Molecular Aging in Human Prefrontal Cortex Is Selective and Continuous Throughout Adult Life

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Background: *Aging leads to morphologic and functional changes in the brain and is associated with increased risk for psychiatric and neurological disorders.*

Methods: *To identify age-related transcriptional changes in the human brain, we profiled gene expression in two prefrontal cortex (PFC) areas in postmortem samples from 39 subjects, ranging in age from 13 to 79 years.*

Results: *Robust transcriptional age-related changes were identified for at least 540 genes. Gene expression correlates of aging were highly specific, and the large majority of the 22,000 transcripts investigated were unaffected by age. Across subjects, changes were progressive throughout adult life and accurately predicted chronological age. Age-upregulated transcripts were mostly of glial origin and related to inflammation and cellular defenses, whereas downregulated genes displayed mostly neuron-enriched transcripts relating to cellular communication and signaling.*

Conclusions: *Continuous changes in gene expression with increasing age revealed a "molecular profile" of aging in human PFC. The restricted scope of the transcript changes suggests cellular populations or functions that are selectively vulnerable during aging. Because age-related gene expression changes begin early in adulthood and are continuous throughout life, our results suggest the possibility of identifying early cellular mechanisms that may be engaged in preventive or detrimental age-related brain functions.*

Key Words: Age, human, microarray, postmortem, prefrontal, cortex

With increased life expectancy resulting from improved medical interventions and living conditions over the last century comes increased risk for the development of psychiatric and neurologic disorders in later life, impairing daily function in terms of cognition, mood, memory, coordination, and gait. Unlike neurodegenerative diseases, however, the mechanisms of normal aging processes are still poorly understood. From an evolutionary standpoint, old age corresponds to a postreproductory phase in life with limited impact for natural selection, suggesting a pleiotropic downregulation of functions (Hughes et al 2002; but see also Hawkes [2003] for multigenerational mentoring role by longer-lived "grandmothers"). The identification of single gene mutations that increase longevity in nematodes, insects, and rodents (Hekimi and Guarente 2003) has shifted attention toward more specific pathways, such as programmed genetic effects or oxidative stress.

In the brain, the course of aging parallels that of peripheral mitotic tissues, although additional mechanisms may reflect the specificities of postmitotic neurons. Morphologic studies reveal a small loss or no change in cortical neurons with age (Morrison and Hof 1997; Pakkenberg and Gundersen 1997) and a thinning of cortical thickness affecting both gray and white matter (Resnick et al 2003; Sowell et al 2003). Oxidative stress and inflammation may mediate an increase in reactive astrocytes (Finch 2002) and neuronal damage, thus increasing cellular senescence and susceptibility to numerous age-related disorders (Morrison and Hof 1997). Instability of nuclear and mitochondrial genomes may be critical in differentiated neurons, where the accumulation of mutations and damaged macromolecules may decrease the efficiency of energy production (Lin et al 2002) and impair cellular and synaptic plasticity. Altered Ca2+ regulation and functions (Thibault and Landfield 1996) may contribute to neuronal deficits underlying the continuous decline of mental and cognitive capacities with age (Park et al 2002; Hedden and Gabrieli 2004).

Recent developments in genome sequencing and microarray technologies allow the simultaneous monitoring of large numbers of genes, offering a global view of biological systems from the perspective of gene expression. Age-related large-scale gene expression changes have been described in the rodent brain (Blalock et al 2003; Jiang et al 2001; Lee et al 1999, 2000; Verbitsky et al 2004), and recently in human subjects with incipient Alzheimer's disease (Blalock et al 2004). Here, using a large sample of well-characterized postmortem human brain samples (Galfalvy et al 2003; Sibille et al 2004) with a combination of statistical and bioinformatic approaches, we report continuous gene expression changes in specific subsets of genes occurring throughout adult life. Transcriptional changes are concordant across two regions of the human PFC and differentially affect glial and neuronal gene expression.

Methods and Materials

Brain Samples

Samples from 39 subjects, ranging from 13 to 79 years of age (44 ± 20 years, Mean ± SD, Supplement 1) were obtained from the brain collection of the Human Neurobiology Core of the Sylvio Conte Center for the Neuroscience of Mental Disorders, at the New York State Psychiatric Institute. The applicable institutional review boards approved all studies. Psychological autopsy (Kelly and Mann 1996) indicated that all cases were free of neurologic disease (see details in Sibille et al 2004). Fixed tissue from all subjects was available for neuropathologic examination, including thioflavine S or immunohistochemical stains for senile plaques and neurofibrillary tangles. Varying degrees of athero-

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sclerosis were present in subjects aged 45 or older. Several cases contained plaques or neurofibrillary tangles, but never in sufficient numbers to indicate a diagnosis of Alzheimer's disease. No other significant abnormalities were observed. Caucasians represented 71%, African Americans 8%, Hispanics 18%, and Asians 2% of the subjects. Average postmortem interval and brain pH were 17 ± 7 hours and 6.53 ± .21, respectively (Supplement 1). As a group, male subjects (n = 30) did not differ significantly from female subjects (n = 9) on age, race, postmortem interval (PMI), or brain pH. We have also previously reported that in our brain collection, PMI, brain pH, and race did not correlate with RNA quality and gene expression and that the effect of gender was restricted to a few sex-chromosome genes (Galfalvy et al 2003).

Twenty subjects committed suicide (psychologic autopsies indicated that 17 of these had a lifetime diagnosis of major depression) and 19 died of causes other than suicide. All cases were selected on the basis of sudden death and absence of prolonged agonal periods. Using body fluids and brain tissue, a toxicologic screen was carried out for the presence of psychotropic or illegal drugs. Only brain samples from psychotropic medication-free cases were included. Other drug exposures were minimal (Supplement 1). Within the current analytical limits, we found no evidence for molecular differences that correlated with depression or suicide (Sibille et al 2004). Because clinical manifestations vary with age, we also observed no interaction between gene expression and experimental, demographic, or clinical parameters and age (Galfalvy et al 2003; Sibille et al 2004). Therefore, the effect of age on gene expression was analyzed across all samples combined in one group, thereby increasing our analytical power.

Oligonucleotide DNA Microarrays

Brodmann areas 9 (dorsolateral PFC, BA9) and 47 (orbital PFC, BA47) were dissected from frozen brain sections warmed from −80°C to −20°C for 2 hours. The blocks were manually dissected avoiding white matter (WM) and collecting only gray matter (GM) containing all cortical layers. Three samples of white matter were processed separately. RNA extraction, microarray samples preparation, and quality control were performed according to the manufacturer protocol (http://www.affymetrix.com) and as described elsewhere (Galfalvy et al 2003). Samples were hybridized to Affymetrix U133A microarrays (22,283 probesets). Signal intensities were extracted with the Robust Multi-array Average algorithm (Irizarry et al 2003). Seventy-one arrays passed quality control and were retained for analysis in BA9 (37 arrays) and BA47 (34 arrays, Supplement 1 and Galfalvy et al 2003). Minimum information about a microarray experiment (MIAME) format is available in Supplement 2.

Real-time polymerase chain reaction (PCR) was performed as described in Galfalvy et al (2003).

