Brain Struct Funct (2008) 213:255–271
DOI 10.1007/s00429-008-0181-5

ORIGINAL ARTICLE

Age-related changes in the expression of schizophrenia susceptibility genes in the human prefrontal cortex

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Received: 23 January 2008 / Accepted: 6 April 2008 / Published online: 10 May 2008

Abstract The molecular basis of complex neuropsychiatric disorders most likely involves many genes. In recent years, specific genetic variations influencing risk for schizophrenia and other neuropsychiatric disorders have been reported. We have used custom DNA microarrays and qPCR to investigate the expression of putative schizophrenia susceptibility genes and related genes of interest in the normal human brain. Expression of 31 genes was measured in Brodmann's area 10 (BA10) in the prefrontal cortex of 72 postmortem brain samples spanning half a century of human aging (18–67 years), each without history of neuropsychiatric illness, neurological disease, or drug abuse. Examination of expression across age allowed the identification of genes whose expression patterns correlate with age, as well as genes that share common expression patterns and that possibly participate in common cellular mechanisms related to the emergence of schizophrenia in early adult life. The expression of GRM3 and RGS4 decreased across the entire age range surveyed, while that of PRODH and DARPP-32 was shown to increase with age. NRG1, ERBB3, and NGFR show expression changes during the years of greatest risk for the development of schizophrenia. Expression of FEZ1, GAD1, and RGS4 showed especially high correlation with one another, in addition to the strongest mean levels of absolute correlation with all other genes studied here. All microarray data are available at http://www.ncbi.nlm.nih.gov/geo/ (accession #: TBA).

Keywords Aging · Disease onset · Schizophrenia · Gene expression · Susceptibility · Postmortem · Prefrontal cortex

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Introduction

Strong evidence for a genetic basis of schizophrenia was first provided by the Danish adoption studies (Kety et al. 1976) and was further verified by studies of the relative concordance rates among monozygotic versus dizygotic twin pairs (Cardno and Gottesman 2000; Sullivan et al. 2003). It is assumed that for most individuals with this disorder, the genetic liability for schizophrenia is due to the interaction of risk alleles for multiple genes, rather than a single genetic defect (Gottesman and Shields 1967). Two recent meta-analyses of genome-wide scans have identified multiple loci of particular significance (Badner and Gershon 2002; Lewis et al. 2003). In recent years, a number of genetic polymorphisms have been identified that impact cognition and risk for neuropsychiatric illness. Genes that have been implicated in schizophrenia include catechol-O-methyl transferase (COMT: Li et al. 1996; Kunugi et al. 1997; Li et al. 1999; Egan et al. 2001; Shifman et al. 2002; Chen et al. 2004), neuregulin I (NRG1: Stefansson et al. 2002, 2003; Yang et al. 2003;

Tang et al. 2004; Corvin et al. 2004), regulator of G-protein signaling 4 (RGS4: Mirnics et al. 2001; Chowdari et al. 2002, Williams et al. 2004; Morris et al. 2004), dysbindin (DTNBP1: Straub et al. 2002; Schwab et al. 2003; Numakawa et al. 2004), G72 (Chumakov et al. 2002; Korostishevsky et al. 2004; Schumacher et al. 2004; Korostishevsky et al. 2005; Zou et al. 2005), disrupted-in-schizophrenia 1 (DISC1: St Clair et al. 1990; Hennah et al. 2003; Callicott et al. 2005; Millar et al. 2005), and metabotropic glutamate receptor-3 (GRM3: Marti et al. 2002; Fujii et al. 2003; Egan et al. 2004; Chen et al. 2005). A number of other genes are also under active investigation, and the total number of potential susceptibility genes continues to rise (Harrison and Weinberger 2005). While genetic association has opened the door to characterizing genetic causation of diseases like schizophrenia, the mechanisms of these associations are uncertain and will require biologic experiments to understand the relationship of variation in gene function with disease pathogenesis.

Using custom DNA microarrays in addition to qPCR, we have measured the expression of many genes potentially involved in schizophrenia in 72 frontal cortical samples (BA10) from normal postmortem human controls spanning approximately a half century of human aging. We chose to interrogate gene expression patterns in the prefrontal cortex, due to its role in schizophrenia, and also because it is one of the last areas of the brain to fully mature (Paus et al. 1999; Barnea-Goraly et al. 2005).

Our first aim here was the identification of genes whose expression patterns correlate with age. The distinct age of onset associated with schizophrenia (Hafner and an der Heiden 1997; Alda et al. 1996; DeLisi 1992; Jayaswal et al. 1988) as well as differences in this age of onset across sex (Leung and Chue 2000; Tamminga 1997; Larsen et al. 1996; Angermeyer and Kuhn 1988) have long been thought to be clues to the molecular etiology of schizophrenia (Weinberger 1987). Hence, we took particular interest in expression changes occurring during the years of schizophrenia disease onset. We also investigated correlations between all the expression patterns studied here to identify genes that share common expression patterns, and hence possible functional connections with one another. This is designed as a first step in investigating possible interactions by which these genes may confer risk for schizophrenia. While enabling insight into the expression patterns of these genes involved in neuropsychiatric illness, how these patterns relate to one another, and how they relate to age, this study design does not provide an approach for adding new genes to the list of those involved in neuropsychiatric illness, as a whole genome expression study might.

Materials and methods

Schizophrenia susceptibility gene list

The list of schizophrenia susceptibility genes was constructed based on a review of the relevant literature and most current research (Harrison and Weinberger 2005). Table 1 lists the 31 genes we selected for investigation in this study.

Human postmortem tissue collection and dissection

Brain tissue was obtained from normal control subjects at autopsy from the Offices of the Chief Medical Examiner of Washington DC and of Northern Virginia (Clinical Brain Disorders Branch, IRP, NIMH collection). All the tissues were donated with informed consent from the next-of-kin (NIMH IRB approved protocol # 90-M-0142), and collection of the tissue conformed to research guidelines of the National Institutes of Health. The demographics for the research cohort are given in Table 2. It was of concern that many of the susceptibility genes under study here had been identified in primarily Caucasian populations (references in Table 1), while 60% of subjects in this study are of African–American descent (Table 2). To investigate the possibility that ethnicity had an impact on gene expression in this study, we calculated expression changes across ethnicity for all the 31 genes studied here, and compared these to both a theoretical and empirically derived (permutation-based) null distribution of expression changes. The distribution of observed expression changes across ethnicity did not significantly exceed either of these null distributions.

Ascertainment of normal control status was established by two psychiatrists conducting independent review of the medical records and with information from family interviews, applying DSM-IV criteria. The cohort consisted of individuals without any history of neurological disease, or psychiatric illness or treatment (including psychotropic medication). Samples showing evidence of intoxication with illicit drugs (including cannabinoids, phencyclidine, amphetamine, cocaine, ketamine, and opiates), or >0.06% blood ethanol levels were excluded. The latter was confirmed by serum and brain toxicology screening. Legal psychotropic drugs including benzodiazepines and barbiturates were also included in toxicological screens. Subjects positive for over-the-counter medications, medications associated with common chronic disease such as diabetes or hypertension, or medications commonly used in the resuscitation of patients were not excluded from this study. The next-of-kin for all subjects were interviewed to gather a history of psychological care, psychiatric admissions, detoxification or drug treatment for the donor, as

Table 1 Thirty-one schizophrenia susceptibility genes and the correlation of their expression levels with age

	Gene symbol	Alternate gene symbols	Gene name	RefSeq transcripts in probe design	Chromosome location	References	Pearson's *r*	*P* value
1*	AKT1	RAC, PKB	v-akt Murine thymoma viral oncogene homolog 1	NM_005163	14q32.32	Emamian et al. 2004; Ikeda et al. 2004; Schwab et al. 2005	0.09	4.7E-01
2*	BDNF		Brain-derived neurotrophic factor	NM_001709, NM_170731, NM_170732, NM_170733, NM_170734, NM_170735	11p13	Hariri et al. 2003; Egan et al. 2003	−0.14	2.3E-01
3*	CHRNA7	NACHRA7	Cholinergic receptor, nicotinic, alpha polypeptide 7	NM_000746	15q14	Freedman et al. 2001; Xu et al. 2001	−0.25	3.3E-02
4	COMT		Catechol-O-methyltransferase	NM_000754	22q11.21	Many—see text	0.02	8.4E-01
5	DAOA	G72	D-amino acid oxidase activator	NM_172370	13q34	Many—see text	−0.02	8.5E-01
6*	DISC1	KIAA0457	Disrupted in schizophrenia 1	NM_018662	1q42.1	Many—see text	−0.06	5.9E-01
7	DLG4	PSD95, SAP90	Discs, large homolog 4	NM_001365	17p13.1	Binds to ERBB's. NRG1 binds DLG4 promoter	0.03	8.0E-01
8*	DTNBP1	Dysbindin	Dystrobrevin binding protein 1	NM_183041	6p22.3	Many—see text	0.00	9.9E-01
9	ERBB2	HER2	v-erb-b2 Erythroblastic leukemia viral oncogene homolog 2	NM_004448	17q21.1	NRG1 receptor. Corfas et al. 2004	0.23	5.6E-02
10	ERBB3	HER3	v-erb-b2 Erythroblastic leukemia viral oncogene homolog 3	NM_001982	12q13	NRG1 receptor. Corfas et al. 2004	0.20	8.5E-02
11	ERBB4	HER4	v-erb-a Erythroblastic leukemia viral oncogene homolog 4	NM_005235	2q33.3-q34	NRG1 receptor. Corfas et al. 2004; Norton et al. 2005	0.03	8.1E-01
12	ESR1	ESRA	Estrogen receptor 1	NM_000125	6q25.1	Perlman et al. 2005	−0.08	5.0E-01
13	FEZ1	Zygin 1	Fasciculation and elongation protein zeta 1	NM_022549	11q24.2	Binds to DISC1. Miyoshi et al. 2003; Yamada et al. 2004	−0.09	4.7E-01
14*	GAD1	GAD, GAD67	Glutamate decarboxylase 1 (brain, 67 kDa)	NM_013445, NM_000817	2q31	Addington et al. 2005	−0.12	3.1E-01
15*	*GRM3*	*MGLUR3, GLUR3*	*Glutamate receptor, metabotropic 3*	*NM_000840*	*7q21.1-q21.2*	*Many—see text*	*−0.34*	*4.0E-03*
16	MUTED	MU	Muted homolog	NM_201280	6p25.1-p24.3	Binds to DTNBP1. Li et al. 2003; Starcevic and Dell'Angelica 2004	0.22	6.2E-02
17	NDEL1	NUDEL	NudE nuclear distribution gene E homolog like 1	NM_030808	17p13.1	Binds to DISC1. Morris et al. 2003; Ozeki et al. 2003; Brandon et al. 2004	−0.08	5.3E-01
18	NGFR	P75(NTR)	Nerve growth factor receptor (TNFR superfamily, member 16)	NM_002507	17q21-q22	Neurotrophin receptor	0.05	7.0E-01
19*	NOS1AP	CAPON	Nitric oxide synthase 1 (neuronal) adaptor protein	NM_014697	1q23.3	Brzustowicz et al. 2004; Zheng et al. 2005	0.05	7.0E-01

