they were not included in subsequent analyses. We used a linear regression model to compute adjusted p values and regression coefficients (r^2) of probesets with age and pH as covariates. Based on the criteria of significance (FDR-adjusted $q < 0.001$ and $r^2 > 0.6$), we identified 2281 probesets (1792 genes) that change expression with age ranging from 1 month to 49 years. Among the 2281 probesets, 1124 probesets showed increased expression, and 1157 probesets showed decreased expression across age.

Functional annotation of age-associated genes

We performed functional annotation of age-associated genes (2281 probesets) using the DAVID 2008 knowledgebase. Table 2 represents several over-represented Gene Ontology (GO) terms in the GO analysis. Significant GO terms include nervous system development ($p < 0.001$, fold enrichment 2.23, FDR < 0.001%), cellular component organization/biogenesis ($p < 0.001$, fold enrichment 1.40, FDR < 0.001%),

Table 1: Demographic characteristics of 46 individuals from whom we obtained postmortem brain tissue samples for analysis

Age group	Age, mean (range) yr	Sex, M:F	Race	Value, mean (SD)		
				pH	Postmortem interval, h	RNA integrity number
Neonate	0.2 (0.1–0.2)	5:2	6B, 1W	6.6 (0.13)	23 (6.34)	9.0 (0.32)
Infant	0.4 (0.3–0.9)	7:2	6B, 3W	6.7 (0.17)	18 (7.21)	8.7 (0.63)
Toddler	3.1 (1.6–4.9)	3:3	3B, 3W	6.7 (0.17)	26 (9.56)	8.2 (0.56)
School age	9.7 (5.4–13.0)	3:3	1B, 5W	6.7 (0.15)	15 (5.43)	8.7 (0.40)
Teenage	16.9 (15.0–17.8)	5:1	2B, 4W	6.8 (0.06)	17 (4.71)	8.0 (1.06)
Young adult	23.2 (20.1–25.4)	4:2	3B, 3W	6.8 (0.19)	12 (5.40)	9.0 (0.35)
Adult	41.9 (36.0–49.2)	4:2	4B, 2W	6.7 (0.22)	14 (5.34)	8.1 (0.42)

B = black; F = female; M = male; RNA = ribonucleic acid; SD = standard deviation; W = white.

transmission of nerve impulse ($p < 0.001$, fold enrichment 2.20, FDR = 0.002%), Golgi apparatus ($p < 0.001$, fold enrichment 1.58, FDR = 0.3%) and mitochondrion ($p < 0.001$, fold enrichment 1.37, FDR = 2.9%). A complete list of significant GO terms is shown in Appendix 3, available at www.cma.ca/jpn.

Next, we mapped disease phenotypes with age-associated genes (2281 probesets) in the Genetic Association Database (GAD). This analysis revealed that genes associated with schizophrenia are over-represented among the age-associated genes ($p < 0.001$, fold enrichment: 1.66, FDR = 1.5%). Thus, 42 schizophrenia susceptibility genes show age-dependent changes in expression in the PFCs of humans. Among the 42 genes, 20 genes show increased expression across age, and 22 genes show decreased expression across age. Figure 1 represents expression profiles of the 42 schizophrenia susceptibility genes across age ranging from 1 month to 49 years.

Genes with increasing expression across age include *DARPP-32* (PPP1R1B), monoamine oxidase B (*MAO*B), regulator of G-protein signaling (*RGS4*) and synapse-related genes (*CPLX1* and *SNAP25*), as shown in the lower part of Figure 1. Genes with decreasing expression across age include reward-related genes (*PNOC* and *CNR1*) and several glutamate receptor genes: NMDA receptor 2D (*GRIN2D*), AMPA receptor 1 (*GRIA1*), Kainate receptor 1 (*GRIK1*), kainate receptor 2 (*GRIK2*), metabotropic glutamate receptor 5 (*GRM5*), metabotropic glutamate receptor 7 (*GRM7*) and glutamate transporter (*SLC1A6*), as shown in the upper part of Figure 1. Detailed information on gene annotation and statistical analysis on the 42 schizophrenia susceptibility genes is shown in Appendix 4, available at www.cma.ca/jpn.