Statistical Analysis and Gene Selection

Univariate statistical tests were used to assess correlations between gene expression levels and age of subjects. Genes detected as expressed in 10% or fewer samples or with coefficient of variation below 2% (based on Log2 scale) were removed, leaving 11,546 genes for statistical testing. Significance values for Pearson correlation coefficients were computed, measuring linear [log2(Age) and log2(Signal)] and exponential [Age and log2(Signal)] relationships between age and gene expression. 80.2% gene expression changes were detected with both approaches. Large-scale gene-by-gene testing can result in low p values even when there is no significant effect, because the probability of a false positive result (Type I error) increases with the number of tests performed. Here, p values were adjusted for multiple testing by the Benjamini–Hochberg method for controlling the false discovery rate (Benjamini and Hochberg 1995) with an experimentwise false discovery rate of 5%. Using sex-chromosome-linked genes as internal controls, we have previously demonstrated that this approach is well suited to separate true differences from false-positive results, with no evidence for false-negative results (Galfalvy et al 2003). Rank-based Spearman correlation coefficients and analysis of variance (averaged over three age-groups) were also applied, but with the exception of marginal changes in midlife for a few genes, these last two techniques did not identify differentially expressed genes beyond those found by Pearson correlation and consequently were omitted for the rest of the analysis. A multifactorial analysis, including demographic (age, gender, race), clinical (psychiatric diagnostic), sample (pH, PMI), and array parameters, was also performed on the 20 most affected probesets per brain area. Not surprisingly, because these probesets were age related, age explained more than 50% of the variation, whereas other variables (e.g., pH, PMI) explained 1%–2%. Array parameters explained 7.2% of the variation in BA47 and 1.2% in BA9.

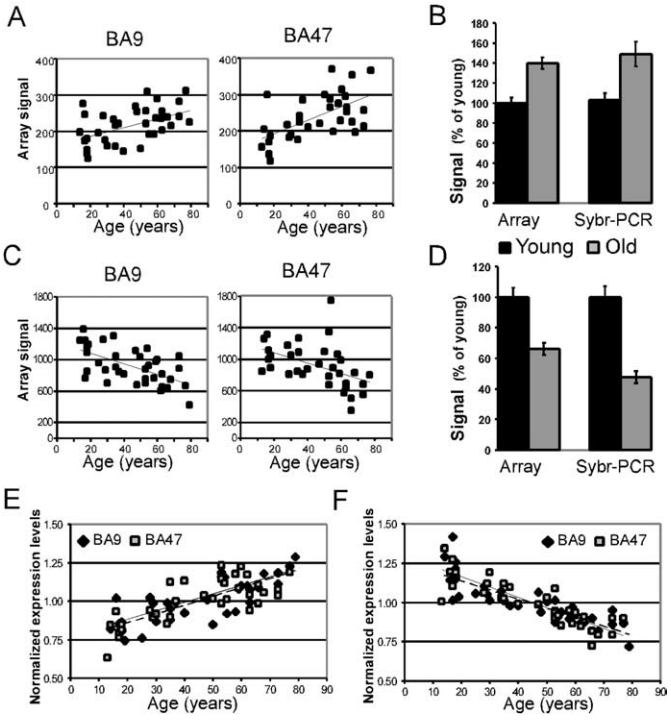

Figure 1. Age-related changes in gene expression and confirmation by quantitative real-time polymerase chain reaction (PCR). Representative microarray signal intensities for BCL2 **(A)** and CRF **(C)** gene transcripts are displayed in Brodmann areas (BA) 9 and 47. **(B, D)** Confirmation of altered expression levels by independent real-time PCR (Sybr-PCR). Fold changes were calculated between older (> 60 years) and younger (< 30 years) age groups. All group differences were significant (t test, $p < .05$). See also Supplements 5 and 6 for additional microarray versus real-time PCR comparison. **(E, F)** The effect of age (y axis : average of Log_2-based normalized and mean-centered gene expression values) per sample and per brain area for all increased (part E, n = 297) or decreased (part F, n = 291) genes was plotted against age (x axis). Lines indicate data trend lines. Coefficient of correlation for increased genes: BA9, r = .80; BA47, r = .75; and decreased genes: BA9, r = .84; BA47, r = .87.

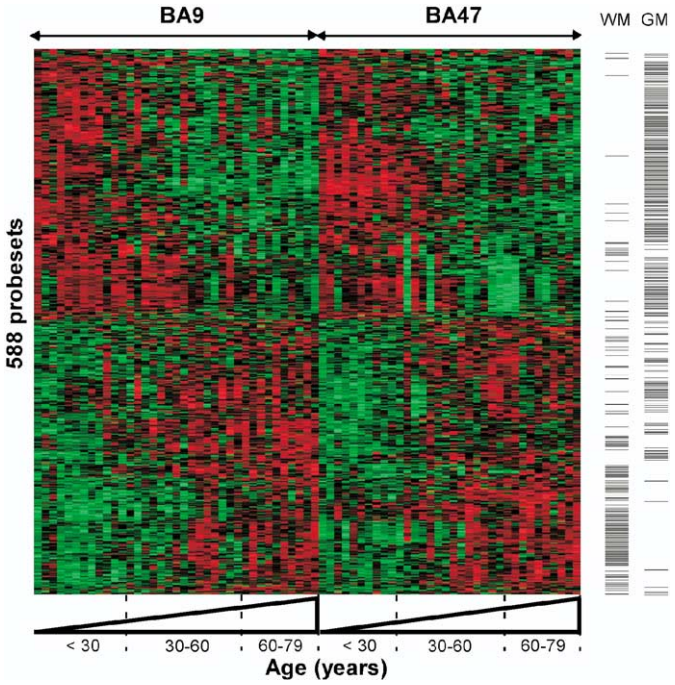

Figure 2. Consistent effect of aging throughout the prefrontal cortex. Expression levels for 588 age-affected probesets are presented together for Brodmann areas (BA) 9 and 47. Each probeset is represented by a row; each array, or brain area, per subject by a column. Samples are organized left to right by brain area and increasing age. Green and red bars indicate decreased and increased gene expression, respectively, versus the averaged signal for these genes across all samples. For example, a horizontal row going from red to green indicates a gene with expression that decreases with age in that brain area. Along the y axis, probesets are clustered (Eisen et al 1998) according to similarities in expression profiles across age. A similar number of probesets were downregulated (n = 291, upper panel) and upregulated (n = 297, lower panel) throughout lifetime. Columns to the right indicate the distribution of genes with glial- (WM) or neuronal-enriched (GM) signals (see text). Notice the high concentration of glial-enriched genes with increased expression with age, whereas most, but not all, neuronal-enriched genes appear to be downregulated with age.

To describe each sample individually in the general aging trend, we devised a one-number summary ("Molecular age") for each sample, describing the "predicted age" of the sample when removed from the analysis. For each sample, the remaining database was analyzed for age-related genes using the same correlation-based methods described earlier, controlling the false discover rate (FDR) at .05. For each selected gene, a linear or exponential regression with age was performed, and the age for the heldout sample was predicted using the resulting function. The resulting genewise predicted values were averaged per sample and rescaled to allow comparison with chronologic ages. A similar technique was used to compute "molecular ages" of depressed suicide victims based on the aging trend of control subjects and vice versa. In this case, the sample group was divided in two. Because considerable power was lost, the top 200 genes were used for the two sample configurations.

Clustering analysis was performed using Cluster and Treeview softwares (Eisen et al 1998).

Functional Class Scoring Analysis

Rather than analyzing genes one at a time, gene functional class scoring (Pavlidis et al 2002) gives scores to classes or groups of genes, representing the overall effect of age on these groups of genes. Genes with white to gray matter (WM/GM) changes less than −1.5-fold were removed from the data set to analyze glial-enriched functions, and genes with WM/GM changes more than 1.5-fold were not used for analyzing neuronal-enriched functions, leaving 8,064 and 8,585 genes, respectively. Gene Ontology groups (GO; Ashburner et al 2000) with greater than 200 or fewer than 8 genes were screened out, leaving 897 groups for glial-enriched and 970 for neuronal-enriched analysis (88% overlap). The GO groups were scored as described (Pavlidis et al 2002, 2004), using age-related p values as gene scores.

