Coordinated Gene Expression of Neuroinflammatory and Cell Signaling Markers in Dorsolateral Prefrontal Cortex during Human Brain Development and Aging

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Abstract

Background: Age changes in expression of inflammatory, synaptic, and neurotrophic genes are not well characterized during human brain development and senescence. Knowing these changes may elucidate structural, metabolic, and functional brain processes over the lifespan, as well as vulnerability to neurodevelopmental or neurodegenerative diseases.

Hypothesis: Expression levels of inflammatory, synaptic, and neurotrophic genes in the human brain are coordinated over the lifespan and underlie changes in phenotypic networks or cascades.

Methods: We used a large-scale microarray dataset from human prefrontal cortex, BrainCloud, to quantify age changes over the lifespan, divided into Development (0 to 21 years, 87 brains) and Aging (22 to 78 years, 144 brains) intervals, in transcription levels of 39 genes.

Results: Gene expression levels followed different trajectories over the lifespan. Many changes were intercorrelated within three similar groups or clusters of genes during both Development and Aging, despite different roles of the gene products in the two intervals. During Development, changes were related to reported neuronal loss, dendritic growth and pruning, and microglial events; TLR4, IL1R1, NFKB1, MOBP, PLA2G4A, and PTGDS2 expression increased in the first years of life, while expression of synaptic genes GAP43 and DBN1 decreased, before reaching plateaus. During Aging, expression was upregulated for potentially pro-inflammatory genes such as NFKB1, TRAF6, TLR4, IL1R1, TSPO, and GFAP, but downregulated for neurotrophic and synaptic integrity genes such as BDNF, NGF, PDGFA, SYN, and DBN1.

Conclusions: Coordinated changes in gene transcription cascades underlie changes in synaptic, neurotrophic, and inflammatory phenotypic networks during brain Development and Aging. Early postnatal expression changes relate to neuronal, glial, and myelin growth and synaptic pruning events, while late Aging is associated with pro-inflammatory and synaptic loss changes. Thus, comparable transcriptional regulatory networks that operate throughout the lifespan underlie different phenotypic processes during Aging compared to Development.

Introduction

The human brain changes structurally, functionally, and metabolically throughout the lifespan [1,2]. Programmed dendritic growth followed by pruning, neuronal loss, shifts in energy metabolism from ketone body to glucose consumption, and rapid myelination occur during development [3,4,5,6]. Many of these changes are completed within the first two decades of life, although myelination can continue into the fourth decade [5,7,8]. In middle age, the brain reaches a level of homeostatic stability, but neuronal and synaptic loss associated with cognitive changes can appear later on [2,9,10,11,12]. Aging also is a risk factor for progressive brain disorders in which neuroinflammation plays a prominent role [13,14,15,16,17]. Neuroinflammation involves activation of resident brain microglia and astrocytes, and can be produced by different internal or external stresses [14,18,19,20]. Microglial activation via toll-like receptors (TLRs) or cluster of differentiation (CD)14 receptors releases cytokines such as interleukin (IL)-1β, IL-6, tumor necrosis factor-α (TNFα) and interferon gamma (IFNγ),...
chemokines such as fractalkine (CX3CL1), and nitric oxide (NO), following activation of inducible nitric oxide synthase (iNOS), thereby creating response cascades that can negatively impact brain structure and function [21]. Downstream activation of IL-1 receptors (IL-1R) and TNFα receptors on astrocytes and other cell types alters levels of transcription factors such as nuclear factor-kappa B (NF-κB) and activator protein (AP)-2, which can increase expression of a number of inflammatory genes [21,22,23,24,25].