Figure 2 illustrates the differential expression patterns for 9 glutamate genes across age. Although *GRIN3A* (NMDA receptor 3A subunit) was not included in the schizophrenia susceptibility gene list obtained from the GAD, it was downregulated across age and therefore included in Figure 2. Genes such as *GRIN3A* ($r^2 = 0.88$, $q < 0.001$), *GRM7* ($r^2 = 0.84$, $q < 0.001$) and *SLC1A6* ($r^2 = 0.91$, $q < 0.001$) showed a pattern of constant downregulation across age. However, genes such as *GRIA1* ($r^2 = 0.75$, $q < 0.001$), *GRIK2* ($r^2 = 0.69$, $q < 0.001$), *GRM5* ($r^2 = 0.73$, $q < 0.001$) and *GRIP*1 ($r^2 = 0.71$, $q < 0.001$) showed a rapid decrease in expression in the first 5 years. In contrast, the expression of *GRIK1* ($r^2 = 0.60$, $q < 0.001$) declined most significantly after 5 years of age.

Quantitative polymerase chain reaction validation of the glutamate-related genes

We selected 8 glutamate receptor genes — *GRIA1*, *GRIK1*, *GRIK2*, *GRIN2D*, *GRIN3A*, *GRM5*, *GRM7* and *SLC1A6* — for qPCR validation based on the microarray analysis. Overall, the microarray and the qPCR data were consistent, with all genes showing the same direction of expression changes in both analyses (Table 3). The qPCR experiment confirmed that the expression levels of these glutamate-related genes are high in early postnatal development and decrease across age in the PFCs of humans. The qPCR results further support the effectiveness of our significance criteria (FDR-adjusted $q < 0.001$ and $r^2 > 0.6$) in minimizing false-positive discovery in the microarray data.

Discussion

Our results indicate that a substantial number of schizophrenia susceptibility genes undergo dynamic changes in expression in the PFC during the early years of development. Thus, interference in the expression changes of schizophrenia susceptibility genes may increase the risk for abnormal brain function that could lead to the onset of schizophrenia. Furthermore, the differential expression of these genes across development may have important implications for the responses to psychiatric medications that are administered to younger individuals.

Age-associated genes

We identified 2281 probesets (1792 genes) with a threshold of $q < 0.001$ and $r^2 > 0.6$ across age from 1 month to 49 years. This corroborates a recent study that found 1513 transcripts showing large expression changes (a more than 2-fold change from the gene's median value).[20] This study also found 130 transcripts ($p < 0.001$) that show expression changes by sex, and we found similar sex effects on gene expression (110 transcripts, $p < 0.001$). Another microarray study examined age-related changes in gene expression in the PFCs of humans and identified 540 transcripts that changed expression in ages ranging from 13 to 79 years.[29] This suggests that many of the age-related changes occur during the first decade of human life and that the inclusion

Table 2: Gene Ontology analysis with age-associated genes*

Category	Term	No. (%) of genes included in each category	*p* value	Fold enrichment	FDR, %
GOTERM_BP_ALL	GO:0007399~nervous system development	132 (7.37)	< 0.001	2.23	< 0.001
GOTERM_BP_ALL	GO:0016043~cellular component organization and biogenesis	289 (16.13)	< 0.001	1.40	< 0.001
GOTERM_BP_ALL	GO:0019226~transmission of nerve impulse	58 (3.24)	< 0.001	2.20	0.002
GOTERM_CC_ALL	GO:0043005~neuron projection	25 (1.40)	< 0.001	2.96	0.028
GOTERM_CC_ALL	GO:0005794~Golgi apparatus	83 (4.63)	< 0.001	1.58	0.289
GOTERM_CC_ALL	GO:0005739~mitochondrion	107 (5.97)	< 0.001	1.37	2.859