Results

Continuous and Extensive Age-Related Transcriptional Changes in the Human Prefrontal Cortex

To assess the effect of age on gene expression, we calculated Pearson coefficients for linear and exponential correlation between transcript levels and age for the 39 subjects (see Methods and Materials). After adjustment for multiple statistical testing, 588 probesets were identified as being significantly affected by age in BA9 or BA47 (465 probesets), or in both brain areas (123 probesets); 48 probesets represented duplicate or triplicate assays for the same genes, bringing the number of age-affected genes to 540. Independent analysis of variance on samples from three age groups (young [< 30 years], adult [30–60 years], and old [> 60 years]; Jarskog and Gilmore 2000; Webster et al 2002) and nonparametric approaches did not detect additional probesets beyond those found by Pearson correlation, indicating that the very large majority of age-related changes are continuous and progressive throughout the 66-year age range investigated here. Normalizing expression levels for all age-related genes per subject and brain area highlighted this cross-subject continuous progression of the effect of age for both increased (297 probesets, 50.5% of changes) or decreased (291 probesets, 49.5% of changes) genes throughout the lifespan (Figure 1).

In gene expression studies, it is difficult to obtain a "final" list of significant genes, because alternative statistical approaches are often applicable, and thresholds for significance, sample selection, and number of arrays vary between studies. Using a systematic "leave-one sample out" approach to attenuate the effect of variability in our sample, an additional 1,074 probesets demonstrated statistically significant changes with aging in one area or both, representing a total of 1,662 transcripts or 7.5% of the probesets tested (listed in Supplements 3 and 4). Quantitative real-time PCR on cDNAs obtained from the original RNA samples confirmed age-related changes in expression for all 14 genes tested (increased: MAOB, BCL2, ERRB2, LPL, CLU; decreased: CALB1, SST, CRH, HTR2A, GABRA5), including four genes identified by the "leave-one out" approach (increased: S100B; decreased: CNR1, VIP, HOMER1; Figure 1 and Supplements 5 and 6).

To determine whether changes in gene expression with age were region-specific within the PFC, we compared expression levels for all 588 age-affected probesets in BA9 and BA47 (based on the full sample) by organizing or clustering genes according to similarities in expression profiles. Genes exhibited comparable changes in the two brain areas. In particular, the similarity in the graphs of expression profiles between BA9 and BA47 (compare right versus left panels in Figure 2) was reflected by high correlation levels within subjects for age-related gene expression changes (averaged correlation, r = .974). Similar correlations were observed within same brain areas for closely aged-matched subjects (averaged correlations, BA9 across sub-

jects, $r = .968$; BA47 across subjects, $r = .969$). Correlation between young and old subjects decreased (averaged correlations, BA9, $r = .879$; BA47, $r = .913$). Together, these results indicate a high consistency of age-related transcriptional changes across the two PFC areas within individuals, and within areas across subjects close in age.

"Molecular" Age Predicts Chronological Age

As an attempt to derive an empirical age predictor based on a panel of age-dependent genes and as a first step in identifying aging biomarkers in the brain, "molecular ages" were calculated in BA9 and BA47 for each subject, based on the distance between expression levels of all age-affected genes in a subject compared with the levels of the same transcript in the remaining subjects (Figure 3). Average differences between "molecular" and chronological age were 11.6 ± 9.6 years in BA9, and 10.1 ± 8.7 years in BA47. Intrasubject differences in BA9 and BA47 molecular ages were 11.4 ± 11.3 years. Although the correlation between chronologic and molecular ages was high for all samples combined (BA9: $r = .735$; BA47: $r = .653$), larger individual deviation was observed for some samples (Figure 3). These samples did not correspond to any known experimental or demographic parameters. Because caloric restriction is known to retard the aging process and to reverse some of the gene expression correlates of aging in the rodent brain (Lee et al 2000), we examined the relationship between body mass index (BMI) and gene expression. No correlation was observed between BMI and age-related gene expression profiles (data not shown), although our analysis was limited by the lack of information on lifelong eating habits.

Despite the absence of discernable transcriptional differences associated with psychiatric diagnosis (Sibille et al 2004), we sought to rule out the possibility of confounding age-diagnosis interactions. Molecular ages for subjects in one group of samples (depressed-suicide or control subjects) were calculated based on age-related gene expression levels in the other group. Average differences between chronologic and molecular ages were not different between controls (BA9: 14.3 ± 10.4 years; BA47: 12.0 ± 11.8 years) and psychiatric subjects (BA9: 12.0 ± 5.4 years; BA47: 14.0 ± 12.6 years), thus indicating that the intersubject progression of the molecular effect of age was not different between clinical samples and nonpsychiatric control subjects.

Glial-Enriched and Neuronal-Enriched Transcripts Are Differentially Affected by Age

Because aging may differentially affect diverse cellular populations, we attempted to characterize age-related gene expression changes in correlation with putative glial or neuronal origin. To estimate glial to neuronal relative transcript levels, a ratio was calculated for each gene between pure white matter signal (WM, averaged over three samples processed on three arrays) and gray matter signal (GM, averaged over all samples per brain area). High WM/GM ratio indicated glial-enrichment. Genes similarly expressed in both cell populations displayed ratios close to one, whereas low WM/GM ratio reflected neuronal enrichment, due to low WM signal. Ratios for known glial markers confirmed these premises (Supplement 4). Using a minimal 1.5-fold level of enrichment, 29% of the genes represented on the arrays displayed neuronal-enrichment, and 21.6% of the genes revealed WM or glial-enriched signals (Supplements 2 and 7). Ratios correlated highly between BA9 and BA47 ($r = .98$) and whether GM samples from young or old subjects were used ($r = .99$).

The direction of change in expression levels with age agreed with the WM/GM ratio for 75% of the 588 age-affected probesets (chi-square; BA9, C2 = 157.4, $p < .0001$; BA47, C2 = 141.5, $p < .0001$) indicating that age-upregulated genes were mostly glial-enriched, whereas age downregulated genes tended to be neuronal-enriched (Figure 2, right columns). A global change in glial/neuronal ratio in the aging brain would preferentially affect genes that are highly enriched in one cellular compartment. Only 6% and 14%, respectively, of the most enriched glial and neuronal genes (100 glial and 500 neuronal genes with more than sevenfold enrichment) were affected by age. Age-affected genes were evenly distributed along the spectrum of glial- or neuronal enrichment (Supplement 7), thus demonstrating that the gene expression correlates of aging were specific rather than reflecting a general change in glia versus neuron transcript ratio.