Table 1 continued

	Gene symbol	Alternate gene symbols	Gene name	RefSeq transcripts in probe design	Chromosome location	References	Pearson's *r*	*P* value
20*	NRG1	ARIA, HRG, SMDF, GGF, NDF	Neuregulin 1	NM_004495, NM_013957, NM_013958, NM_013959, NM_013960	8p21-p12	Many—see text	−0.08	5.1E-01
21	NTRK1	TRKA	Neurotrophic tyrosine kinase, receptor, type 1	NM_002529	1q21-q22	Neurotrophin receptor	0.11	3.5E-01
22	NTRK2	TRKB	Neurotrophic tyrosine kinase, receptor, type 2	NM_006180	9q22.1	Neurotrophin receptor. BDNF receptor	−0.14	2.5E-01
23	NTRK3	TRKC	Neurotrophic tyrosine kinase, receptor, type 3	NM_002530	15q25	Neurotrophin receptor	−0.04	7.4E-01
24*	OFCC1	MRDS1	Orofacial cleft 1 candidate 1	NM_153003	6p24.3	See reviews referenced in text	−0.09	4.7E-01
25	PLDN		Pallidin homolog	NM_016081	4q32.3	Binds to DTNBP1. Li et al. 2003; Starcevic and Dell'Angelica 2004	0.23	5.3E-02
26*	*PPP1R1B*	*DARPP-32*	*Protein phosphatase 1, regulatory (inhibitor) subunit 1B (dopamine and cAMP regulated phosphoprotein)*	*NM_032192, NM_181505*	*17q12*	*Albert et al. 2002*	*0.41*	*3.8E-04*
27*	PPP3CC	CALNA3	Protein phosphatase 3 (formerly 2B), catalytic subunit, gamma isoform (calcineurin A gamma)	NM_005605	8p21.3	Gerber et al. 2003	−0.06	6.0E-01
28	*PRODH*	*PIG6*	*Proline dehydrogenase (oxidase) 1*	*NM_016335*	*22q11.21*	*Hoogendoorn et al. 2004; Li et al. 2004*	*0.52*	*3.3E-06*
29*	*RGS4*	*RGP4*	*Regulator of G-protein signalling 4*	*NM_005613*	*1q23.3*	*Many—see text*	*−0.32*	*6.8E-03*
30	SLC1A2	EAAT2, GLT-1	Solute carrier family 1 (glial high affinity glutamate transporter), member 2	NM_004171	11p13-p12	Ohnuma et al. 1998; Ohnuma et al. 2000; Smith et al. 2001; Lauriat et al. 2005	0.02	8.8E-01
31	SLC6A4	5HTT, SERT	Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	NM_001045	17q11.1-q12	Dubertret et al. 2005	0.02	9.0E-01

This list of genes that have been implicated in the molecular pathology of schizophrenia was assembled through comprehensive review of literature and current research. Two microarray probes were designed for each gene on the list not marked with an *asterisk* (*). An *asterisk* indicates genes for which two probes were designed for each exon of the gene. *Italicized rows* indicate genes depicted in Fig. 2. *P* values are not corrected for multiple comparisons

well as for a history of chronic medical conditions such as hypertension, diabetes, strokes, seizures, and other neurological conditions. Based on these interviews, subjects with prolonged severe medical illnesses likely to impact neuronal function were not included in the study. Further detail on cohort selection has been reported previously (Lipska et al. 2006).

Additionally, all the brains were subjected to macroscopic and microscopic neuropathological evaluations by a neuropathologist, examining multiple cortical areas with hematoxylin–eosin stain and the Bielschowsky's silver impregnation method adapted for paraffin-embedded tissue (modified from Mallory 1961; Prophet et al. 1992). Any subjects with significant neuropathological abnormalities

Table 2 Cohort demographic and RNA quality information

Cohort details ($N = 72$)	Mean ± SD
Age (range: 18–67 years)	43.4 ± 14.5
Ethnicity: African–American (%)	60
Ethnicity: Caucasian (%)	35
Ethnicity: Hispanic (%)	4
Ethnicity: Asian (%)	1
Sex: female (%)	32
Postmortem interval (h)	29.8 ± 14.3
Brain pH	6.60 ± 0.23
RNA integrity number (RIN)	7.45 ± 0.79

Information is expressed as mean ± SD. Total cohort $N = 72$

were excluded from the study. Records of general autopsy findings were also reviewed.

Postmortem cortical tissue samples from 92 normal controls that fulfilled the above criteria were assembled from the Clinical Brain Disorders Branch, NIMH collection. A total of 72 of these eventually passed all tissue, RNA, and microarray data quality criteria (14 samples were excluded secondary to not qualifying as normals, including positive toxicological results, and a further 6 were dropped in the RNA/microarray quality control steps, see below). Two cortical regions were selected for their involvement in higher cognition and particularly in neuropsychiatric disorders: BA10 and BA9/46.

Cortical gray matter was identified on a frozen coronal slab from the superior aspect of the frontal pole and dissected using a dental drill as previously described (Lipska et al. 2006; drill: Cat# UP500-UG33, Brasseler, USA). Briefly, each slab was removed from the freezer and placed on a frozen plastic cutting board. Using the hand-held dental drill the arachnoid-pia was shaved off and the cortical ribbon was removed from the slab and underlying white matter rapidly without thawing either the slab or the dissected specimen. Each dissection took less than 1 min. The RIN from each specimen shows that this method does not excessively damage or degrade the RNA.

Anatomically, BA10 specimens were taken from the mid-point of the frontal pole, conforming to the macroscopic delineations from anatomical analyses of this brain region (Brodmann 1909 as depicted in "The Human Nervous System" edited by Paxinos, and Semendeferi et al. 2001). For the BA9/46 (dorsolateral prefrontal cortex, DLPFC) dissections, grey matter tissue from the crown of the middle frontal gyrus was obtained from the coronal slab corresponding to the middle one-third immediately anterior to the genu of the corpus callosum (Brodmann 1909 as depicted in "The Human Nervous System" edited by Paxinos, and Rajkowska et al. 1995).

A portion of data in Fig. 4 were obtained using an independent cohort subjected to the same exclusion criteria as described above for the primary cohort (labeled as "new cohort" in Fig. 4). This second cohort contained samples from 16 subjects (mean ± SD): age 40.7 years ± 16.0 (range 18–63), PMI 34.1 ± 14.9 h, pH 6.60 ± 0.23, 19% female, 56% African–American, and 39% Caucasian.

RNA extraction and quality assessment

All the tissues were pulverized and stored at 80°C. Total RNA was extracted using TRIZOL Reagent (Life Technologies Inc., Grand Island, NY, USA). RNA quality was assessed using the Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA, USA). Approximately 700 ng RNA was applied to a RNA 6000 Nano LabChip without prior heating. RNA integrity number (RIN) was obtained from the Agilent software and was used as a measure of RNA quality. RINs are generated using not only the quantification of ribosomal RNA bands, but the entire electrophoretic trace—including potential degradation products. Further information can be found at http://www.chem.agilent.com/temp/rad19E63/00047692.pdf. No RNA samples with RIN values <5.4 were included in this study.

Microarray design

A custom microarray (Illumina Sentrix Array Matrix or SAM) was designed and constructed to interrogate the expression of the selected genes (Table 1) in addition to many other genes relevant to neural function. The total number of unique probes included in the microarray was 1,379. The arrays consist of randomly assembled arrays of 3-micron bead features, each coated with a 50-nucleotide DNA probe. All the features are quality controlled by a sequential hybridization process called array decoding. Two unique probes were designed for each target sequence, and each probe was included on the array an average of 30 times.

Each target sequence for which probes were to be synthesized was subjected to a filtering process that masked regions unsuitable for probe design, based on complexity and cross-homology thresholds, as determined by DUST and BLAST (Altschul et al. 1990) algorithms, respectively. All the possible 50-mer probes were identified within unmasked regions, and were ranked by a formula that takes into account distance from the 3′ end of the transcript,

melting temperature, and self-complementarity. The two highest scoring probes were then linked to 23-nucleotide identifier sequences by use of a sequence-matching program that minimizes the probability of interactions between the probe and identifier sequence and prevents the creation of junction sequences with cross-homology to the genome in question.

Microarray probe design approaches to transcriptional complexity

To limit the number of statistical comparisons performed here, we analyzed only data for each gene averaged over all probes for that gene, not probe-by-probe, or probe combination data. For this study, we attempted to design two probes for each of 18 genes in addition to two probes per exon of an additional 13 genes that we found to be of particular interest in neuropsychiatry, i.e., those marked with an asterisk (*) in Table 1. Given the requirements of the massively parallel hybridization events that microarrays entail, it was not always possible to design two distinct probes for every exon of these genes. The primary limitation in this respect was finding sequences in small exons that were sufficiently specific such that no cross-hybridization would occur with other DNA species. The figures of this report contain data from 7 of the 31 genes in Table 1. Of these, two had two probes designed to measure their expression (PRODH, and TRKC). The attempt was made to design probes to each individual exon in the five additional genes: for DARPP-32: 10 probes were designed in 8 of 8 exons, NRG1: 22 probes in 13 of 25 exons, RGS4: 7 probes in 4 or 5 exons, GRM3: 12 probes in 6 of 6 exons, BDNF: 15 probes in 8 of 8 exons. It should be kept in mind when considering the data presented here, that all the data reported are the average of all probes designed for an individual gene. The exact sequence of all gene- or exon-specific probe(s) is available along with all expression data from this microarray experiment at http://www.ncbi.nlm.nih.gov/geo/ (accession #: TBA).

Microarray use

Experiments were carried out on a 96-array matrix system (SAM) that requires 100 ng of total RNA per sample and used standard microtiter plates to process samples in parallel. In this manner all the samples analyzed in this study could be labeled, hybridized and quantified in parallel. Sample labeling consisted of a modification of a previously reported technique (Eberwine et al. 1992). mRNA was reverse transcribed into cDNA followed by an amplification and labeling step mediated by T7 DNA polymerase. Labeling and amplification of the total RNA samples were performed according to the MessageAmp aRNA kit (Ambion Cat. #1750) using oligo-dT as the primer and bio-16-UTP (Roche Cat. #1388908) as the label. This protocol was modified for use in a 96-well microtiter plate.