FDR = false discovery rate†; GO = Gene Ontology.
*Gene Ontology analysis was performed using genes associated with age (2281 probesets). Examples of significant GO terms that are related to neuronal development and cell signalling are shown, *p* values are based on the Fisher exact test, and a complete list of significant GO terms is shown in Appendix 3, available at www.cma.ca/jpn.
†Based on Benjamini-Hochberg method.

of the younger individuals (1 mo to 12 yr) improves the power to detect gene-expression changes in the current analysis. With the younger age groups in our study, we identified 1792 genes that showed large expression changes across age. The details on the genes associated with age ($q < 0.001$ and $r^2 > 0.6$) are shown in Appendix 5, available at www.cma.ca/jpn. Moreover, the GO analyses confirmed that the genes were primarily involved in the developmental processes, such as central nervous system development, cellular component organization and biogenesis, cell adhesion and cell motility (Appendix 3).

Schizophrenia susceptibility genes

Mapping age-associated genes to disease phenotypes using the GAD revealed schizophrenia as the only significant disease class at an FDR-adjusted threshold of $p < 0.001$ (fold enrichment 1.66, FDR 1.5%), with 42 susceptibility genes included in this analysis. This is striking because we used an unbiased approach to map age-associated genes to various disease phenotypes in the GAD. Among the 42 schizophrenia susceptibility genes, 20 genes showed increased expression across age, and 22 genes showed decreased expression across