The term “inflamm-aging” has been proposed to describe the progressive increase in proinflammatory status in the brain with senescence [26]. Inflamm-aging may prime brain microglia and astrocytes to respond excessively to different stressors, including neurodegenerative, traumatic or infectious insults [27,28,29,30,31]. Increased inflammatory response markers with late-state brain aging have been documented in rodents [27,32], nonhuman primates [33], and humans [34]. Increases have been noted in proinflammatory cytokines IL-1α, IL-1β, IL-18 and IFNγ, major histocompatibility complex class II (MHC II), CD11b, scavenger receptors CD68, CD86 and CD40, and TLRs 1, 2, 4, 5, 7, and 9. In the human brain, increased TNFα and interferon gamma-inducible protein 16 (IFI16) were reported, as were increased mRNA and protein levels of CD11b, gliab fibrially associated protein (GFAP), IL-1β, iNOS, NF-kB p50, cytosolic phospholipase A2 (cPLA2) Type IVA and cyclooxygenase (COX)-2, while levels of brain derived neurotropic factor protein (BDNF) and synaptophysin (SYP) were reduced [34,35].

Cytokines, chemokines, growth and other microglial and astrocytic factors that change with age in the adult brain also have important regulatory actions during neurodevelopment [36,37]. For example, microglia participate postnatally in synaptic pruning and apoptosis, and produce nerve growth factor (NGF), BDNF, neurotrophin (NT)-3 and cytokines that influence neuronal path finding, synaptogenesis and experience dependent plasticity [38,39,40,41].

Multiple metabolic and protein networks have been described that underlie brain structure and function, and brain vulnerability to disease [42,43,44,45,46]. The extent to which these phenotypic networks or “cascades” are regulated at the transcriptional level, particularly during brain development, maturity, and aging are not well understood. To address this limitation, in the present study we analyzed age changes over the lifespan in brain mRNA levels of 39 genes whose protein products have been reported to be involved in neuroinflammation, synaptic integrity, neurotrophic effects, and related processes. As in our prior report on age expression of brain lipid metabolic markers [47], we employed the large-scale microarray dataset called BrainCloud, which contains genome-wide expression levels in frontal cortex from non-pathological individuals, in the fetal period to postnatal 78 years of age [48]. Similarly, and consistent with the literature, we considered gene expression in two distinct postnatal age intervals, Development (0 to 21 years) and Aging (22 to 78 years) [35,47,49,50], henceforth identified by capitalizations.

Based on prior studies, we hypothesized that expression of genes linked to neuroinflammation would increase with age in the Aging interval [29,34,31,32], while expression of genes coding for synaptic integrity and plasticity would decrease [9]. Furthermore, expression of these same genes would change during Development to reflect reported roles of their protein products in synaptic and neuronal growth, pruning, myelination, and other events in this period [1].

We also hypothesized that expression of genes coding for products that belong to common growth and neuroinflammatory cascades would be coordinately regulated during the lifespan, in relation to the specific phenotypic networks in which their proteins interacted. Coordinated or synchronized gene transcription underlying changes in phenotype networks has been demonstrated in cell culture, rodent brain, and artificial systems [53,54,55], and in the human brain in relation to age [47,48,56,57,58].
and Aging periods. Expression of CX3CR1 declined with age whereas CX3CL1 increased during both intervals, while expression of CX3CR1 decreased. Expression of GFAP declined with age whereas NGF increased significantly with age in the Development and Aging periods. As illustrated in Figure 5a, Aging clusters were: Cluster 1: MOBP, MAP2, APP, SNCA; Cluster 2: BACE1, TLR4, PTGS1, CX3CR1; Cluster 3: TSPO, TNFRSF1A, MYD88, IL1R1, NFKB1, AIF1, CD68, CASP1, TLR2. As illustrated in Figure 5b, Aging clusters were: Cluster 1: NOS2, CX3CL1, SYP, TRAP1, GAP43, BDNF, SNCA; Cluster 2: MAP2, APP, ILIRAP, PTGS1, CX3CR1, PLA2G4A; Cluster 3: GFAP, TSPO, TNFRSF1A, IL1R1, MYD88, CD68, AIF1, CASP1, TLR2.