All the steps of hybridization, washing, blocking and signal generation were performed by sequential transfer of a SAM from one microtiter plate (ThermoLab Systems, Cat. #95040000) to the next with the wells of each step containing 40 μL of the appropriate solution. All incubations were carried out without agitation and, with the exception of the hybridization, at room temperature. Amplified, biotin-labeled RNA samples were prepared in a solution of Hyb E1 buffer (Illumina, Part #11166381) and 25% (v/v) formamide at a final concentration of 25 ng/μL. An array matrix was then mated to the hybridization plate using a sealed alignment fixture. Hybridization proceeded at 55°C, for 16–20 h. After hybridization, the array matrix was washed by a 5-min incubation in Illumina Wash E1 buffer, followed by a 10-min wash in fresh Wash E1 buffer (Illumina, Part #11165898). Arrays were then blocked for 5 min in 1% (w/v) casein-PBS, Hammerstein grade (Pierce, Cat. #37528). Array signal was developed by a 10-min incubation in a 1 μg/mL solution of Streptavidin-Cy3 (Amersham, Cat. #PA43001) in 1% casein-PBS blocking solution. The array matrix was washed a final time for 5 min in Wash E1 buffer and then dried. Arrays were scanned on an Illumina BeadArray reader—a confocal imaging system with ~0.8 μm resolution. Bead signals were computed with weighted averages of pixel intensities and local background subtraction.

This system is optimized for specific and sensitive analysis of mammalian RNA and has the following performance details: specificity of 1:250,000 in mammalian poly(A+) mRNA; limit of detection 0.13 pM; dynamic range 3.2 logs; and sufficient precision to detect 1.3-fold differences with 95% confidence within this dynamic range. Additional details on array design, construction, and use are available (Kuhn et al. 2004).

Microarray data processing

All the data processing was performed using the R statistical language (http://www.r-project.org). Following local background correction upon scanning, microarray data were transformed into the log base 2 scale. Log2 intensities for all genes were converted to log2 ratios relative to the grand mean for each gene across all arrays. These log2 ratios were then normalized to the local mean log2 ratio across expression level within each array using the loess

function in R (Colantuoni et al. 2002). The local variance correction described in this reference was not necessary for the Illumina array data presented here. Instead, a global standard deviation calculated across log2 ratios within each array was used to generate the final gene expression Z-scores used in this analysis. As described above, there exist multiple probes for individual genes on the Illumina arrays. All gene expression measures reported here reflect within-gene mean-centered and Z-transformed data averaged from all probes within each gene.

Data quality control took place at all steps of microarray data normalization and processing. The distribution of gene expression intensities obtained from each sample was inspected via density plots and box plots. Similarly, the distributions of log2 ratios were examined with density plots, box plots, and intensity versus ratio to grand mean scatter plots. The variance of these within-array log2 ratios to the grand mean was also systematically inspected, as were the correlations of the intensities form each sample to the grand mean. Dimension reduction algorithms, including MDS and principal components analysis (PCA) were applied to identify possible outliers.

Of primary interest in this report is the association between gene expression and age. In an attempt to remove unwanted artifacts from the data, all gene expression measures (microarray and qPCR), as well as age, were adjusted for PMI, brain pH, and RIN using multiple linear regression. That is, effects of these covariates on expression were removed from the data so that the effects of age would be more clearly observable. Ethnicity and sex were the factors not included in this model as they did not appear to have an impact on gene expression when assessed via *T* tests and permutation testing: the distribution of observed statistics did not differ from the distribution obtained with permuted covariate data.

The residuals from these models represent adjusted measures of gene expression and age. While unadjusted metrics are used in the plotting of all data in this report to visualize data across actual age in years, all Pearson's product moment correlation coefficients along with the associated *P* values were calculated from adjusted measures of gene expression and age. All statistics and *P* values reported here have not been corrected for multiple comparisons. The exact number of subjects in data reported for each assay varies slightly due to the failure of individual assays and/or problems with individual samples.

In Fig. 5, linear spline regression models were used to quantify the linear association between gene expression and age separately during early (18–30) and late (>30–67) adulthood. Specifically, the models estimate one slope (i.e., change in gene expression per year of life) during early adulthood, and a separate slope during late adulthood. Wald tests were used to test the equality of these two slopes. An age of 30 was used as an a priori turning point because of its clinical significance as the end of the period of most disease onset in schizophrenia. These models were also adjusted for covariates using multiple linear regression as described above.

In Fig. 6, a multidimensional scaling (MDS) algorithm was used to visualize the expression patterns of each of the 31 genes under study across all subjects in two-dimensional space. The MDS algorithm takes a matrix of pair-wise distances between all genes as its starting point. The distance metric used for this was derived from the absolute value of the pair-wise Pearson correlation coefficients: $1 - \mathrm{ABS}(r)$.

Quantitative real-time PCR (qPCR)

A total of 4 μg of total RNA was used in a 50 μL reverse transcription reaction to synthesize cDNA for use in qPCR (SuperScript First-Strand Synthesis kit, Invitrogen, Carlsbad, CA, USA). The ABI Prism 7900 sequence detection system in combination with TaqMan probes and primers (Applied Biosystems, Foster City, CA, USA) were used to carry out qPCR on sample cDNAs. ABI Assays-on-Demand were used for the majority of genes: β-actin (ABI cat.# Hs99999903), porphobilinogen deaminase (PBGD; Hs00609297), β-2-microglobulin (B2 M; Hs99999907) and β-glucuronidase (GUSB; Hs99999908), RGS4 (Hs00194501), DARPP-32 (1) assay designed to measure all transcripts and which spans the final 2 exons of the gene: Hs00938415, and (2) assay spanning the exon2–3 junction, designed to measure only one of the known RefSeq transcripts NM_181505: (Hs00938416), PRODH (Hs01574357). Custom primers and probe were used to measure GRM3 levels: Forward primer ACACACAGACTGCACCTCAACAG, reverse primer TGCACACCGTTGGCACAT, probe CTCT CAGTCCTCTGCAAG (spans the junction of exons 5 and 6).

Each 20 μL reaction contained 900 nM of primer, 250 nM of probe and Taqman Universal PCR Mastermix (Applied Biosystems) containing Hot Goldstar DNA Polymerase, dNTPs with dUTP, uracil-N-glycosylase, passive reference and 200 ng of cDNA template. PCR cycle parameters were 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, and 59°C or 60°C for 1 min.

qPCR data were acquired from the Sequence Detector Software (SDS version 2.0, Applied Biosystems) and quantified by a standard curve method using serial dilutions of pooled cDNA derived from RNA obtained from many brain regions from many normal control subjects. In each experiment the R^2 value of the curve was more than 0.97, the slope was between 3.2 and 3.5 (amplification efficiency 96–101%) and controls comprising no-template cDNA resulted in no detectable signal. All the measurements were

performed in triplicate and gene expression levels calculated as the average of the log2 of the three measurements. Following mean centering, target gene expression levels were normalized to the mean level of all control genes measured in each sample, and then expressed as within-gene Z-scores. Multiple linear regression was used to correct for effects of covariates as described above in the "Microarray data processing" section. All *P* value calculation in Figs. 3 and 4 were conducted using a one-sided alternative hypothesis, as these data represented confirmations of effects already observed in Fig. 2.

Results

DNA microarray analysis of 31 schizophrenia susceptibility genes in BA10

The expression of a group of 31 genes (Table 1: schizophrenia susceptibility genes as well as other genes implicated by physical interaction with these) was measured in a cohort of 72 normal control postmortem human frontal cortex (BA10) RNA samples (Table 2) using a custom Illumina DNA microarray (see "Materials and methods").

The overall impact of age on gene expression in this study was assessed by calculating the correlation of each gene's expression with age (Table 1, columns at right) and subsequent analysis of the distribution of these correlations. Figure 1 depicts the distribution of age to expression correlations (solid line) as compared to the distribution of correlations obtained when age information is permuted across subjects (dashed line). It is clear that the observed distribution of correlations contains more extreme values (both positive and negative) than would be expected by chance (as estimated by the permuted data). Other covariate data were also investigated for effects on gene expression. While postmortem interval (PMI), brain pH levels, and RNA integrity number (RIN) all showed effects on gene expression, all of these effects were of a magnitude less than that of the age effect. Sex and ethnicity showed no effect on expression when assessed via *T* tests and similar permutation analysis. Therefore, in calculating statistics and *P* values throughout this report we use data adjusted for PMI, brain pH, and RIN, but not sex and ethnicity (see "Gene expression data processing" section in "Materials and methods" for details).

Of the 31 genes interrogated, 4 showed a statistically significant correlation with age (Fig. 2, and highlighted genes in Table 1). The expression of two genes decreased with age: RGS4 and GRM3. The expression of two genes increased with age: DARPP-32 and PRODH. Each of these genes' expression shows even, gradual change across the entire age range examined in this study (18–67 years). The correlation of CHRNA7 expression with age reached an alpha value of $P < 0.05$ (Table 1), however, we considered this effect too modest for follow-up in the context of an investigation of 31 genes (*P* value calculations do not include multiple testing correction).

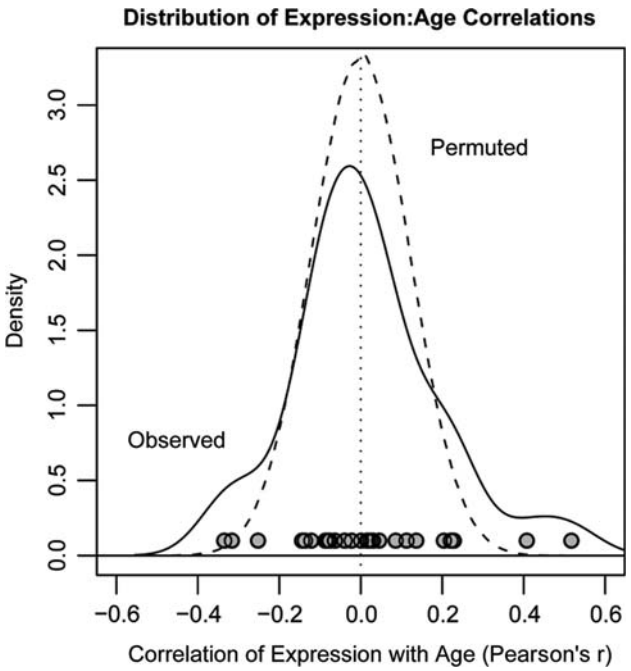

Fig. 1 Density estimate of the distribution of expression to age correlations (Pearson's *r*'s). The observed correlations (*solid line*, $N = 31$ genes from Table 1, also shown as *individual points* along the bottom of the density plot) are shown along with correlations obtained from many permutations of the ages of subjects (*dashed line*, $N = 1{,}379{,}000$). The observed distribution of correlations contains more extreme values than would be expected by chance. The four genes focused on in Figs. 2 and 3 are the two most extreme points on either side of the distribution

qPCR in BA10

Using the same RNA resources generated for the microarray analysis, qPCR was performed to specifically verify the expression pattern of all 4 genes whose expression was found to be correlated with age in BA10. Figure 3 shows the qPCR verification of the microarray data in Fig. 2. In both microarray and qPCR data, the expression of RGS4, GRM3, DARPP-32, and PRODH all showed a significant correlation with age. The correlation of DARPP-32 expression with age was only observed when using an ABI Taqman assay spanning exons 2 and 3, targeting transcript(s) which include adjacent exons #2 and #3 (RefSeq transcript NM_181505). No correlation was observed when using an ABI Taqman assay targeting all known transcripts, i.e., transcripts which include the final 2 adjacent exons of the gene.