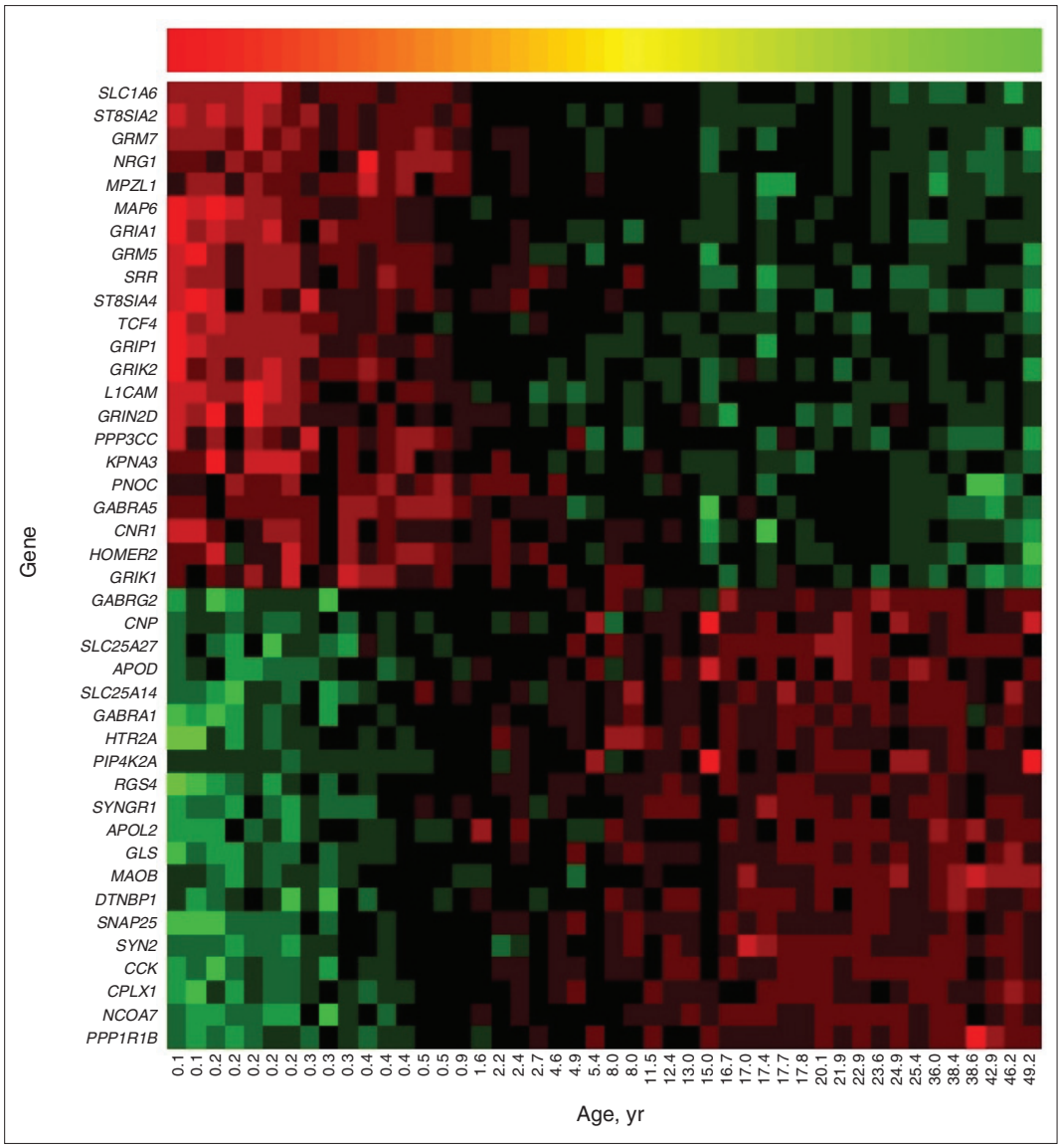

Fig. 1: Heat map of schizophrenia susceptibility genes showing age-related changes in the prefrontal cortices (PFCs) of humans. The disease-association analysis using the Genetic Association Database (GAD) revealed schizophrenia as a significant disease class, with 42 susceptibility genes included in this class. In this pseudo-colour heat map, increasing red intensities indicate genes with high expression levels, and increasing green intensities indicate genes with low expression levels across age. Colour bar scale: hybridization intensity (log base 2) from 2.18 to 11.47. Please see Appendix 4, available at www.cma.ca/jpn, for detailed information on the genes and statistical analysis.

age. However, the individual gene-expression patterns between 1 month and 49 years were quite different for each gene. For example, some genes (*GRIA1*, *GRIK2*, *GRM5* and *GRIP1*) showed the most dramatic expression changes in the first 5 years, whereas *GRIK1* showed the most dynamic changes after 5 years.