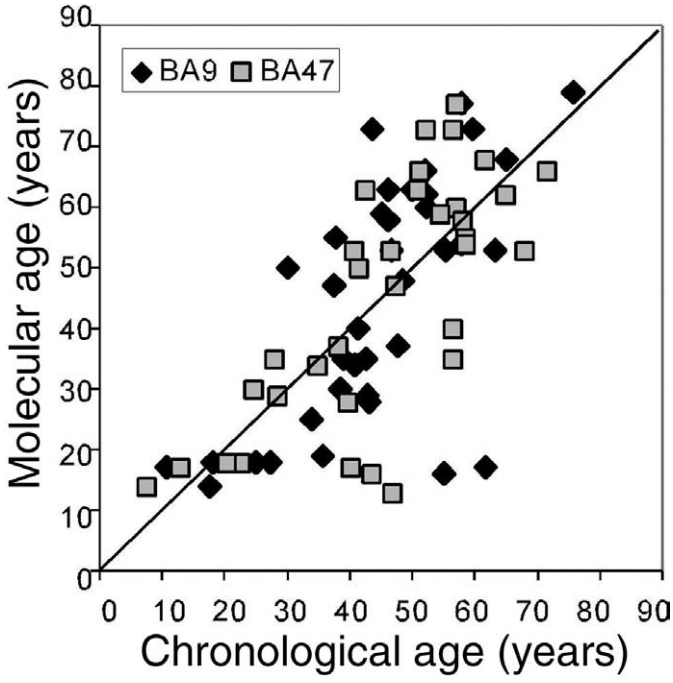

Figure 3. "Molecular" age versus chronological age. The "molecular" age, or age as defined by gene expression levels, represents a summary number for each sample that depicts the average deviation in gene expression within this sample when compared with age-affected transcripts in all other subjects (see Methods and Materials). Overall, there was a high correlation between "molecular" and chronological ages (Brodmann area [BA] 9: $r = .65$; BA47, $r = .73$). A few samples demonstrated larger deviation than average at both young and older ages. These subjects did not correspond to any identifiable clinical, demographic, or experimental parameters.

Age-Related Gene Expression Changes Correspond to Different Cellular Functions in Glia and Neurons

To assess whether changes in transcript levels with age were functionally related, the nature and interrelationship of genes with differential expression were examined. Genes were organized according to the gene ontology (GO) classification (Ashburner et al 2000). Although incomplete, GO annotations provide overall information covering most biological processes, cellular components, and molecular functions. The cumulated effect of aging on individual groups of genes was separately assessed for glial- and neuronal-enriched transcripts. Although 88% of gene groups were assessed in both analyses, two sets of almost nonoverlapping functional groups were identified (Figure 4). For neuronal-enriched transcripts, 22 of the top 25 age-

Gene Ontology (GO) groups	Neuronal-enriched (ranks)			Glial-enriched (ranks)		
Glial-enriched-top 25 age-affected GO classes	BA9	BA47	BA9/47	BA9	BA47	BA9/47
humoral_immune_response_GO:0006959	30	31	26	1	9	1
microtubule_associated_complex_GO:0005875	255	37	121	9	1	2
structural_constituent_of_cytoskeleton_GO:0005200	124	153	118	4	7	3
cytoskeleton_organization_and_biogenesis_GO:0007010	148	43	79	6	6	4
complement_activation_GO:0006956	40	7	17	2	16	5
cytoskeletal_protein_binding_GO:0008092	28	17	16	7	14	6
intermediate_filament_GO:0005882	431	431	425	17	5	7
chromatin_assembly/disassembly_GO:0006333	53	96	62	8	15	8
TM_receptor_protein_TYR_kinase_docking_protein_activity_GO:0005069	393	152	242	23	2	9
establishment_and/or_maintenance_of_chromatin_architecture_GO:0006325	239	104	143	16	13	10
nuclear_organization_and_biogenesis_GO:0006997	369	126	212	21	10	11
tubulin_binding_GO:0015631	79	40	46	18	20	12
protein_tyrosine_kinase_activity_GO:0004713	14	3	4	29	12	13
chromatin_GO:0005717	128	65	82	39	3	14
perception_of_light_GO:0009583	102	321	184	20	22	15
DNA_packaging_GO:0006323	231	106	139	26	18	16
microtubule_binding_GO:0008017	196	117	127	22	28	17
chromosome_organization_and_biogenesis_(sensu_Eukarya)_GO:0007001	364	137	215	34	23	18
cellular_morphogenesis_GO:0000902	64	280	144	3	56	19
TM_receptor_protein_TYR_kinase_signaling_protein_activity_GO:0005066	429	221	303	49	11	20
SNAP_receptor_activity_GO:0005484	97	148	100	15	47	21
response_to_light_GO:0009416	110	349	204	27	37	22
enzyme_inhibitor_activity_GO:0004857	479	199	319	45	21	23
receptor_signaling_protein_activity_GO:0005057	177	151	133	10	58	24
microtubule_cytoskeleton_GO:0015630	222	180	173	52	26	25
Neuronal-enriched-top 25 age-affected GO classes						
voltage-gated_ion_channel_activity_GO:0005244	3	6	1	454	302	363
transmission_of_nerve_impulse_GO:0019226	2	9	2	194	438	284
synaptic_transmission_GO:0007268	1	11	3	271	389	298
protein_tyrosine_kinase_activity_GO:0004713	14	3	4	29	12	13
metal_ion_transport_GO:0030001	4	19	5	643	508	616
calmodulin_binding_GO:0005516	13	13	6	206	522	341
G-protein_signaling,_coupled_to_cyclic_nucleotide_2nd_messenger_GO:0007187	9	20	7	408	594	522
TM_receptor_protein_TYR_phosphatase_signaling_pathway_GO:0007185	22	8	8	59	87	47
auxiliary_transport_protein_activity_GO:0015457	32	2	9	69	129	71
channel_regulator_activity_GO:0016247	31	4	10	62	134	68
diacylglycerol_binding_GO:0019992	26	10	11	107	8	35
second-messenger-mediated_signaling_GO:0019932	7	30	12	382	598	505
calcium_ion_transport_GO:0006816	16	23	13	318	346	303
G-protein_signaling,_coupled_to_cAMP_nucleotide_2nd_messenger_GO:0007188	23	16	14	198	458	295
phosphoric_ester_hydrolase_activity_GO:0042578	8	34	15	51	485	233
cytoskeletal_protein_binding_GO:0008092	28	17	16	7	14	6
complement_activation_GO:0006956	40	7	17	2	16	5
di-,_tri-valent_inorganic_cation_transport_GO:0015674	11	38	18	230	385	278
regulation_of_cell_proliferation_GO:0042127	29	21	19	37	107	46
cation_channel_activity_GO:0005261	6	44	20	690	505	647
positive_regulation_of_cell_proliferation_GO:0008284	50	1	21	585	248	407
receptor_binding_GO:0005102	46	5	22	315	586	458
transmembrane_receptor_protein_tyrosine_phosphatase_activity_GO:0005001	38	15	23	48	48	29
neuropeptide_signaling_pathway_GO:0007218	5	52	24	544	758	696
ion_channel_activity_GO:0005216	12	47	25	732	536	683

Figure 4. Glial- and neuronal-enriched functional analysis of aging. Top 25 ranked Gene Ontology (GO) families most affected during aging (dark gray). Analyses were highly similar between Brodmann areas (BA) 9 and 47 (top 25 in light gray). For the combined effect in BA9 and BA47, GO families were ranked based on the averaged results in both areas. Blue, synaptic and/or receptor activity, signal transduction. Red, cellular defenses. Green, microtubule, filaments. Black, chromatin structure and organization. Two GO families relating to the "perception of light" (GO 0009583 and 0009416) were represented in the top glial-related GO groups, mostly because of the presence within these GO groups of genes with roles in light perception, but also in more general structural and signaling functions.

affected gene groups related to synaptic and signal transduction (Figure 4, lower panel), including ion channel, transmembrane receptor, calcium regulation, synaptic structure, and signal transduction. In contrast, altered glial-related gene groups (Figure 4, upper panel) mostly concerned cellular defenses (immune system, complement activation), structural filaments (microtubule function and cytoskeleton), chromatin organization, and receptor signaling and transduction. Selected genes are listed in Figure 5. In agreement with the correlation between the effect of age and the WM/GM ratio previously described, most genes displayed age-related changes in opposite directions for glial- and neuronal-enriched transcripts. Neuronal-enriched transcripts for synaptic transmission, signaling and transduction genes were overwhelmingly downregulated with age (e.g., CALB1), whereas glial-enriched transcripts affected by age were mostly increased (i.e., GFAP). A subgroup of neuronal-enriched genes displayed increased expression with age. Functions supported by these genes include oxidative stress (MAOB, GPX3) and putative reactive processes against age-associated damage to macromolecules, such as modulation of immune response (SERPING1, STAT4), protein, and DNA repair (Heatshock protein [CLGN, MDN1], DNA polymerase [POLD3]), and lipid and protein degradation (LPL, USP20, PRODH, CTSD).