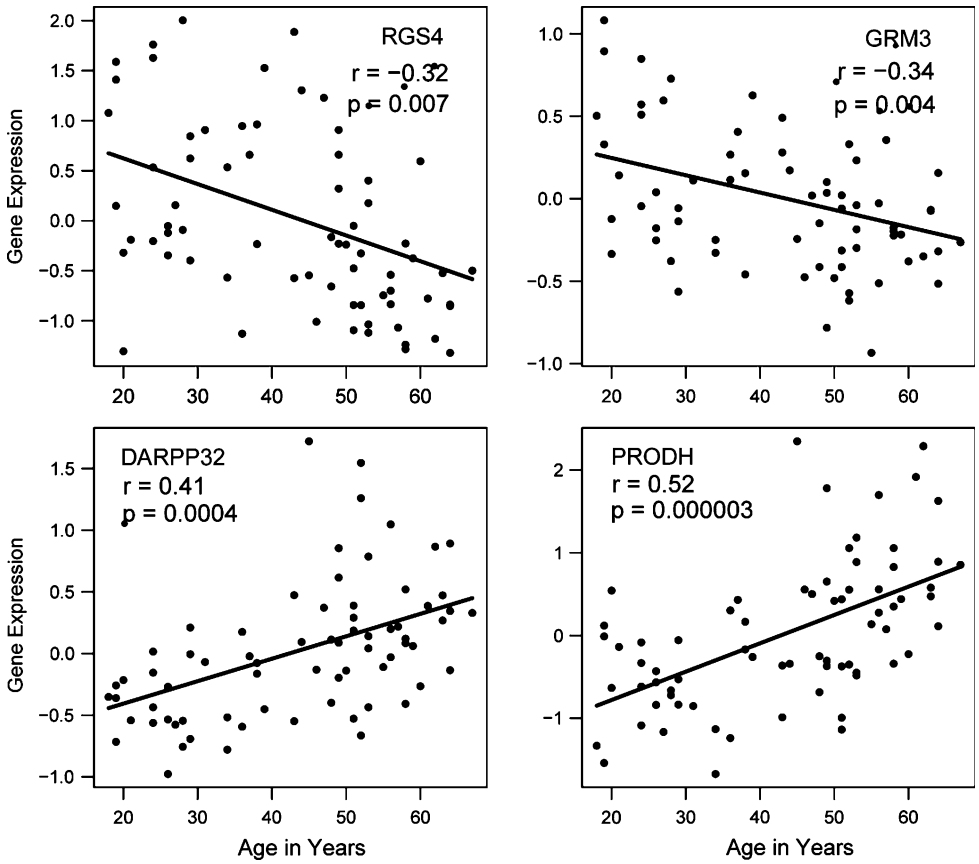

Fig. 2 DNA microarray data in BA10. Expression of 4 of the 31 genes interrogated showed significant correlation with age: RGS4, GRM3, DARPP-32, and PRODH. Mean-centered gene expression measures in the log base 2 scale are on the *y*-axis and age is on the *x*-axis. The *solid line* depicts a linear fit through the data. Pearson's correlation coefficients (*r*) and associated *P* values (*P*) calculated from adjusted data (see "Materials and methods") are listed on each plot

qPCR in BA9/46

To examine these age-related expression changes in another cortical area especially relevant to neuropsychiatric disorders, RNA was isolated from BA9/46 (DLPFC) of the same subjects used in the BA10 studies detailed above. qPCR in BA9/46 revealed that, as observed in BA10 (Figs. 2, 3), GRM3 gene expression was negatively correlated with age and DARPP-32 expression was positively correlated with age (Fig. 4). As in BA10, correlation of DARPP-32 expression with age in the BA9/46 was observed only when qPCR assays spanned exons 2 and 3 (RefSeq transcript NM_181505).

To assess if such expression changes over age are biologically replicable in new cases, the expression of GRM3 was measured via qPCR in BA9/46 in a novel cohort of 16 subjects (see "Materials and methods" for cohort details). The decrease in expression of GRM3 with age was clearly found in this new cohort (Fig. 4 "new cohort").

Expression differences during the ages of schizophrenia disease onset

Late neurodevelopmental processes occurring in early adulthood are of particular interest in schizophrenia research as disease onset occurs primarily during these years. In this analysis, we attempted to identify genes among the 31 under study in the BA10 microarray dataset whose expression pattern across age during early adulthood differs from that during late adulthood. Here, we defined early adulthood as ages 18–30 years, and late adulthood as >30–67 years. This analysis was conducted by comparing the slope of a linear fit of gene expression across age during early adulthood to that during late adulthood. Only three genes showed a pattern in which the change in gene expression across age in early adulthood was significantly different than that in late adulthood: ERBB3, NRG1, and NGFR (Fig. 5). Expression of both ERBB3 and NRG1 (a receptor–ligand pair) decreases from age 18 to 30 years, and then remains constant or increases over the remainder of the lifespan. NGFR shows the opposite pattern, increasing from 18 to 30 years and remaining essentially unchanged during the rest of life. See "Discussion" for additional information.

We also analyzed the expression of these three genes separately in females and males because the age of schizophrenia onset is often different across gender (Leung and Chue 2000; Tamminga 1997; Larsen et al. 1996; Angermeyer and Kuhn 1988). No statistically significant gender differences were observed: decreasing expression during early adulthood was observed in both females and

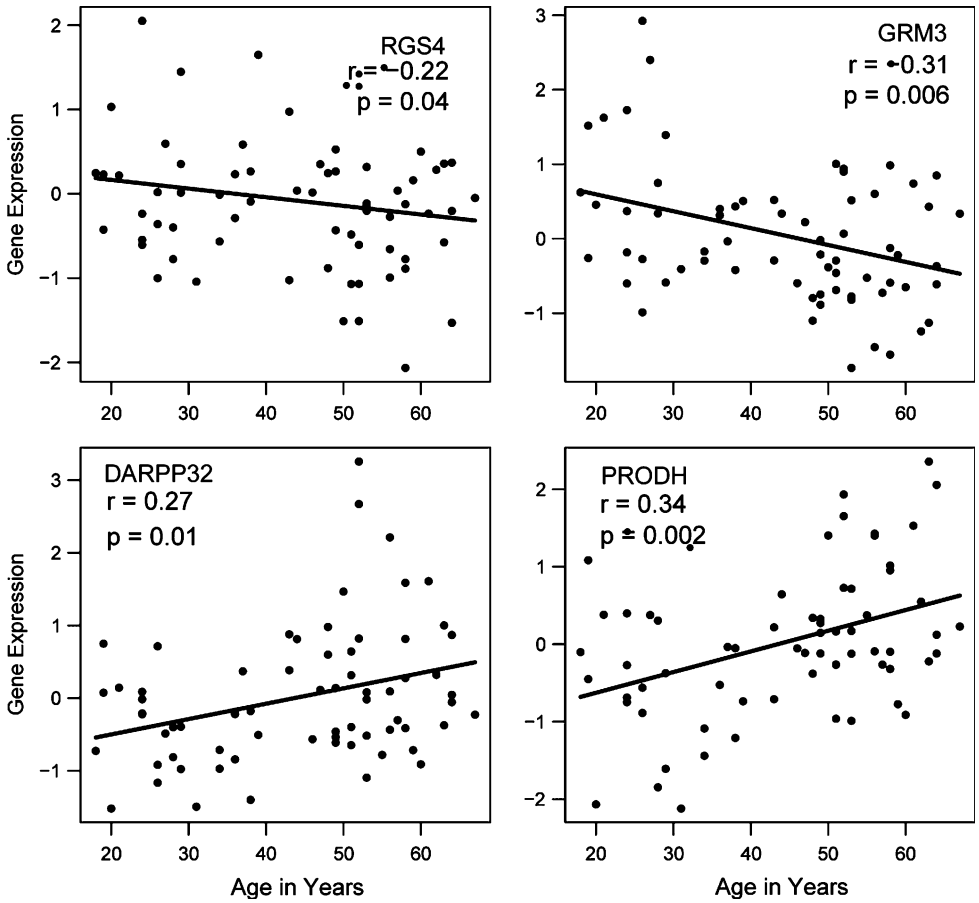

Fig. 3 qPCR measurement of gene expression in BA10. qPCR results on identical RNA samples reproduced correlations of gene expression with age as seen in the microarray data (Fig. 2). The *solid line* depicts a linear fit of gene expression across age. Pearson's correlation coefficient (r) and associated P value (P) calculated from adjusted data (see "Materials and methods") are listed in each plot

males for all three of these genes. Perhaps most notably, there were no observable expression differences later in life when females often show a perimenopausal increase in disease onset.

Gene subgroups showing similarity in expression patterns

To examine known and possibly novel relationships between the 31 genes under study here, we inspected pair-wise correlations between all expression profiles. Among the genes investigated here are several that are known to physically interact: DISC1 binds to FEZ1 (Miyoshi et al. 2003) and NUDEL (Ozeki et al. 2003), DTNBP1 binds to muted and pallidin (Falcon-Perez et al. 2002; Starcevic and Dell'Angelica 2004), and BDNF binds to its receptors TRKB and TRKC. Together this produces eight pair-wise interactions. Only two of these eight pairs showed significant positive correlation between expression patterns: FEZ1 and NUDEL: $r = 0.32$, $P = 0.007$, and BDNF and TRKC: $r = 0.32$, $P = 0.006$. In addition to these two strong positive correlations, several modest negative correlations in this group of eight result in the modest proximity of these binding partners in Fig. 6, where each gene is visualized as a single point in two-dimensional space. The distance between each pair of genes in the plot was derived from the absolute level of correlation between their expression profiles [distance $= 1 - \mathrm{ABS}(r)$, where r is the Pearson's correlation coefficient]: greater positive OR negative correlation = lesser distance. A multidimensional scaling (MDS) algorithm was used to represent each of these pair-wise distances in Fig. 6. Just under 86% of 100,000 groups of 8 random gene pairs drawn from the list of 31 genes yielded lesser summed correlations than the 8 gene pairs that are known to interact (empirical $P = 0.14082$). Hence, although it is clear that binding partners in this dataset are more highly correlated with one another than randomly selected genes, this effect is neither statistically significant, nor sufficient to identify novel pairs of interacting proteins.