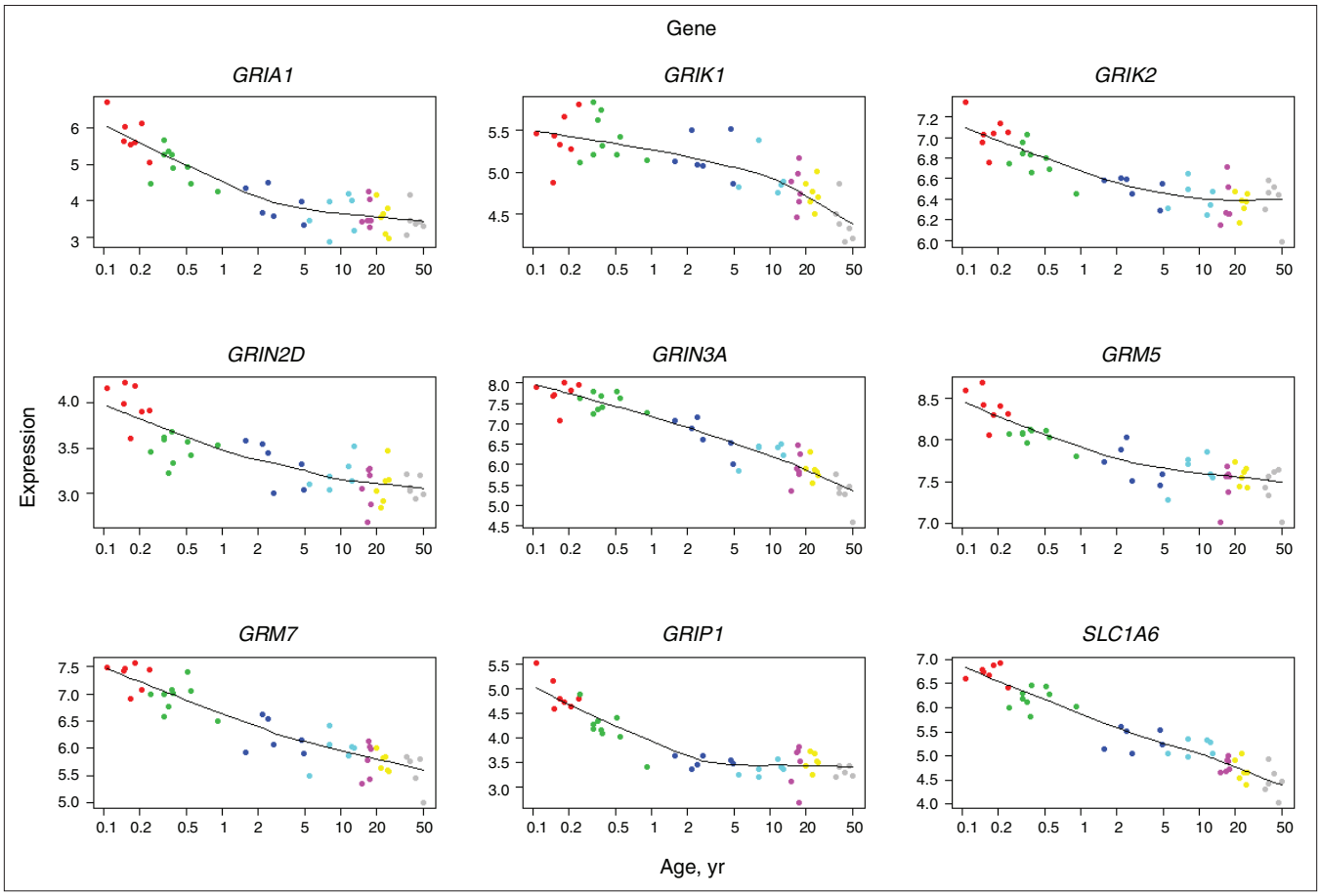

Fig. 2: Nine glutamate-related genes show consistent downregulation across age from 1 month to 49 years. The scatter plot demonstrates that each gene shows different patterns of expression across age ($q < 0.001$ and $r^2 > 0.6$). Points are coloured by the predefined age groups: red = neonate, green = infant, blue = toddler, light blue = school age, pink = teenage, yellow = young adult, gray: adult. *GRIA1* = AMPA receptor subunit 1; *GRIK1* = Kainate receptor subunit 1; *GRIK2* = Kainate receptor subunit 2; *GRIN2D* = NMDA receptor subunit 2D; *GRIN3A* = NMDA receptor subunit 3A; *GRM5* = metabotropic glutamate recptor 5; *GRM7* = metabotropic glutamate recptor 7; *GRIP*1 = glutamate receptor interacting protein; *SLC1A6* = high affinity aspartate/glutamate transporter.