Discussion

Aging, a Continuous Process Throughout Adult Life

Using gene expression profiling in two areas of the prefrontal cortex in a large cohort of human subjects, a "molecular profile" of aging was identified, consisting of progressive changes in expression of a large number of genes (at least 540, and possibly as many as 1662, or 7.5%, of transcripts tested when using the "leave-one out" approach). Although longitudinal studies of molecular changes are not possible in postmortem brain samples, this cross-sectional study in subjects covering a 66-year age range (13–79 years) suggests a continuous process of aging in the brain as reflected by changes in gene transcriptome during adult life. Adolescent samples fit the curve describing the age-related progression of transcriptional changes across subjects, and their inclusion increased the analytical power of the study (data not shown), as would be expected by increasing the number of samples with similar overall effects. These observations suggest that some of the gene expression changes occurring during late-maturation processes in adolescence may be continuous with aging processes throughout adult life. We have previously shown that in our cohort of subjects, and under our experimental conditions, most demographic, clinical, and experimental parameters do not correlate with RNA quality and transcript levels (Galfalvy et al 2003; Sibille et al 2004). For example, gender affects few genes, postmortem delay was short in our sample and has no detectable effect, and the presence of a psychiatric diagnosis of a mood disorder or dying by suicide was not associated with altered patterns of expression. Although we cannot exclude the possibility of undetected confounding effects, the robustness of age-related transcriptional changes is highlighted by the large number of genes being similarly affected across two PFC areas in a heterogeneous group of human subjects. Furthermore, transcript levels for age-related genes accurately predicted chronologic age of subjects.

The continuous aspect of age-related transcriptional changes throughout adult life is surprising in light of the commonly held belief that negative aspects of physical and cognitive aging may not appear until the later decades of life but are consistent with studies describing early onset and constant rates of morphological changes (Resnick et al 2003) and subtle cognitive decline (Hedden and Gabrieli 2004; Park et al 2002) across the life span. A recent gene expression profiling study (Lu et al 2004) focused on the role of oxidative stress and DNA damage and suggested that gene expression changes initiate after age 40. Here, by investigating two brain regions and by identifying the cellular origin of changes, we show that age-related changes in transcript levels differentially affect neurons and glia and are initiated as early as during adolescence. Our results are also consistent with gene expression profiling studies of the aging hippocampus in rodents, describing progressive changes in transcript levels between young, middle-aged, and old animals (Blalock et al 2003; Verbitsky et al 2004).

Our transcriptome analysis of the aging human PFC confirms results from numerous prior studies on individual gene expression (Weindruch and Prolla 2002) but also offers an unbiased survey of cellular mechanisms during aging from the vantage point of the transcriptome. Altered gene expression was confirmed by real-time PCR for 14 of 14 genes selected, further supporting the microarray results reported here. Currently, large-scale protein surveys are more limited in the number of peptides identified and thus do not offer overviews of cellular functions as RNA surveys do. Nonetheless, although RNA levels may not correlate with protein levels in all cases, numerous studies corroborate our age-related RNA results at the protein or function levels, including, among others, astrocyte markers (GFAP [Finch 2002; Porchet et al 2003], S100B [Kato et al 1990; Katoh-Semba and Kato 1994]), neurotransmitter receptors (HTR2A [Arango et al 1990; Meltzer et al 1998; Sheline et al 2002], ADRA2A [Sastre and Garcia-Sevilla 1993, 1994]), Ca2+-binding proteins (CALB1/2 [Bu et al 2003]), enzymes (MAOB [Sastre and Garcia-Sevilla 1993]), CA4/10 [Sun and Alkon 2002]), and trophic factors (IGF1 [reviewed in Ghigo et al 1996], CLU [Trougakos and Gonos 2002], NTRK3 [Torres et al 1995]; see Figure 5 for gene descriptions).

Glial-Mediated Inflammation, Neuronal Downregulation, and Putative Resistance to Cell Death in the Aging Brain

Assessing the relative contribution of neurons and glia to age-related gene expression indicated an upregulation of glial-enriched transcripts and a downregulation of neuronal-enriched transcripts. Because age-related genes represented only a minority of the most enriched glial and neuronal genes, our results suggest a specificity of gene expression changes with age, as opposed to a widespread change in glia/neuron transcript ratio. This specificity could reflect changes restricted to particular cellular functions or to distinct cellular populations (or both). For instance, although we examined transcript and not protein levels, our results are consistent with reports of increase in

Figure 5. Gene expression changes in the aging human prefrontal cortex (selected genes). Of the 540 genes, 193 were significantly affected by age (see Supplements 3 and 4 for complete lists of genes). Although not identified in the top 25 GO groups, age-affected genes involved in several aspects of cellular metabolism were included. "Age" indicates the fold change between older ($\geq$ 60) and younger ($\leq$ 30) age groups (red, increased expression; green, decreased expression with age). "WM/GM" indicates signal intensity fold changes between white matter and gray matter for each gene: dark gray, neuronal-enriched expression (WM/GM fold change $<$ –1.5); light gray, neuronal and glial mixed expression (–1.5 $<$ WM/GM fold change $<$ 1.5); white, glial-enriched expression (WM/GM fold change $>$ 1.5).