We also inspected correlations among genes whose expression was identified to change across age in Figs. 2, 3,and 4. Not surprisingly, the two genes which showed positive correlation with age (Figs. 2, 3: DARPP-32 and PRODH) showed highly correlated expression patterns: $r = 0.62$, $P = 4.6e9$. Similarly, the two genes showing negative correlation with age (Figs. 2, 3: RGS4 and GRM3) were also correlated with one another: $r = 0.49$,

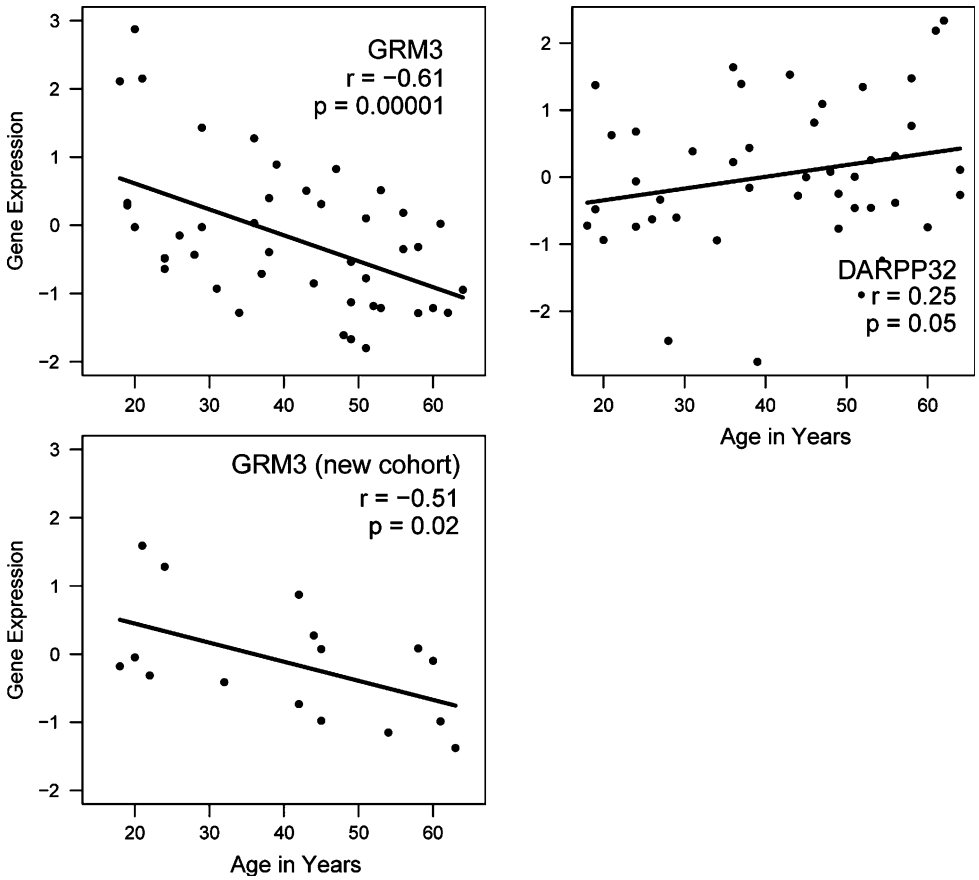

Fig. 4 qPCR measurement of gene expression in BA9/46. Similar to the BA10 microarray data (Fig. 2) and qPCR in BA10 (Fig. 3), qPCR in BA9/46 (DLPFC) revealed a negative correlation of GRM3 expression with age and a positive correlation of DARPP-32 expression with age. In addition, the GRM3 finding was also replicated in an independent cohort of 18 new subjects ("new cohort"). The *solid line* depicts a linear fit of gene expression across age. Pearson's correlation coefficient (r) and associated P value (P) are listed in the plot

$P = 1.4\text{e-}05$. However, by far the most highly correlated subgroup of genes among the 31 investigated, consisted of 3 genes that share no obvious molecular interactions with one another: FEZ1, GAD1, and RGS4: mean pair-wise $r = 0.66$. The functional biological meaning of this high level of similarity in expression patterns is unclear.

Another interesting characteristic of this group of three genes was revealed when the absolute values of the Pearson correlation coefficients were examined. In using the absolute value of the correlation coefficients, any correlation between expression profiles, either positive or negative, is considered to be an indication of functional relation. These 3 genes showed the strongest average absolute correlations across all the genes investigated here: mean absolute correlation across all 31 genes for FEZ1, GAD1, and RGS4 = 0.24. This group of three genes is highlighted in Fig. 6.

The high mean absolute correlation across all 31 genes observed for FEZ1, GAD1, and RGS4 demonstrates a high overall level of interconnectedness (whether reflected by a positive or negative correlation) to all other genes in this network. This is apparent in Fig. 6 where each gene is visualized as a single point in two-dimensional space, and where absolute correlation determines distance. FEZ1, GAD1, and RGS4 lie near the center of the plot. GRM3 is also highly correlated with this gene group, while TRKC is strongly negatively correlated with the group (hence both lie proximal to this group of three genes). This central position is a result of the MDS algorithm attempting to minimize the distance of all the other diverse gene expression patterns to this group of genes that share the highest correlation (positive or negative) across all genes.

To assess the statistical significance of (1) the high correlation between the expression patterns of the FEZ1, GAD1, and RGS4 genes (mean pair-wise correlation amongst this group = 0.66), and (2) their high mean absolute correlation genes across the other 28 susceptibility genes (mean absolute correlation of these genes across the other 28 = 0.24), we conducted the following sampling scheme: we investigated the correlation structure of 100,000 randomly sampled groups of 31 probes from the 1,379 unique probes included on the custom microarray (as there are 31 susceptibility genes investigated in this report—see "Materials and methods" for additional details). For each of the 100,000 groups of 31 random probes we calculated, (1) the mean pair-wise correlation amongst the three most highly positive correlated probes, for comparison to our observed pair-wise correlation of 0.66, (2) the mean absolute correlation for the three probes that showed the highest absolute correlations across all 31 random probes, for comparison to our observed mean absolute correlation of 0.24, and (3) how many times

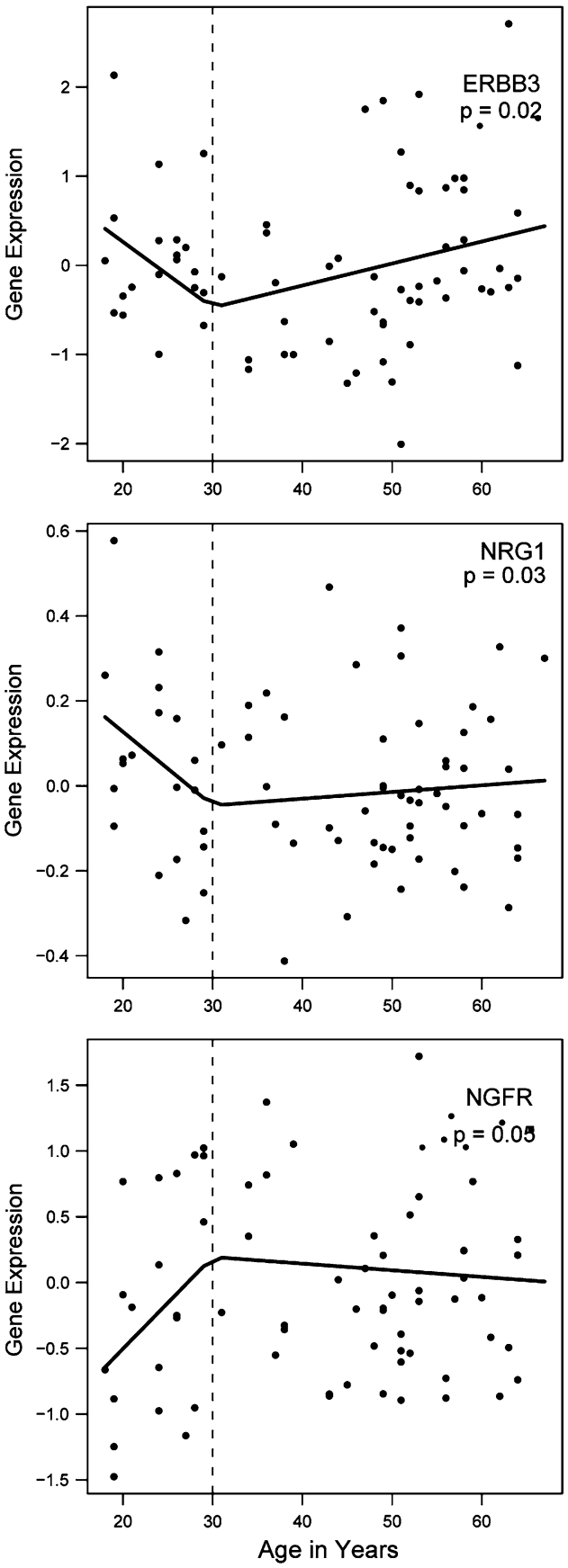

Fig. 5 Differential gene expression during the years of schizophrenia disease onset. Expression of ERBB3 and NRG1 decreases during early adulthood (18–30 years) followed by constant or increasing expression across late adulthood (>30–67 years). NGFR expression shows the opposite pattern. The *solid line* depicts the average linear fit of gene expression across age before and after the age of 30 years (averaged over the subject covariates, see "Materials and methods" for details). The *P* value (*P*) listed in each plot corresponds to the comparison of the slopes of these two lines. All panels depict microarray data from BA10

the three probes yielding the highest positive pair-wise correlation (point #1 above) were the same probes as those which yielded the highest mean absolute correlation across all 31 randomly selected genes (point #2 above)—as was the case in our observations here (FEZ1, GAD1, and RGS4 fulfilled both of these criteria). In only 2,083 of the 100,000 sets of 31 randomly selected probes were all 3 of the following criteria met. Hence, we can assign an empirical *P* value of 0.02083 to this collection of observations involving FEZ1, GAD1, and RGS4.

Discussion

Differential expression of schizophrenia susceptibility genes across age

Here we have investigated the expression of several schizophrenia-related genes across adulthood in the normal human cerebral cortex. The expression of several of these genes showed strong correlation with age across the entire age range investigated (18–67 years): RGS4 and GRM3 decreased with age, while DARPP-32 and PRODH increased with age (Figs. 2, 3, 4). Because the qPCR data for DARPP-32 in both BA10 and BA9/46 only showed correlation with age when probes specific for transcripts including adjacent exons 2 and 3, it is likely that this effect of age on expression is specific to DARPP-32 transcript NM_181505. This collection of expression changes was demonstrated in a large cohort, with some findings across multiple cortical regions (GRM3 and DARPP-32) and multiple cohorts (GRM3). Given the gradual expression changes over lifespan, these genes are likely involved in what we would consider the normal molecular aging of the human brain.