Mean expression level differences between Aging and Developmental Periods
As summarized in Table 1, mean expression levels were significantly (adjusted p<0.05) lower during Aging than Development for CX3CR1, CX3CL1, PTGS2, BDNF, CASP1, CD68, AIF1, MYD88, NGF, PDGFA, DBN1, IL1B, and SYP, and significantly higher for GFAP, TSPO, MOBP, TRAF6, and NFKB1 (highlights show significance after correction for multiple comparisons).

Correlated group expression changes during Development and Aging
Pearson’s correlation matrices relating all combinations of the genes were visualized using unsupervised hierarchical clustering and heat maps within the Development (Figure 5a) and Aging (Figure 5b) intervals. Gene order based on hierarchical clustering are not conserved between Development and Aging heat maps, as they represent the highest probability of correctly clustering genes based on Pearson’s r correlation in the individual intervals. In Figures 5a and 5b, genes that are highly positively correlated within a cluster are highlighted in green; those that are negatively correlated in red.

Three different clusters of genes whose expression levels were highly intercorrelated were identified in both the Development and Aging periods. As illustrated in Figure 5a, Development clusters were: Cluster 1: GAP43, SYP, BDNF, ILIRAP, NOS2, MAPK14, MAP2, APP, SNCA; Cluster 2: BACE1, TRL4, PTGS1, CX3CR1; Cluster 3: TSPO, TNFRSF1A, MYD88, IL1R1, NFKB1, AIF1, CD68, CASP1, TLR2. As illustrated in Figure 5b, Aging clusters were: Cluster 1: NOS2, CX3CL1, SYP, TRAP1, GAP43, BDNF, SNCA; Cluster 2: MAP2, APP, ILIRAP, PTGS1, CX3CR1, PLA2G4A; Cluster 3: GFAP, TSPO, TNFRSF1A, IL1R1, MYD88, CD68, AIF1, CASP1, TLR2.

Figures S1a and S1b show pairwise correlation values between gene expression levels in the Development and Aging periods, respectively. Green coloring highlights statistical significance at p<0.0001. The global clusters identified in Figures 5a and 5b, and the highly significant pairwise correlations illustrated in Figures S1a and S1b, show plausible synchronization of gene transcription within clusters or networks throughout the lifespan. The most significant pairwise correlations taken from Figures S1a and S1b, at p<10^{-10} and r>=0.6, are given in Table 2.

Gene Expression and Chromosome Proximity
Four pairs of genes have the same band number on a chromosome: PLA2G4A and PTGS2 (1q25); IL1β and IL1RN (2q14); CX3CR1 and MOBP (3p21.3); and AIF1 and MAPK14 (6p21.3) (cf. Table S1). At p<0.001, expression levels of PLA2G4A and PTGS2 were correlated positively during both Development and Aging, while expression levels of IL1β and IL1RN were correlated positively during Development only. Expression levels of the other two gene pairs were not correlated significantly in either period.

Discussion
We used the BrainCloud database for human prefrontal cortex [48] to examine age variations in mRNA levels of 39 genes reported to be involved in pathways of neuroinflammation, cytokine signaling, arachidonic acid metabolism, neuronal and
myelin integrity, synaptic function, neurotrophic action, and related processes. We divided the postnatal lifespan into Development (0–21 years) and Aging (22 to 78 years) intervals, on the basis of reported distinct functional and structural brain changes in these periods [4,5,47,49,72].

Confirming this division, expression patterns and age correlations of many of the chosen genes frequently differed significantly between the two intervals. Genes with higher expression during Aging include TSPO, associated with microglial activation and cholesterol transport [73,74]; GFAP, associated with glial activation [71]; TRAF6, associated with TNFα signaling; MOBP, associated with myelin integrity; and NFKB1, coding for NF-kB, a major transcription factor of inflammatory genes involved in innate immunity [25]. More genes were expressed at lower levels...
Figure 2. Statistically significant correlations with age in Development and/or Aging intervals. Graphical identification of genes with statistically significant (p < 0.05) Pearson r correlations in expression level during Development (x axis) and Aging (y axis) intervals. Gene expressions negatively correlated with age during Development are to the left, while genes positively correlated are to the right of the vertical line. Genes that were negatively correlated with age in the Aging group are below the horizontal line, while genes positively correlated are above line. Development: n = 87; Aging: n = 144.