Gene	Name	Age	WM / GM
Cellular defenses			
	Apoptosis		
BAD	BCL2-antagonist of cell death	-1.22	-1.54
PIG11	p53-induced protein	-1.05	-4.89
CLU	clusterin	1.30	-1.37
BCL2	B-cell CLL/lymphoma 2	1.40	-1.16
DFFB	DNA fragmentation factor, 40 kD, beta polypeptide	1.15	1.59
	Immune System - Complement system - Inflammation		
CX3CL1	small inducible cytokine subfamily D , member 1	-1.34	-5.73
MMD	monocyte to macrophage differentiation-associated	-1.32	-2.87
IL12RB2	interleukin 12 receptor, beta 2	-1.15	-3.58
SERPING1	serine (or cysteine) proteinase inhibitor, clade G , member 1	1.23	-2.29
STAT4	signal transducer and activator of transcription 4	1.36	-6.84
CXCL12	chemokine (C-X-C motif) ligand 12	-1.32	-1.11
IRF3	interferon regulatory factor 3	1.11	-1.21
STAT1	signal transducer and activator of transcription 1, 91kD	1.38	-1.02
AQP1	aquaporin 1	2.00	6.37
C4B	complement component 4B	1.61	3.40
C4A	complement component 4A	1.40	3.51
C1QA	complement component 1, q subcomponent, alpha polypeptide	-1.34	1.61
C3	complement component 3	-1.60	3.23
	Heat-shock protein - Chaperone		
CLGN	calmegin	1.32	-3.21
MDN1	Midasin homolog	1.20	1.36
	Oxidative stress		
MAOB	monoamine oxidase B	1.28	-1.97
GPX3	glutathione peroxidase 3	1.22	-6.61
NDUFB8	NADH dehydrogenase 1 beta subcomplex, 8	1.20	1.23
Structure - Microtubule regulation - Filaments			
COL5A2	collagen, type V, alpha 2	-1.42	-14.16
CDH8	cadherin 8, type 2	-1.39	-2.78
JAM2	junctional adhesion molecule 2 (integrin alpha 4 Beta1 ligand)	-1.36	-1.59
TUBB	tubulin, beta polypeptide	-1.32	-4.99
NPTXR	dynein, axonemal, light polypeptide 4	-1.28	-16.23
LAMB1	laminin, beta 1	-1.25	-33.4
MYO1B	myosin IB	-1.24	-4.07
BAI2	brain-specific angiogenesis inhibitor 2	-1.22	-4.75
SPTBN1	spectrin, beta, non-erythrocytic 1	-1.19	-1.91
CSPG5	chondroitin sulfate proteoglycan 5	-1.18	-2.23
TUBB4	tubulin, beta, 4	-1.13	-2.92
HD	huntingtin	-1.10	-1.80
ANK1	ankyrin 1, erythrocytic	1.11	-3.19
TNNT2	troponin T2	1.12	-5.06
CDH11	cadherin 11, type 2, OB-cadherin	-1.17	-1.49
MAPRE2	microtubule-associated protein, RP/EB family, member 2	-1.15	1.15
FN1	fibronectin 1	-1.12	-1.35
CHI3L1	chitinase 3-like 1	1.47	1.10
GFAP	glial fibrillary acidic protein	1.82	1.91
KIF5B	kinesin family member 5B	1.34	2.68
ITGB4	integrin, beta 4	1.34	1.56
ANXA4	annexin A4	1.32	2.43
TNS	Tensin	1.29	3.12
KIF13B	kinesin family member 13B	1.27	5.41
MAP4	microtubule-associated protein 4	1.25	3.25
MAP7	microtubule-associated protein 7	1.20	4.06
KTN1	kinectin 1 (kinesin receptor)	1.18	2.49
EML2	microtubule-associated protein like echinoderm EMAP	1.08	2.04
Chromatin structure - Transcription regulation			
MAPK4	mitogen-activated protein kinase 4	-1.29	-2.74
NFKBIE	NF kappa light polypeptide gene enhancer in B-cells inhibitor	-1.14	-1.59
HDAC9	histone deacetylase 9	-1.15	-1.17
POLD3	polymerase (DNA directed), delta 3	1.07	1.16
NAP1L1	nucleosome assembly protein 1-like 1	1.25	1.35
H2BFT	H2B histone family, member T	-1.26	1.90
H2BFA	H2B histone family, member A	-1.15	3.28
MXI1	MAX interacting protein 1	1.21	1.92
TCFL5	transcription factor-like 5	1.39	2.56
Metabolism			
	Energy Metabolism		
CA4	carbonic anhydrase IV	-1.54	-3.98
CA10	Carbonic anhydrase-related protein 10	-1.42	-6.04
ATP6V0B	ATPase, H+ transporting, lysosomal	-1.24	-1.16
GAA	glucosidase, alpha; acid	1.08	1.49
SLC6A8	solute carrier family 6 (neurotransmitter, creatine), member 8	1.21	2.40
	Lipid metabolism		
HMGCS1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	-1.61	-2.69
LPL	lipoprotein lipase	1.52	-1.94
LPIN1	lipin 1	1.25	1.26
	Protein metabolism		
B3GNT1	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 1	-1.50	-2.10
UST	uronyl 2-sulfotransferase	-1.42	-1.82
MGAT4B	mannosyl glycoprotein acetylglucosaminyltransferase B	-1.26	-2.01
CTSB	cathepsin B	-1.20	-2.01
SIAT8C	sialyltransferase 8C	-1.19	-11.16
B4GALT2	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase 2	-1.17	-1.61
USP20	ubiquitin specific protease 20	1.07	-2.35
NMT1	N-myristoyltransferase 1	1.13	-2.66
PPARGC1	peroxisome proliferative activated receptor, gamma, coactivator 1	1.19	-3.83
PRODH	proline oxidase homolog	1.26	-1.63
CTSD	cathepsin D	1.05	1.14
RPS27L	40S ribosomal protein S27 isoform	1.40	1.84
RPS6KA5	ribosomal protein S6 kinase, 90kD, polypeptide 5	1.24	3.14
Cell Signaling - Transduction			
	Cell cycle - Growth factors		
CCNG2	cyclin G2	-1.33	-1.70
CCND2	cyclin D2	-1.30	-2.90
EGR4	early growth response 4	-1.27	-3.51
CDK5	cyclin-dependent kinase 5	-1.21	-5.47
NTRK3	neurotrophic tyrosine kinase, receptor, type 3	-1.19	-16.06
CENTG1	cyclin-dependent kinase 4	-1.19	-7.37
IGF1	insulin-like growth factor 1	-1.17	-1.79
VEGFB	vascular endothelial growth factor B	1.16	-1.67
FRZB	frizzled-related protein	-1.26	-1.39
TGFBI	transforming growth factor, beta-induced, 68kD	-1.17	-1.29
FGFR1	fibroblast growth factor receptor 1	1.25	-1.05