Table 3: Quantitative polymerase chain reaction validation of glutamate receptor genes

Gene symbol	Probeset	Gene name	Microarray		qPCR	
			*r**	*q* value†	*r**	*p* value
GRIA1	215634_at	Glutamate receptor, ionotropic, AMPA 1	–0.87	< 0.001	–0.45	0.002
GRIK1	214611_at	Glutamate receptor, ionotropic, kainate 1	–0.78	< 0.001	–0.55	< 0.001
GRIK2	213845_at	Glutamate receptor, ionotropic, kainate 2	–0.83	< 0.001	–0.34	0.025
GRIN2D	229883_at	Glutamate receptor, ionotropic, *N*-methyl-D-aspartate 2D	–0.81	< 0.001	–0.56	< 0.001
GRIN3A	233220_at	Glutamate receptor, ionotropic, *N*-methyl-D-aspartate 3A	–0.94	< 0.001	–0.51	< 0.001
GRM5	214217_at	Glutamate receptor, metabotropic 5	–0.86	< 0.001	–0.46	0.002
GRM7	207548_at	Glutamate receptor, metabotropic 7	–0.91	< 0.001	–0.45	0.003
SLC1A6	206882_at	High affinity aspartate/glutamate transporter	–0.95	< 0.001	–0.48	0.001

FDR = false discovery rate; qPCR = quantitative polymerase chain reaction.
*Correlation coefficient from linear regression model.
† FDR-adjusted *q* value (FDR = 5%).

Using a candidate gene approach, Colantuoni and colleagues[16] examined the expression profiles of 31 schizophrenia susceptibility genes in the PFCs of humans ranging in age from 18 to 67 years. The authors found that *PPP1R1B* (*DARPP-32*) expression increased across age, whereas neuregulin-1 (*NRG1*) expression decreased across age, particularly between 18 and 30 years.[16] This is consistent with our findings, and our results further suggest that *PPP1R1B* ($r^2 = 0.84$, $q < 0.001$) and *NRG1* ($r^2 = 0.81$, $q < 0.001$) undergoes more dramatic expression changes during the younger ages (1 mo–17 yr). Moreover, the different isoforms of *NRG1* appear to have varying developmental expression patterns across age and will require further investigation.[19]

Glutamate receptor genes implicated in schizophrenia

Previous studies have suggested abnormal glutamate neurotransmission in the pathophysiology of schizophrenia,[30–33] and the glutamate receptors, including the NMDA, AMPA/kainate and metabotropic receptors, have been studied at both gene and protein levels in people with schizophrenia. Moreover, the glutamate receptors have also been implicated in the biological processes that are important in brain development.[34–37] We identified 9 glutamate genes that are consistently downregulated in the PFCs of humans aged 1 month to 49 years.

The AMPA receptor subunit GluR1 (*GRIA1*) and kainate receptor subunit *GRIK2* are expressed at high levels at birth, decrease rapidly until age 5 years and then stabilize throughout the adolescent and young adult years. People with schizophrenia appear to maintain the high levels of *GRIA1* throughout life[38] and thus may not experience the decrease in *GRIA1* expression that occurs in the early developmental period. Whether the maintenance of high *GRIA1* levels in the PFCs of people with schizophrenia results from single nucleotide polymorphisms in the gene or from the downstream effects of other abnormalities, it would be expected to result in subsequent changes in brain development and function. In contrast, expression levels of the kainate receptor *GRIK1* gene decrease rapidly after 5 years of age through the adolescent and young adult years. The *GRIK1*-containing kainate receptors have been implicated in modulating the release of both glutamate[39] and γ-aminobutyric acid (GABA)[40] and are important for mediating neuronal oscillations in the gamma frequency band.[41] Therefore, a disruption in the expression changes of *GRIK1* during development may lead to disruption of the inhibitory tone and abnormalities in the gamma synchrony as observed in schizophrenia.[42,43]

The NMDA receptor subunits 2D (*GRIN2D*) and 3A (*GRIN3A*) are expressed at high levels after birth then decline progressively through to adulthood, which is consistent with previous findings for *GRIN3A*.[44] Changes in the NMDA receptor subunit composition are known to occur during development and contribute to the NMDA receptor variation and to the changing synaptic plasticity that is observed during cortical development.[45,46] The *GRIN3A* subunits, which can suppress dendritic spine formation,[47–49] appear to be maintained at high levels throughout adulthood in the PFCs of people with schizophrenia,[50] and therefore this gene may have an important role in regulating cell survival and activity-dependent synaptic plasticity in the PFCs of patients with schizophrenia.