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Figure 3. Significant linear regressions of gene expression during both Development and Aging intervals (top), and Aging interval alone (bottom). Scatterplots illustrating log2 gene expression over age in years. An increase or decrease of 1 on the log2 scale (y-axis) represents a two-fold change in gene expression in the positive or negative direction, respectively. Each data point represents observation from one brain (Development: n = 87; Aging: n = 144). Gene name (p-value during Development, p-value during Aging) - CX3CR1 (p < 0.0001, p < 0.0001), NGF (p = 0.006, p = 0.002), MOBP (p < 0.0001, p = 0.02), NFkB1 (p = 0.01, p = 0.01), SNCA (p < 0.0001, p = 0.02). Genes significant in only Aging interval – GFAP (p < 0.0001), TSPO (p = 0.006), BDNF (p < 0.0001), PTGS2 (p = 0.0003), CX3CR1 (p = 0.03).

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during Aging than Development, reflecting a reduced intensity of
critical developmental events during Aging. These genes are related
to synaptic integrity, neuronal growth, neurotrophic, glial changes
and other development modifications (CX3CR1, CX3CL1, PTGS2, BDNF, CASP1, CD68, AIF1, MYD88, NGF, PDGFA, DBN1, IL1B, SYP, and BACE1).

Neurodevelopment is influenced largely by programmed
transcriptional changes involving neuronal, glial and synaptic
integrity, myelination, whereas after 21 years of age, gene
expression reaches a homeostatic state that depends more on
factors such as health status, environment, and nutrition
[48,57,75,76]. Late senescence becomes a risk factor for neuro-
degenerative diseases such as Alzheimer’s and Parkinson’s disease,
when the brain shows increased inflammatory, apoptotic, and
arachidonic cascade markers, but reduced neurotrophic and
synaptic markers [9,13,15,77,78,79]. Our data in the Aging
interval indicate many significant positive age correlations in
expression of genes in the former category (GFAP, TSPO,
PTGS2, BDNF, CASP1, CD68, AIF1, MYD88, NGF, PDGFA,
DBN1, IL1B, SYP, and BACE1).

The significant age changes in mRNA levels during the Aging
interval do not always correspond to reported protein changes.
TNFα and IFI-16 protein levels were reported to increase between
26 to 106 years in postmortem human brain [35], whereas we did
not find age increases in TNFRSF1A or IFI16 expression in the
Aging interval (Figure 2), or on average between Aging and
Development (Table 1). On the other hand, and consistent with
our expression changes, positive age correlations between 42 and
70 years were reported in brain mRNA and protein levels of
GFAP, IL-1β, iNOS, NF-kB p50, cPLA2 IVA and COX-2, while
levels of BDNF and SYP declined in this period [34]. Some of the
changes correlated with promoter hypermethylation of BDNF and
cylic AMP responsive element binding protein (CREB), and
hypomethylation of Bcl-2 associated X protein (BAX), suggesting
epigeneic influence [80]. Another study also reported a decrease

Figure 4. Nonlinear fits for expression levels with age of eight genes during Development. Fitted line added to expression data following
equation for 0 to 21 years, $Y = (Y_0 - \text{Plateau}) \cdot \exp(-K \cdot A) + \text{Plateau}$, where $Y$ = expression level at age $A$, and $Y_0$ = expression level at $A = 0$ years). An
increase or decrease of 1 on the log2 scale (y-axis) represents a two-fold change in expression in the positive or negative direction, respectively.
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in BDNF protein during the Aging interval [B1], while another reported decreased protein levels of DBN1, GAP-43, and SYN [9]. In general agreement, we found significant age increases in expression of GFAP, IL1R1, and NFKB1, and reductions in expression of BDNF and SYP (Table 1).