Gene	Name	Age	WM / GM
Cell Signaling - Transduction			
	Cell cycle - Growth factors		
FGF1	fibroblast growth factor 1 (acidic)	1.44	5.60
GDF1	growth differentiation factor 1	1.20	2.14
TGFB3	transforming growth factor, beta 3	1.20	3.06
LTBP3	latent transforming growth factor beta binding protein 3	1.14	1.70
	Calcium homeostasis - Regulation		
CALB1	calbindin 1, (28kD)	-2.34	-19.75
ATP2B2	ATPase, Ca++ transporting, plasma membrane 2	-1.71	-100.7
CPNE6	copine VI	-1.47	-6.65
HPCA	hippocalcin	-1.38	-27.7
CAMK4	calcium/calmodulin-dependent protein kinase IV	-1.34	-3.98
CAMK1	calcium/calmodulin-dependent protein kinase I	-1.31	-5.68
SLC24A3	solute carrier family 24 (Na/K/Ca exchanger), member 3	-1.31	-3.83
CACNB2	calcium channel, voltage-dependent, beta 2 subunit	-1.29	-7.49
CACNA1G	calcium channel, voltage-dependent, alpha 1G subunit	-1.27	-6.50
CACNG3	calcium channel, voltage-dependent, gamma subunit 3	-1.24	-27.8
CACNB3	calcium channel, voltage-dependent, beta 3 subunit	-1.23	-11.72
SLC8A2	solute carrier family 8 (sodium-calcium exchanger), member 2	-1.11	-6.83
CAMK2G	calcium/calmodulin-dependent protein kinase II gamma	1.12	-4.40
S100A6	S100 calcium binding protein A6	1.14	-2.11
	Channel		
GABRA5	Gamma-aminobutyric acid A receptor, alpha 5	-1.79	-17.02
KCNV1	neuronal potassium channel alpha subunit	-1.54	-40.7
KCNK3	potassium channel, subfamily K, member 3	-1.39	-11.55
SCN3B	voltage-gated sodium channel beta-3 subunit	-1.39	-11.28
KCNG1	potassium voltage-gated channel, subfamily G, member 1	-1.36	-8.21
MGC21688	chloride channel 2	-1.35	-3.85
GABRA4	Gamma-aminobutyric acid A receptor, alpha 4	-1.29	-4.24
KCNH1	potassium voltage-gated channel, subfamily H, member 1	-1.22	-7.56
KCNF1	potassium voltage-gated channel, subfamily F, member 1	-1.22	-5.63
KCNQ2	potassium voltage-gated channel, KQT-like subfamily, member 2	-1.15	-1.98
KCNS1	potassium voltage-gated channel, delayed-rectifier, S, 1	1.21	-5.66
	GPCR		
HTR2A	serotonin 2A receptor	-1.55	-9.84
ADRA1D	adrenergic, alpha-1D-, receptor	-1.46	-4.26
ADRA2A	adrenergic, alpha-2A-, receptor	-1.39	-3.39
GRM2	glutamate receptor, metabotropic 2	-1.31	-4.74
HTR1E	serotonin 1E receptor	1.17	-3.26
EDG2	endothelial differentiation, lysophosphatidic acid GPCR, 2	1.58	6.82
GRINL1A	glutamate receptor, ionotropic, N-methyl D-aspartate-like 1A	1.24	3.71
GRM3	glutamate receptor, metabotropic 3	-1.21	3.01
GPR17	G protein-coupled receptor 17	-1.45	4.18
	GPCR - Neuropeptide		
PENK	proenkephalin	-1.92	-2.83
SST	somatostatin	-1.50	-3.32
CRH	corticotropin releasing hormone	-1.50	-16.68
NMU	neuromedin U	-1.50	-21.96
SSTR1	somatostatin receptor 1	-1.41	-1.71
TAC3	tachykinin 3 (neuromedin K, neurokinin beta)	-1.32	-3.05
PNOC	prepronociceptin	-1.30	-4.80
EDN3	endothelin 3	-1.24	-1.69
PTHLH	parathyroid hormone-like hormone	-1.23	-7.69
CRHR1	corticotropin releasing hormone receptor 1	-1.22	-7.13
GRP	gastrin-releasing peptide	-1.22	-3.33
NPPA	natriuretic peptide precursor A	-1.16	-2.20
PDYN	prodynorphin	-1.16	-8.95
PPP2R5A	protein phosphatase 2, regulatory subunit B (B56), alpha isoform	-1.12	1.35
AGTRL1	angiotensin receptor-like 1	1.23	5.71
	Kinase - Phosphatase		
ITPKA	inositol 1,4,5-trisphosphate 3-kinase A	-1.40	-23.81
AKAP5	A kinase anchor protein 5	-1.34	-29.6
PRKCD	protein kinase C, delta	-1.32	-2.75
PAK6	p21(CDKN1A)-activated kinase 6	-1.27	-13.47
PAK7	p21(CDKN1A)-activated kinase 7	-1.26	-7.42
PTPRR	protein tyrosine phosphatase, receptor type, R	-1.25	-6.53
PCTK1	PCTAIRE protein kinase 1	-1.23	-2.62
PRKCB1	protein kinase C, beta 1	-1.21	-5.22
PTPRT	protein tyrosine phosphatase, receptor type, T	1.21	-6.64
PTK2B	protein tyrosine kinase 2 beta	1.32	-6.27
PTPRA	protein tyrosine phosphatase, receptor type, A	-1.44	1.09
DYRK2	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	-1.20	1.15
PTPRF	protein tyrosine phosphatase, receptor type, F	-1.19	-1.04
PPM1A	protein phosphatase 1A, magnesium-dependent, alpha isoform	1.12	-1.24
AKAP1	A kinase (PRKA) anchor protein 1	1.18	1.03
DGKG	diacylglycerol kinase, gamma (90kD)	1.20	-1.37
PPP3CB	protein phosphatase 3, catalytic subunit, beta isoform	1.22	2.90
PCTK3	PCTAIRE protein kinase 3	1.24	4.55
ITPKB	inositol 1,4,5-trisphosphate 3-kinase B	1.58	3.43
	Misc. Signal transduction - Synaptic		
ARPP-21	cyclic AMP-regulated phosphoprotein, 21 kD	-1.46	-27.8
ARPP-19	cyclic AMP phosphoprotein, 19 kD	-1.42	-2.52
YKT6	SNARE protein	-1.41	-1.88
RGS12	regulator of G-protein signalling 12	-1.38	-2.14
ABI-2	abl-interactor 12	-1.35	-2.07
GNAL	G protein, alpha activating activity polypeptide, olfactory type	-1.33	-49.4
RASGRP1	RAS guanyl releasing protein 1 (calcium and DAG-regulated)	-1.31	-7.24
ADCY2	adenylate cyclase 2	-1.30	-6.74
STX1A	syntaxin 1A	-1.24	-7.94
PDE2A	phosphodiesterase 2A, cGMP-stimulated	-1.19	-5.39
RGS6	regulator of G-protein signalling 6	-1.17	-16.95
PLCB1	phospholipase C, beta 1 (phosphoinositide-specific)	-1.14	-8.07
MAPK11	mitogen-activated protein kinase 11	-1.14	-15.44
CALCYON	calcyon; D1 dopamine receptor-interacting protein	1.17	-10.99
PDE4A	phosphodiesterase 4A, cAMP-specific B122	1.18	-5.72
GNAS	GNAS complex locus	1.21	-3.37
GNA14	G protein, alpha 14	1.24	-2.00
VAMP1	vesicle-associated membrane protein 1	1.52	-2.06
SNAP29	synaptosomal-associated protein, 29kD	-1.18	-1.12
PDE7B	phosphodiesterase 7B	-1.15	-1.43
RGS10	regulator of G-protein signalling 10	-1.45	2.07
PDE8A	phosphodiesterase 8A	1.38	9.44
ERBB2IP	erbb2 interacting protein	1.49	3.90

number and reactivity of astrocytes during aging (i.e., increased GFAP expression) and overall confirm the apparently universal occurrence of inflammatory processes during aging (reviewed in DeVellis 2002). Elevated levels of the C4A/B components of the circulating complement system suggest an increasing presence of pro-inflammatory agents in the aging brain (Pasinetti et al 1999), whereas increased RNA levels for transcription factors and elements of signal transduction cascades (IRF3, STAT1 and 4) suggest increased cytokines and pro-inflammatory signaling. We also observed expression changes for selected genes with functions suggesting a possible homeostatic response, blunting inflammatory cascades. Such evidence included decreased transcript levels coding for cytokines (CX3CL1/12), cytokine receptor (IL12RB2), and elements of the complement system (C1QA, C3). Because microglial and oligodendrocyte markers were unaffected or slightly downregulated (MMD, CSF1R) with age, the upregulation of numerous glial-enriched genes involved in cytoskeleton, microtubule-related functions, cellular processes, and anterograde transport (Figures 4 and 5) could support the increased number and reactivity of astrocytes during aging.

Genes associated with the structure, morphology, and function of neurons represented the largest proportion of all age-affected genes (35.5% of genes with known function) and were mostly downregulated with age (Figure 4). For instance, decreased neuronal-enriched transcripts coding for cellular structure included among others, components of the cytoskeleton and microtubules (TUBB, TUBB4, MYO1B), cell–cell interaction (CDH8, CDH11), junctional adhesion molecule (JAM2), and cell–matrix interaction (COL5A2, LAMB1). Decreased CALB1 transcript has been reported as a marker of vulnerable neuronal populations in the basal forebrain, hippocampus, and cortex during aging (Bu et al 2003; Geula et al 2003; Iacopino and Christakos 1990; and confirmed here). Thus, the identification of a group of selectively downregulated neuronal-enriched genes may reflect changes in cellular programs within CALB1-positive cortical neurons, or alternatively within other not-yet-identified neuronal populations. The large number of putative age-related cellular markers that we report here will facilitate the identification and characterization of such selectively vulnerable neuronal populations in future studies.