In addition, a group of genes showed differential expression change with age before and after age 30 years: ERBB3, NRG1, and NGFR (Fig. 5). Unlike the genes identified in Figs. 2, 3, 4, it is clear that the expression patterns here do not represent part of the gradual, directly progressive molecular aging process. It is interesting that ERBB3 and NRG1, a receptor–ligand pair (Buonanno and Fischbach 2001 for review) show a similar expression pattern across age: decreasing during early adulthood and increasing or flat thereafter. It is surprising, however, that

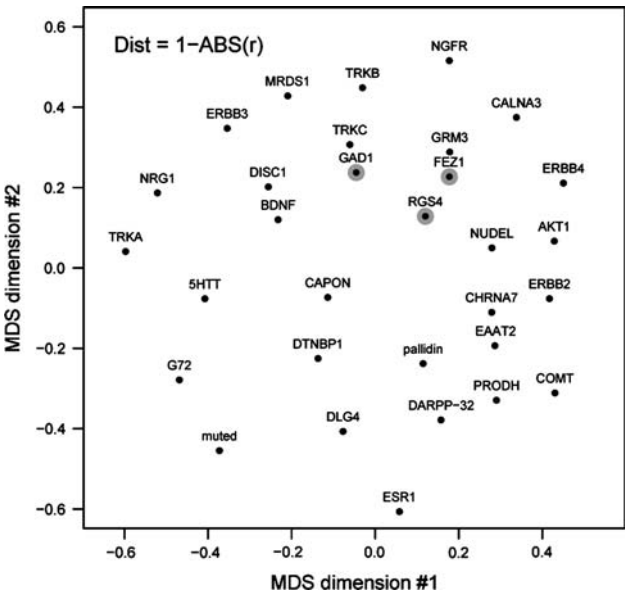

Fig. 6 Expression profiles of 31 schizophrenia susceptibility genes. Using an MDS algorithm, the expression pattern of each gene across all subjects is depicted as a single point in two-dimensional space. One minus the absolute value of the Pearson's correlation coefficient [1 ABS(r)] is used as a distance metric. Hence, genes with highly similar or dissimilar expression patterns lie proximal to one another. RGS4, GAD1, and FEZ1 are *highlighted*. See text for details

correlation between the expression of these two genes is slightly negative when assessed directly ($r = -0.14$, N.S.). In addition to the genetic implication of NRG1 in the pathology of schizophrenia (see "Introduction" for references), ERBB3 expression has been shown to be decreased in the frontal cortex of schizophrenic patients (Hakak et al. 2001; Tkachev et al. 2003). These decreases in ERBB3 expression were observed along with decreases in the expression of many myelin-related genes. Along with the known role of NRG1 and ERBB3 in myelin development (Corfas et al. 2004 for review), this decrease in the expression of these genes during early adulthood may represent the conclusion or slowing of late neurodevelopmental processes involving myelination, which could be involved in the molecular etiology of schizophrenia. These data are consistent with a commonly proposed neurodevelopmental model of schizophrenia in which late neurodevelopmental processes in the cortex, possibly including synapse pruning and myelination, are disrupted (Woo and Crowell 2005 for review).

NGFR showed a different pattern of expression, increasing prior to age 30 years and leveling off after this age. Disruption of NGF signaling in the developing rodent frontal cortex has been shown to result in dopaminergic hyperresponsivity, impaired prepulse inhibition, and impaired social behavior (all measures that have previously been associated with schizophrenic pathology) in adult animals (Rajakumar et al. 2004; Lazar et al. 2008).

It must be kept in mind here that although expression changes during the ages of disease onset may be of interest in the etiology of schizophrenia, expression changes prior to this period (conception—18 years of age) are not investigated here, and may be important. Further studies including RNA from brains of younger subjects are required to investigate this important time period in brain growth and maturation.

Functionally interacting networks of genes

In the analysis of correlation between gene expression patterns, several subgroups of genes were shown to share expression patterns that are highly similar to one another. Although intriguing, it is unclear whether this high level of similarity in expression is indicative of common transcriptional control mechanisms, involvement in common cellular pathways, expression in a common cell type, or some other molecular commonality, including mechanisms connected to perimortem factors. Further investigation is required to shed light on molecular mechanisms that connect these various gene subgroups. It is also important to note that ligand–receptor pairs and proteins known to interact with one another do not, in general, show greater positive correlation at the expression profile level. Only when absolute correlations are considered do these genes show modestly elevated correlations with one another.

Expression levels of FEZ1, GAD1, and RGS4 showed especially high levels of correlation with one another. In addition, when absolute correlations were averaged across all genes studied here, it was found that these same three genes also showed the highest mean absolute correlations. GRM3 expression was also highly correlated with this gene group. The precise meaning of this high expression correlation is not clear and requires additional study. Given this finding, it is particularly interesting that RGS4, GAD1, GRM3, and DISC1 (also proximal to this gene group in Fig. 6, and a binding partner of FEZ1) have all been found to be in epistasis with another gene: COMT (Nicodemus et al. 2006; Straub et al. 2007). FEZ1 was not tested for epistasis in this study. Additionally, differential expression of RGS4 has been associated with genetic polymorphism in COMT (Lipska et al. 2006). While its expression does not correlate with that of these other genes, genetic variants of the COMT gene could be driving the observed correlation of expression in this gene group.

Conclusion

While it is not surprising that many genes involved in neural function, cognition, and mental illness change their expression over the human lifespan, it is important to

describe which genes these are, their expression patterns across age, and what other genes they may be functioning in concert with in order to produce a common disease phenotype. These are the questions we begin to address here. Further molecular investigation is required to elucidate the functional details of the molecular pathways that these expression findings begin to outline, as well as their role in the neuropathology of schizophrenia.

Acknowledgments This research was supported by funding solely provided by the Intramural Research Program of the National Institute of Mental Health. The authors would also like to thank Llewellyn B. Bigelow, MD., Vesna Imamovic, Yeva Snitkovsky, and Jewell King for their contributions to the collection and diagnosis of the brain specimens used in this research, the Offices of the Chief Medical Examiner of Washington DC and of Northern Virginia, as well as families of the deceased whose donation of this tissue made this research possible.

Disclosure/Conflicts of interest statement We, the authors declare that, except for income received from our primary employer, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

References

Addington AM, Gornick M, Duckworth J, Sporn A, Gogtay N, Bobb A, Greenstein D, Lenane M, Gochman P, Baker N, Balkissoon R, Vakkalanka RK, Weinberger DR, Rapoport JL, Straub RE (2005) GAD1 (2q31.1), which encodes glutamic acid decarboxylase (GAD67), is associated with childhood-onset schizophrenia and cortical gray matter volume loss. Mol Psychiatry 10(6):581–588

Albert KA, Hemmings HC Jr, Adamo AI, Potkin SG, Akbarian S, Sandman CA, Cotman CW, Bunney WE Jr, Greengard P (2002) Evidence for decreased DARPP-32 in the prefrontal cortex of patients with schizophrenia. Arch Gen Psychiatry 59(8):705–712

Alda M, Ahrens B, Lit W, Dvorakova M, Labelle A, Zvolsky P, Jones B (1996) Age of onset in familial and sporadic schizophrenia. Acta Psychiatr Scand 93(6):447–450

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215(3):403–410

Angermeyer MC, Kuhn L (1988) Gender differences in age at onset of schizophrenia. An overview. Eur Arch Psychiatry Neurol Sci 237(6):351–364

Badner JA, Gershon ES (2002) Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. Mol Psychiatry 7(4):405–411

Barnea-Goraly N, Menon V, Eckert M, Tamm L, Bammer R, Karchemskiy A, Dant CC, Reiss AL (2005) White matter development during childhood and adolescence: a cross-sectional diffusion tensor imaging study. Cereb Cortex 15(12):1848–1854

Brandon NJ, Handford EJ, Schurov I, Rain JC, Pelling M, Duran-Jimeniz B, Camargo LM, Oliver KR, Beher D, Shearman MS, Whiting PJ (2004) Disrupted in Schizophrenia 1 and Nudel form a neurodevelopmentally regulated protein complex: implications for schizophrenia and other major neurological disorders. Mol Cell Neurosci 25(1):42–55

Brzustowicz LM, Simone J, Mohseni P, Hayter JE, Hodgkinson KA, Chow EW, Bassett AS (2004) Linkage disequilibrium mapping of schizophrenia susceptibility to the CAPON region of chromosome 1q22. Am J Hum Genet 74(5):1057–1063

Buonanno A, Fischbach GD (2001) Neuregulin and ErbB receptor signaling pathways in the nervous system. Curr Opin Neurobiol 11:287–296

Callicott JH, Straub RE, Pezawas L, Egan MF, Mattay VS, Hariri AR, Verchinski BA, Meyer-Lindenberg A, Balkissoon R, Kolachana B, Goldberg TE, Weinberger DR (2005) Variation in DISC1 affects hippocampal structure and function and increases risk for schizophrenia. Proc Natl Acad Sci USA 102(24):8627–8632

Cardno AG, Gottesman II (2000) Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet 97(1):12–17

Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM, Herman MM, Apud J, Egan MF, Kleinman JE, Weinberger DR (2004) Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 75(5):807–821

Chen Q, He G, Chen Q, Wu S, Xu Y, Feng G, Li Y, Wang L, He L (2005) A case–control study of the relationship between the metabotropic glutamate receptor 3 gene and schizophrenia in the Chinese population. Schizophr Res 73(1):21–26

Chowdari KV, Mirnics K, Semwal P, Wood J, Lawrence E, Bhatia T, Deshpande SN, Thelma BK, Ferrell RE, Middleton FA, Devlin B, Levitt P, Lewis DA, Nimgaonkar VL (2002) Association and linkage analyses of RGS4 polymorphisms in schizophrenia. Hum Mol Genet 11(12):1373–1380

Chumakov I, Blumenfeld M, Guerassimenko O, Cavarec L, Palicio M, Abderrahim H, Bougueleret L, Barry C, Tanaka H, La Rosa P, Puech A, Tahri N, Cohen-Akenine A, Delabrosse S, Lissarrague S, Picard FP, Maurice K, Essioux L, Millasseau P, Grel P, Debailleul V, Simon AM, Caterina D, Dufaure I, Malekzadeh K, Belova M, Luan JJ, Bouillot M, Sambucy JL, Primas G, Saumier M, Boubkiri N, Martin-Saumier S, Nasroune M, Peixoto H, Delaye A, Pinchot V, Bastucci M, Guillou S, Chevillon M, Sainz-Fuertes R, Meguenni S, Aurich-Costa J, Cherif D, Gimalac A, Van Duijn C, Gauvreau D, Ouellette G, Fortier I, Raelson J, Sherbatich T, Riazanskaia N, Rogaev E, Raeymaekers P, Aerssens J, Konings F, Luyten W, Macciardi F, Sham PC, Straub RE, Weinberger DR, Cohen N, Cohen D (2002) Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. Proc Natl Acad Sci USA 99(21):13675–13680

Colantuoni C, Henry G, Zeger S, Pevsner J (2002) SNOMAD (Standardization and NOrmalization of MicroArray Data): web-accessible gene expression data analysis. Bioinformatics 18(11):1540–1541

Corfas G, Roy K, Buxbaum JD (2004) Neuregulin 1-erbB signaling and the molecular/cellular basis of schizophrenia. Nat Neurosci 7(6):575–580

Corvin AP, Morris DW, McGhee K, Schwaiger S, Scully P, Quinn J, Meagher D, Clair DS, Waddington JL, Gill M (2004) Confirmation and refinement of an "at-risk" haplotype for schizophrenia suggests the EST cluster, Hs.97362, as a potential susceptibility gene at the Neuregulin-1 locus. Mol Psychiatry 9(2):208–213

DeLisi LE (1992) The significance of age of onset for schizophrenia. Schizophr Bull 18(2):209–215

Dubertret C, Hanoun N, Ades J, Hamon M, Gorwood P (2005) Family-based association study of the 5-HT transporter gene and schizophrenia. Int J Neuropsychopharmacol 8(1):87–92