Age correlations often differed between the Development and Aging intervals, indicating different roles for the gene products over the lifespan (Figures 2 and 3, Table 1). Expression of SNCA increased during Development but decreased during Aging; expression of PLA2G4A, PTGS1, TRAF6, TLR4, APP, and IL1R1 increased and expression of DBN1, MYD88, RELA, PDGF, and IFI16 decreased during Development alone. Expression of MOBP increased during both intervals, consistent with continued myelination into the fourth decade in frontal cortex [5], as did expression of NFKB1, while expression of NGF decreased during both periods, suggesting reduced neuroplasticity [82]. The NFκB1 system can be stimulated by a number of cell surface receptors (Figure 1), as well as by oxidative stress, hypoxia, and genotoxic stress [25]. In non-stimulated cells, NF-κB complexes are bound in cytoplasm to inhibitory I-kappa-B (IκB) proteins. Stimulation phosphorylates IκB proteins, which are ubiquitinated and broken down, allowing the NF-κB complex to enter the nucleus and activate transcription of multiple genes, particularly related to inflammatory cascades [25].

In the first six months of life, a number of genes showed nonlinear expression changes that later reach a plateau (Figure 4). In this same period, neuronal density in layers 2–3 of human frontal cortex falls by 80% [3]. However, dendritic spine densities at different levels of prefrontal cortical pyramidal neurons rise from birth to about 5–10 years of age, and then decline [56,83]. Metabolic changes also occur, as the brain shifts from using ketone bodies to glucose in the first months of life [6]. Expression of GAP43 and DBN1, coding for presynaptic GAP43 and postsynaptic dendritic spine drebrin, decreased nonlinearly. As dendritic spine density increases in the first 5–10 years (see above), DBN1 and GAP43 likely change in this period. Expression increased for PLA2G4A and PTGS2 coding for postsynaptic functionally-coupled cPLA2 Type IVA and COX-2 [84,85,86] suggesting a growing role for arachidonic acid signaling in neurotransmission at dendritic spines [87,88].

Increases in TLR4 and IL1R1 in the first years of life may reflect increased receptivity of microglia, on which these receptors are located [89], as microglia participate postnatally in synaptic pruning and apoptosis [38,39,40,41]. In this regard, higher (2-fold) levels of expression of CX3CR1 (chemokine-(C-X3-C motif) receptor 1) and of its ligand fractalkine (CX3CL1) during Development than Aging highlight the importance their protein products in early neuronal-glial interactions. CX3CR1 is expressed exclusively by microglia in brain [90], whereas CX3CL1 is highly expressed in neurons. Knocking out CX3CR1 reduced neuron loss [91] and amyloid-beta deposition [92] in Alzheimer’s disease mouse models, and interfered with formation of thalamic-cortical synapses during development, when fractalkine is overexpressed.

Gene expression clusters in the heat map matrices of Figures 5a and 5b identify genes having high intercorrelated expression patterns as the brain ages. The clusters were similar in the Development and Aging intervals. Thus, comparable transcriptional regulatory networks operate throughout the life span, but underlie different phenotypic processes during Aging compared to Development. Gene products within Cluster 1 of both Development and Aging (GAP43, SYN, BDNF, NOS2, SNCA) are involved in synaptic signaling and integrity, cellular stress, and neurogenesis. Gene products in Cluster 2 of both periods (BACE1, CX3CR1, PTGS1, and PLA2G4A) are involved in the arachidonic acid cascade, protease activity, APP processing, and inflammatory processing. Gene products in Cluster 3 of both groups (TSPO, TNFRSF1A, IL1R1, MYD88, CD68, AIF1, CASP1, and TLR2) are involved in microglial, inflammasome, NF-κB signaling, and various neuroinflammatory responses.
Pairwise correlations, whether positive or negative, are more frequently significant during the Aging than Development interval (Figures S1a and S1b, Table 2). This may reflect our selective choice of genes, but if confirmed would suggest a more stable state of synchronized gene expression in the Aging than Development interval. This is likely since genome-wide promoter DNA methylation of CpG dinucleotides in human prefrontal cortex changes less in adulthood than early childhood [57]. Mean cortical global methylation is increased in the Aging interval, which may reflect some gene silencing [57], but global methylation is higher in late senescence [34].