Transcriptional downregulation with age was prominent for several voltage-gated ion channels and G-protein coupled receptors (GPCRs) systems, confirming previous reports, but also providing numerous novel findings. Significant decreases in transcript levels were observed for serotonin (HTR2A [Arango et al 1990; Meltzer et al 1998; Sheline et al 2002]), adrenergic (ADRA1D/2A [Bigham and Lidow 1995]), and metabotropic glutamate (GRM2) receptors. Transcript levels for numerous GPCR-peptide systems were also negatively affected by age, including pain-related functions (PNOC, PDYN, and PENK), the corticotropin system (CRH, CRH1), and more general hormonal functions (GRP, NMU, PTHLH, TAC3, SST, and SSTR1). This extensive downregulation of genes coding for neuromodulatory peptides is unexpected and may reflect a selective vulnerability of neuropeptide systems to dysregulated protein metabolism in the aging brain, although protein levels and functions were not assessed here. Downregulated transcript levels coding for ionotropic channels included subtypes of GABA-A receptors (GABRA4 and 5) and numerous voltage-gated Ca2+ (CACNB2/3, CACNG3, CACNB3, CACNA1G), Na+ (SCN3B), and K+ (KCNV1/G1/H1/F1/Q2/S1) channels.

Increased L-type Ca2+ channel activity has been reported in the aging brain (Thibault et al 1998, 2001) and has been hypothesized as a primary deficit in aging causing physiologic impairments and cognitive decline. Here, we observed mostly downregulation of neuronal-enriched transcripts coding for proteins involved in Ca2+ buffering (CALB1), voltage-gated Ca2+ channels of the L- (CACNA1G, CACNB2/3) and T-types (CACNG3), Ca2+ transport (ATP2B2), exchange (SLC8A2, SLC24A3), and Ca2+-mediated functions (CAMK1/4, CPNE6, CADH8, HPCA). Changes in transcript levels during aging may represent compensatory mechanisms for altered protein levels or functions, which were not assessed here. For instance, the observed downregulation of numerous genes involved in Ca2+ regulation may reflect compensatory mechanisms for increased L-type Ca2+ channel activity during aging because of increased protein phosphorylation (Davare and Hell 2003).

This extensive downregulation of a large number of neuronal genes may be, at least in part, responsive to increased glial activity, including changes in trophic support. Indeed, this notion of selective glial and neuronal reorganization during aging is supported by altered transcript levels coding for growth factors and associated signaling pathways. Several glial-enriched pleiotropic growth factors and related receptors were upregulated with age (FGF1, FGF1R, TGFB3, GDF1) whereas neuronal-enriched transcript levels for other growth factors and cell growth mediators were decreased (IGF1 [Carter et al 2002], NTRK3, EGR4, CDK5, CCNG2, CCND2). Brain-derived neurotrophic factor (BDNF) transcript levels also decreased with age, although it was statistically significant only in BA9, using the "leave-one sample out" approach (Supplements 3 and 4). GO families relating to protein tyrosine kinase (TYR) activity were in the top 25 glial- and neuronal-enriched gene classes (Figure 4) included elements of transduction pathways associated with trophic factor signaling such as transcripts coding for SH2/SH3 adaptor proteins (GAB2, ITSN2, APS, ABI-2) and numerous downstream protein kinases (PRKCD, PRKCB1, NEK9, NEK2, PCTK1, PCTK3, CIT, LYN, DYRK2). Together, our results suggest a complex reorganization of trophic support provided to neurons and glia in the aging brain.

Finally, increased transcript levels of anti-apoptotic genes (BCL2 and CLU) and downregulation of pro-apoptotic genes (BAD) suggest an increased resistance to cell death. These observations are reminiscent of cell culture studies of peripheral mitotic tissues and of primary neural tissue in which replicative and cellular senescence is associated with resistance to apoptosis (Romero et al 2003; Wang 1997). Resistance to cell death would account for the modest neuronal loss reported by stereological cell counts during the course of normal aging (Morrison and Hof 1997; Pakkenberg and Gundersen 1997).

Gene Expression Changes Corresponding to Oxidative Stress and Macromolecule Turnover

A contributory mechanism to cell injury associated with aging includes oxidative damage to DNA, lipids, and proteins. At the transcript level, our data provided only limited evidence for increased free radical production through upregulation of two neuronal-enriched genes located in the mitochondrion: NADH dehydrogenase (NDUFB8), and monoamine oxidase B (MAOB) (Mason et al 2000; Shemyakov 2001). Indirect evidence for increased oxidative stress was restricted to a putative increase in free radical detoxification through elevated glutathione peroxidase 3 (GPX3) transcripts. Furthermore, increase in transcripts associated with repair or replacement of compromised macromolecules was limited here to increased transcript levels of two chaperones (MDN1 and CLGN). Expression levels of numerous

other chaperone proteins were either marginally altered or unchanged. Some of the effects of aging on heat-shock proteins may have been masked by induction of these same proteins during the early phase of postmortem delay, although no systematic correlation with postmortem interval was observed in our samples (Galfalvy et al 2003).

Evidence for age-related altered protein, lipid, and DNA repair or turnover was mixed. Elevated transcript levels coding for lipoprotein lipase (LPL) and lipin 1 (LPIN1) suggested increased lipid turnover, possibly linked to elevated lipid peroxidation through enhanced MAOB activity (Shemyakov 2001). Several transcripts coding for protein glycosylation enzymes were downregulated with age (B3GNT1, UST, MGAT4B, SIAT8C, B4GALT2), whereas evidence suggesting changes in neuronal protein turnover was sparse and mixed (increased PRODH, CTSD; decreased USP20, CTSB). These results differ from rodent studies of the aging brain, in which robust increases in transcripts coding for protein degradation enzymes are consistently reported (Blalock et al 2003; Jiang et al 2001; Lee et al 2000). Discrepancies extend to other genes investigated (e.g., C1Q, increased in rodent [Lee et al 2000], decreased in human) and may originate in differences in experimental design but may also reflect species variations in neuronal processes and their susceptibility to aging. For instance, protein turnover may be regulated at the mRNA level in rodents, whereas posttranslational activities may mostly regulate the function of proteolytic enzymes in humans. Future studies will require thorough rodent–human comparisons at the RNA and protein levels to further characterize these putative differences.

In summary, using large-scale gene expression profiling in the human PFC as a guide to cellular activity and functional status, our results suggest an extensive yet selective reorganization of glial and neuronal functions during aging, for which we provide here putative molecular markers. BA9 and BA47 presented very similar age-related "molecular profiles," and our data did not support the claim of a region specificity of age effect. Extrapolating from our partial survey of the genome (~50% genes investigated and ~25%–30% detected), the transcriptional correlates of aging appear to implicate less than 10% of all genes in the brain. This observation is tempered by the fact that mRNA differences restricted to small number of cells may be considerably diluted and indistinguishable in the overall RNA pool extracted from whole brain areas. Furthermore, changes in protein levels, modifications, or functions were not assessed here. Nevertheless, despite continuous cognitive (Hedden and Gabrieli 2004; Park et al 2002) and structural (Resnick et al 2003; Sowell et al 2003) declines throughout life, our results support the notion that aging correlates with specific molecular changes in the brain, as opposed to widespread and nonspecific alterations. Whereas increased inflammation appears to be a universal feature of aging across tissues and organisms, an important unresolved question concerns the origins of the selective vulnerability that is suggested here for specific neuronal functions or putative neuronal subpopulations. Because age-related gene expression changes begin early in adulthood and are continuous throughout adult life, our results suggest the possibility of early identification of mechanisms that are either preventive or detrimental to age-related brain functions.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, atdoi:10.1016/j.biopsych.2004.10.034.