Eberwine J, Yeh H, Miyashiro K, Cao Y, Nair S, Finnell R, Zettel M, Coleman P (1992) Analysis of gene expression in single live neurons. Proc Natl Acad Sci USA 89(7):3010–3014

Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D, Weinberger DR (2001) Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci USA 98(12):6917–6922

Egan MF, Weinberger DR, Lu B (2003) Schizophrenia, III: brain-derived neurotropic factor and genetic risk. Am J Psychiatry 160(7):1242

Egan MF, Straub RE, Goldberg TE, Yakub I, Callicott JH, Hariri AR, Mattay VS, Bertolino A, Hyde TM, Shannon-Weickert C, Akil M, Crook J, Vakkalanka RK, Balkissoon R, Gibbs RA, Kleinman JE, Weinberger DR (2004) Variation in GRM3 affects cognition, prefrontal glutamate, and risk for schizophrenia. Proc Natl Acad Sci USA 101(34):12604–12609

Emamian ES, Hall D, Birnbaum MJ, Karayiorgou M, Gogos JA (2004) Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. Nat Genet 36(2):131–137

Falcon-Perez JM, Starcevic M, Gautam R, Dell'Angelica EC (2002) BLOC-1, a novel complex containing the pallidin and muted proteins involved in the biogenesis of melanosomes and platelet-dense granules. J Biol Chem 277(31):28191–28199

Freedman R, Leonard S, Gault JM, Hopkins J, Cloninger CR, Kaufmann CA, Tsuang MT, Farone SV, Malaspina D, Svrakic DM, Sanders A, Gejman P (2001) Linkage disequilibrium for schizophrenia at the chromosome 15q13–14 locus of the alpha7-nicotinic acetylcholine receptor subunit gene (CHRNA7). Am J Med Genet 105(1):20–22

Fujii Y, Shibata H, Kikuta R, Makino C, Tani A, Hirata N, Shibata A, Ninomiya H, Tashiro N, Fukumaki Y (2003) Positive associations of polymorphisms in the metabotropic glutamate receptor type 3 gene (GRM3) with schizophrenia. Psychiatr Genet 13(2):71–76

Gerber DJ, Hall D, Miyakawa T, Demars S, Gogos JA, Karayiorgou M, Tonegawa S (2003) Evidence for association of schizophrenia with genetic variation in the 8p21.3 gene, PPP3CC, encoding the calcineurin gamma subunit. Proc Natl Acad Sci USA 100(15):8993–8998

Gottesman II, Shields J (1967) A polygenic theory of schizophrenia. Proc Natl Acad Sci USA 58(1):199–205

Hakak Y, Walker JR, Li C, Wong WH, Davis KL, Buxbaum JD, Haroutunian V, Fienberg AA (2001) Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. Proc Natl Acad Sci USA 98:4746–4751

Hennah W, Varilo T, Kestila M, Paunio T, Arajarvi R, Haukka J, Parker A, Martin R, Levitzky S, Partonen T, Meyer J, Lonnqvist J, Peltonen L, Ekelund J (2003) Haplotype transmission analysis provides evidence of association for DISC1 to schizophrenia and suggests sex-dependent effects. Hum Mol Genet 12(23):3151–3159

Hafner H, an der Heiden W (1997) Epidemiology of schizophrenia. Can J Psychiatry 42(2):139–151

Hariri AR, Goldberg TE, Mattay VS, Kolachana BS, Callicott JH, Egan MF, Weinberger DR (2003) Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. J Neurosci 23(17):6690–6694

Harrison PJ, Weinberger DR (2005) Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol Psychiatry 10(1):40–68

Hoogendoorn B, Coleman SL, Guy CA, Smith SK, O'Donovan MC, Buckland PR (2004) Functional analysis of polymorphisms in the promoter regions of genes on 22q11. Hum Mutat 24(1):35–42

Ikeda M, Iwata N, Suzuki T, Kitajima T, Yamanouchi Y, Kinoshita Y, Inada T, Ozaki N (2004) Association of AKT1 with schizophrenia confirmed in a Japanese population. Biol Psychiatry 56(9):698–700

Jayaswal SK, Adityanjee, Khandelwal SK (1988): Age of onset of schizophrenia. Br J Psychiatry. 152:428

Kety SS, Rosenthal D, Wender PH, Schulsinger F, Jacobsen B (1976) Mental illness in the biological and adoptive families of adopted individuals who have become schizophrenic. Behav Genet 6:219–225

Korostishevsky M, Kaganovich M, Cholostoy A, Ashkenazi M, Ratner Y, Dahary D, Bernstein J, Bening-Abu-Shach U, Ben-Asher E, Lancet D, Ritsner M, Navon R (2004) Is the G72/G30 locus associated with schizophrenia? Single nucleotide polymorphisms, haplotypes, and gene expression analysis. Biol Psychiatry 56(3):169–176

Korostishevsky M, Kremer I, Kaganovich M, Cholostoy A, Murad I, Muhaheed M, Bannoura I, Rietschel M, Dobrusin M, Bening-Abu-Shach U, Belmaker RH, Maier W, Ebstein RP, Navon R (2005) Transmission disequilibrium and haplotype analyses of the G72/G30 locus: suggestive linkage to schizophrenia in Palestinian Arabs living in the North of Israel. Am J Med Genet B Neuropsychiatr Genet 141:91–95

Kuhn K, Baker SC, Chudin E, Lieu MH, Oeser S, Bennett H, Rigault P, Barker D, McDaniel TK, Chee MS (2004) A novel, high-performance random array platform for quantitative gene expression profiling. Genome Res 14(11):2347–2356

Kunugi H, Vallada HP, Sham PC, Hoda F, Arranz MJ, Li T, Nanko S, Murray RM, McGuffin P, Owen M, Gill M, Collier DA (1997) Catechol-O-methyltransferase polymorphisms and schizophrenia: a transmission disequilibrium study in multiply affected families. Psychiatr Genet 7(3):97–101

Larsen TK, McGlashan TH, Moe LC (1996) First-episode schizophrenia: I. Early course parameters. Schizophr Bull 122(2):241–256

Lauriat TL, Dracheva S, Chin B, Schmeidler J, McInnes LA, Haroutunian V (2005) Quantitative analysis of glutamate transporter mRNA expression in prefrontal and primary visual cortex in normal and schizophrenic brain. Neuroscience 137:843–851

Lazar NL, Rajakumar N, Cain DP (2008) Injections of NGF into neonatal frontal cortex decrease social interaction as adults: a rat model of schizophrenia. Schizophr Bull 34:127–136

Leung A, Chue P (2000) Sex differences in schizophrenia, a review of the literature. Acta Psychiatr Scand Suppl 401:3–38

Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I, Williams NM, Schwab SG, Pulver AE, Faraone SV, Brzustowicz LM, Kaufmann CA, Garver DL, Gurling HM, Lindholm E, Coon H, Moises HW, Byerley W, Shaw SH, Mesen A, Sherrington R, O'Neill FA, Walsh D, Kendler KS, Ekelund J, Paunio T, Lonnqvist J, Peltonen L, O'Donovan MC, Owen MJ, Wildenauer DB, Maier W, Nestadt G, Blouin JL, Antonarakis SE, Mowry BJ, Silverman JM, Crowe RR, Cloninger CR, Tsuang MT, Malaspina D, Harkavy-Friedman JM, Svrakic DM, Bassett AS, Holcomb J, Kalsi G, McQuillin A, Brynjolfson J, Sigmundsson T, Petursson H, Jazin E, Zoega T, Helgason T (2003) Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: schizophrenia. Am J Hum Genet 73(1):34–48

Li T, Sham PC, Vallada H, Xie T, Tang X, Murray RM, Liu X, Collier DA (1996) Preferential transmission of the high activity allele of COMT in schizophrenia. Psychiatr Genet 6(3):131–133

Li T, Ball D, Zhao J, Murray RM, Liu X, Sham PC, Collier DA (2000) Family-based linkage disequilibrium mapping using SNP marker haplotypes: application to a potential locus for schizophrenia at chromosome 22q11. Mol Psychiatry 5(1):77–84

Li W, Zhang Q, Oiso N, Novak EK, Gautam R, O'Brien EP, Tinsley CL, Blake DJ, Spritz RA, Copeland NG, Jenkins NA, Amato D, Roe BA, Starcevic M, Dell'Angelica EC, Elliott RW, Mishra V, Kingsmore SF, Paylor RE, Swank RT (2003) Hermansky–Pudlak syndrome type 7 (HPS-7) results from mutant dysbindin,

a member of the biogenesis of lysosome-related organelles complex 1 (BLOC-1). Nat Genet 35(1):84–89

Li T, Ma X, Sham PC, Sun X, Hu X, Wang Q, Meng H, Deng W, Liu X, Murray RM, Collier DA (2004) Evidence for association between novel polymorphisms in the PRODH gene and schizophrenia in a Chinese population. Am J Med Genet B Neuropsychiatr Genet 129(1):13–15

Lipska BK, Mitkus S, Caruso M, Hyde TM, Chen J, Vakkalanka R, Straub RE, Weinberger DR, Kleinman JE (2006) RGS4 mRNA expression in postmortem human cortex is associated with COMT val158met genotype and COMT enzyme activity. Hum Mol Genet 15:2804–2812

Mallory FB (1961) Pathological technique. Hafner, New York, pp 158–180

Marti SB, Cichon S, Propping P, Nothen M (2002) Metabotropic glutamate receptor 3 (GRM3) gene variation is not associated with schizophrenia or bipolar affective disorder in the German population. Am J Med Genet 114(1):46–50

Millar JK, Pickard BS, Mackie S, James R, Christie S, Buchanan SR, Malloy MP, Chubb JE, Huston E, Baillie GS, Thomson PA, Hill EV, Brandon NJ, Rain JC, Camargo LM, Whiting PJ, Houslay MD, Blackwood DH, Muir WJ, Porteous DJ (2005) DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. Science 310(5751):1187–1191

Mirnics K, Middleton FA, Stanwood GD, Lewis DA, Levitt P (2001) Disease-specific changes in regulator of G-protein signaling 4 (RGS4) expression in schizophrenia. Mol Psychiatry 6(3):293–301

Miyoshi K, Honda A, Baba K, Taniguchi M, Oono K, Fujita T, Kuroda S, Katayama T, Tohyama M (2003) Disrupted-In-Schizophrenia 1, a candidate gene for schizophrenia, participates in neurite outgrowth. Mol Psychiatry 8(7):685–694

Morris JA, Kandpal G, Ma L, Austin CP (2003) DISC1 (Disrupted-In-Schizophrenia 1) is a centrosome-associated protein that interacts with MAP1A, MIPT3, ATF4/5 and NUDEL: regulation and loss of interaction with mutation. Hum Mol Genet 12(13):1591–1608

Morris DW, Rodgers A, McGhee KA, Schwaiger S, Scully P, Quinn J, Meagher D, Waddington JL, Gill M, Corvin AP (2004) Confirming RGS4 as a susceptibility gene for schizophrenia. Am J Med Genet B Neuropsychiatr Genet 125(1):50–53