The G protein-coupled metabotropic glutamate receptor 5 (*GRM5*) and 7 (*GRM7*) mRNA levels also decrease across age. There is evidence that the metabotropic receptors are involved in fundamental developmental processes that occur before synatogenesis such as proliferation, differentiation and survival of neural stem/progenitor cells.[34] Thus, it is possible that the peak in GRM expression occurs before birth, at an earlier stage of development than examined here. The high levels of *GRM7* expression in the first postnatal year may reflect the critical role of the GRMs in very early stages of brain development.

Limitations

Numerous genes show robust changes in expression during the development of the PFC. However, it is possible that some of these changes may be confounded by demographic and clinical factors that are inherent to postmortem brain tissues. Thus, it will be necessary to corroborate our findings with other types of data such as functional brain imaging and neuropsychological and cognitive testing to better understand PFC function during critical periods of brain development.

Conclusion

There are substantial molecular-, cellular- and circuitry-level changes that occur throughout the development of the PFC.[51–53] These changes involve the complex orchestration of events that include the precise timing of excitatory glutamatergic inputs,[54] inhibitory GABAergic connections and modulation by DA and neuropeptide inputs. Disturbances in any of these processes during development could lead to the symptoms of schizophrenia.[55] The high expression levels of the glutamate receptor genes associated with schizophrenia during early development highlight their important contribution to PFC development. Thus, any disruptions that affect the expression changes of the schizophrenia susceptibility genes during brain development may have profound effects on brain function. The different expression profiles of the neurotransmitter-related genes suggest that younger individuals may have quite different responses to psychotropic medications compared with adults. Understanding brain development and the time frame in which the schizophrenia susceptibility genes undergo dynamic changes in expression may lead to early intervention strategies before the onset of schizophrenia and benefit treatment strategies in schizophrenia.

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Contributors: Drs. Choi, Weickert and Webster designed the study. Ms. Zepp acquired the data, which Drs. Choi, Higgs, Weickert and Webster analyzed. Dr. Choi wrote the article. All authors reviewed the article and gave final approval for publication.

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JPN's top 10 Articles, October 2009 (based on Web views on PubMed Central)

1. **Candidate genes and neuropsychological phenotypes in children with ADHD: review of association studies**
   Kebir et al.
   J Psychiatry Neurosci 2009;34(2):88–101

2. **Brand versus generic medications: the money, the patient and the research**
   Blier
   J Psychiatry Neurosci 2003;28(3):167–8

3. **Lifestyle drugs, mood, behaviour and cognition**
   Young
   J Psychiatry Neurosci 2003;28(2):87–9

4. **Amygdala and insula response to emotional faces in patients with generalized social anxiety disorder**
   Shah et al.
   J Psychiatry Neurosci 2009;34(4):296–302

5. **Influence of sleep-wake and circadian rhythm influences in psychiatric disorders**
   Boivin
   J Psychiatry Neurosci 2000;25(5):446–58

6. **What is the best way to treat bipolar depression?**
   Silverstone
   J Psychiatry Neurosci 2005;30(5):384

7. **Antidepressants as analgesics: an overview of central and peripheral mechanisms of action**
   Sawynok et al.
   J Psychiatry Neurosci 2001;26(1):21–9

8. **The role of neurotensin in central nervous sytem pathophysiology: What is the evidence?**
   St-Gelais et al.
   J Psychiatry Neurosci 2006;31(4):229–45

9. **Long-term lithium treatment and thyroid antibodies: a controlled study**
   Baethge et al.
   J Psychiatry Neurosci 2005;30(6):423–27

10. **The use of medroxyprogesterone acetate for the treatment of sexually inappropriate behaviour in patients with dementia**
    Anderson Light and Holroyd
    J Psychiatry Neurosci 2006;31(2):132–4