Nicodemus KK, Kolachana BS, Vakkalanka R, Straub RE, Giegling I, Egan MF, Rujescu D, Weinberger DR (2006) Evidence for statistical epistasis between catechol-O-methyltransferase (COMT) and polymorphisms in RGS4, G72 (DAOA), GRM3, and DISC1: influence on risk of schizophrenia. Hum Genet 120(6):889–906

Norton N, Moskvina V, Morris DW, Bray NJ, Zammit S, Williams NM, Williams HJ, Preece AC, Dwyer S, Wilkinson JC, Spurlock G, Kirov G, Buckland P, Waddington JL, Gill M, Corvin AP, Owen MJ, O'donovan MC (2005) Evidence that interaction between neuregulin 1 and its receptor erbB4 increases susceptibility to schizophrenia. Am J Med Genet B Neuropsychiatr Genet 141:96–101

Numakawa T, Yagasaki Y, Ishimoto T, Okada T, Suzuki T, Iwata N, Ozaki N, Taguchi T, Tatsumi M, Kamijima K, Straub RE, Weinberger DR, Kunugi H, Hashimoto R (2004) Evidence of novel neuronal functions of dysbindin, a susceptibility gene for schizophrenia. Hum Mol Genet 13(21):2699–2708

Ohnuma T, Augood SJ, Arai H, McKenna PJ, Emson PC (1998) Expression of the human excitatory amino acid transporter 2 and metabotropic glutamate receptors 3 and 5 in the prefrontal cortex from normal individuals and patients with schizophrenia. Brain Res Mol Brain Res 56(1–2):207–217

Ohnuma T, Tessler S, Arai H, Faull RL, McKenna PJ, Emson PC (2000) Gene expression of metabotropic glutamate receptor 5 and excitatory amino acid transporter 2 in the schizophrenic hippocampus. Brain Res Mol Brain Res 85(1–2):24–31

Ozeki Y, Tomoda T, Kleiderlein J, Kamiya A, Bord L, Fujii K, Okawa M, Yamada N, Hatten ME, Snyder SH, Ross CA, Sawa A (2003) Disrupted-in-Schizophrenia-1 (DISC-1): mutant truncation prevents binding to NudE-like (NUDEL) and inhibits neurite outgrowth. Proc Natl Acad Sci USA 100(1):289–294

Paus T, Zijdenbos A, Worsley K, Collins DL, Blumenthal J, Giedd JN, Rapoport JL, Evans AC (1999) Structural maturation of neural pathways in children and adolescents: in vivo study. Science 283(5409):1908–1911

Perlman WR, Tomaskovic-Crook E, Montague DM, Webster MJ, Rubinow DR, Kleinman JE, Weickert CS (2005) Alteration in estrogen receptor alpha mRNA levels in frontal cortex and hippocampus of patients with major mental illness. Biol Psychiatry 58:812–824

Prophet EB, Mills B, Arrington JB, Sobin LH (1992) Laboratory methods in histotechnology. American Registry of Pathology, Washington DC

Rajakumar N, Leung LS, Ma J, Rajakumar B, Rushlow W (2004) Altered neurotrophin receptor function in the developing prefrontal cortex leads to adult-onset dopaminergic hyperresponsivity and impaired prepulse inhibition of acoustic startle. Biol Psychiatry 55:797–803

Rajkowska G, Goldman-Rakic PS (1995) Cytoarchitectonic definition of prefrontal areas in the normal human cortex: I. Remapping of areas 9 and 46 using quantitative criteria. Cereb Cortex 5:307–322

Schwab SG, Hoefgen B, Hanses C, Hassenbach MB, Albus M, Lerer B, Trixler M, Maier W, Wildenauer DB (2005) Further evidence for association of variants in the AKT1 gene with schizophrenia in a sample of European sib-pair families. Biol Psychiatry 58(6):446–450

Schwab SG, Knapp M, Mondabon S, Hallmayer J, Borrmann-Hassenbach M, Albus M, Lerer B, Rietschel M, Trixler M, Maier W, Wildenauer DB (2003) Support for association of schizophrenia with genetic variation in the 6p22.3 gene, dysbindin, in sib-pair families with linkage and in an additional sample of triad families. Am J Hum Genet 72(1):185–190

Schumacher J, Jamra RA, Freudenberg J, Becker T, Ohlraun S, Otte AC, Tullius M, Kovalenko S, Bogaert AV, Maier W, Rietschel M, Propping P, Nothen MM, Cichon S (2004) Examination of G72 and D-amino-acid oxidase as genetic risk factors for schizophrenia and bipolar affective disorder. Mol Psychiatry 9(2):203–207

Semendeferi K, Armstrong E, Schleicher A, Zilles K, Van Hoesen GW (2001) Prefrontal cortex in humans and apes: a comparative study of area 10. Am J Phys Anthropol 114:224–241

Shifman S, Bronstein M, Sternfeld M, Pisante-Shalom A, Lev-Lehman E, Weizman A, Reznik I, Spivak B, Grisaru N, Karp L, Schiffer R, Kotler M, Strous RD, Swartz-Vanetik M, Knobler HY, Shinar E, Beckmann JS, Yakir B, Risch N, Zak NB, Darvasi A (2002) A highly significant association between a COMT haplotype and schizophrenia. Am J Hum Genet 71(6):1296–1302

Smith RE, Haroutunian V, Davis KL, Meador-Woodruff JH (2001) Expression of excitatory amino acid transporter transcripts in the thalamus of subjects with schizophrenia. Am J Psychiatry 158(9):1393–1399

St Clair D, Blackwood D, Muir W, Carothers A, Walker M, Spowart G, Gosden C, Evans HJ (1990) Association within a family of a balanced autosomal translocation with major mental illness. Lancet 336(8706):13–16

Starcevic M, Dell'Angelica EC (2004) Identification of snapin and three novel proteins (BLOS1, BLOS2, and BLOS3/reduced pigmentation) as subunits of biogenesis of lysosome-related organelles complex-1 (BLOC-1). J Biol Chem 279(27):28393–401

Stefansson H, Sarginson J, Kong A, Yates P, Steinthorsdottir V, Gudfinnsson E, Gunnarsdottir S, Walker N, Petursson H, Crombie C, Ingason A, Gulcher JR, Stefansson K, St Clair D (2002) Association of neuregulin 1 with schizophrenia confirmed in a Scottish population. Am J Hum Genet 72(1):83–87

Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, Brynjolfsson J, Gunnarsdottir S, Ivarsson O, Chou TT, Hjaltason O, Birgisdottir B, Jonsson H, Gudnadottir VG, Gudmundsdottir E, Bjornsson A, Ingvarsson B, Ingason A, Sigfusson S, Hardardottir H, Harvey RP, Lai D, Zhou M, Brunner D, Mutel V, Gonzalo A, Lemke G, Sainz J, Johannesson G, Andresson T, Gudbjartsson D, Manolescu A, Frigge ML, Gurney ME, Kong A, Gulcher JR, Petursson H, Stefansson K (2003) Neuregulin 1 and susceptibility to schizophrenia. Am J Hum Genet 71(4):877–892

Straub RE, Jiang Y, MacLean CJ, Ma Y, Webb BT, Myakishev MV, Harris-Kerr C, Wormley B, Sadek H, Kadambi B, Cesare AJ, Gibberman A, Wang X, O'Neill FA, Walsh D, Kendler KS (2002) Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. Am J Hum Genet 71:337–348

Straub RE, Lipska BK, Egan MF, Goldberg TE, Callicott JH, Mayhew MB, Vakkalanka RK, Kolachana BS, Kleinman JE, Weinberger DR (2007) Allelic variation in GAD1 (GAD67) is associated with schizophrenia and influences cortical function and gene expression. Mol Psychiatry 12:854–869

Sullivan PF, Kendler KS, Neale MC (2003) Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 60(12):1187–1192

Tamminga CA (1997) Gender and schizophrenia. J Clin Psychiatry 58(Suppl 15):33–37

Tang JX, Chen WY, He G, Zhou J, Gu NF, Feng GY, He L (2004) Polymorphisms within 5′ end of the Neuregulin 1 gene are genetically associated with schizophrenia in the Chinese population. Mol Psychiatry 9(1):11–12

Tkachev D, Mimmack ML, Ryan MM, Wayland M, Freeman T, Jones PB, Starkey M, Webster MJ, Yolken RH, Bahn S (2003) Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. Lancet 362:798–805

Webster MJ, Weickert CS, Herman MM, Kleinman JE (2002) BDNF mRNA expression during postnatal development, maturation and aging of the human prefrontal cortex. Dev Brain Res 139:139–150

Weinberger DR (1987) Implications of normal brain development for the pathogenesis of schizophrenia. Arch Gen Psychiatry 44(7):660–669

Williams NM, Preece A, Spurlock G, Norton N, Williams HJ, McCreadie RG, Buckland P, Sharkey V, Chowdari KV, Zammit S, Nimgaonkar V, Kirov G, Owen MJ, O'Donovan MC (2004) Support for RGS4 as a susceptibility gene for schizophrenia. Biol Psychiatry 55(2):192–195

Woo TU, Crowell AL (2005) Targeting synapses and myelin in the prevention of schizophrenia. Schizophr Res 73(2–3):193–207

Xu J, Pato MT, Torre CD, Medeiros H, Carvalho C, Basile VS, Bauer A, Dourado A, Valente J, Soares MJ, Macedo AA, Coelho I, Ferreira CP, Azevedo MH, Macciardi F, Kennedy JL, Pato CN (2001) Evidence for linkage disequilibrium between the alpha 7-nicotinic receptor gene (CHRNA7) locus and schizophrenia in Azorean families. Am J Med Genet 105(8):669–674

Yamada K, Nakamura K, Minabe Y, Iwayama-Shigeno Y, Takao H, Toyota T, Hattori E, Takei N, Sekine Y, Suzuki K, Iwata Y, Miyoshi K, Honda A, Baba K, Katayama T, Tohyama M, Mori N, Yoshikawa T (2004) Association analysis of FEZ1 variants with schizophrenia in Japanese cohorts. Biol Psychiatry 56(9):683–690

Yang JZ, Si TM, Ruan Y, Ling YS, Han YH, Wang XL, Zhou M, Zhang HY, Kong QM, Liu C, Zhang DR, Yu YQ, Liu SZ, Ju GZ, Shu L, Ma DL, Zhang D (2003) Association study of neuregulin 1 gene with schizophrenia. Mol Psychiatry 8(7):706–709

Zheng Y, Li H, Qin W, Chen W, Duan Y, Xiao Y, Li C, Zhang J, Li X, Feng G, He L (2005) Association of the carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase gene with schizophrenia in the Chinese Han population. Biochem Biophys Res Commun 328(4):809–815

Zou F, Li C, Duan S, Zheng Y, Gu N, Feng G, Xing Y, Shi J, He L (2005) A family-based study of the association between the G72/G30 genes and schizophrenia in the Chinese population. Schizophr Res 73(2–3):257–261