Figure 5. Similarity matrices (hierarchically clustered heat maps) of Pearson’s r correlations of gene expression levels with age in Development (A) and Aging (B) groups. Red indicates negatively correlated associations; green are positively correlated associations, while black represents non-significant associations between gene pairs. Genes are clustered hierarchically along the left y-axis, which is mirrored above in each heat map.
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Table 2. Highly significant ($r \geq 0.6$) pair-wise correlations in age-related gene expression during Development and Aging intervals.

| Development | Gene1 | Gene2 | Correlation | p-value | Aging | Gene1 | Gene2 | Correlation | p-value |
|-------------|-------|-------|-------------|---------|-------|-------|-------|-------------|---------|
| MAP2 | APP | 0.77 | 1.53E-18 | | MAP2 | APP | 0.80 | 5.61E-34 | |
| AIF1 | CD68 | 0.75 | 3.66E-17 | | IL1R1 | TNFRSF1A | 0.76 | 3.38E-29 | |
| TLR2 | CASP1 | 0.67 | 8.13E-13 | | AIF1 | CD68 | 0.75 | 1.49E-27 | |
| APP | SNCA | 0.67 | 9.95E-13 | | AIF1 | CASP1 | 0.71 | 4.82E-24 | |
| MOBP | DBN1 | -0.63 | 3.20E-11 | | TLR2 | CASP1 | 0.69 | 1.84E-22 | |
| MAP2 | SNCA | 0.63 | 3.37E-11 | | CD68 | CASP1 | 0.68 | 1.54E-21 | |
| TSP0 | MYD88 | 0.63 | 5.75E-11 | | CX3CR1 | PTGS1 | 0.68 | 1.68E-21 | |
| IL1R1 | NFkB1 | 0.61 | 2.88E-10 | | TLR2 | IL1R1 | 0.68 | 3.63E-21 | |
| CX3CR1 | PTGS1 | 0.60 | 4.02E-10 | | IL1RAP | APP | 0.67 | 1.25E-20 | |
| MYD88 | CASP1 | 0.60 | 6.18E-10 | | IL1RAP | MAP2 | 0.67 | 1.87E-20 | |
| IL1R1 | TNFRSF1A | 0.60 | 7.41E-10 | | TSPO | TNFRSF1A | 0.66 | 6.52E-20 | |
| TSPO | MYD88 | 0.66 | 6.98E-20 | | TLR2 | CD68 | 0.66 | 1.16E-19 | |
| AIF1 | CX3CR1 | 0.66 | 2.20E-19 | | TNFRSF1A | MYD88 | 0.65 | 5.94E-19 | |
| AIF1 | TLR2 | 0.66 | 8.64E-13 | | IL1R1 | IL1R1 | 0.64 | 1.12E-18 | |
| TSPO | IL1R1 | 0.64 | 2.63E-18 | | TSPO | GFAP | 0.63 | 1.26E-17 | |
| MAP2 | SNCA | 0.63 | 1.64E-17 | | TLR2 | MYD88 | 0.62 | 3.05E-17 | |
| TLR2 | MYD88 | 0.60 | 7.67E-17 | | TSPO | MAP2 | -0.60 | 1.10E-15 | |
| MYD88 | CASP1 | 0.60 | 1.10E-15 | | IL1R1 | MYD88 | 0.60 | 1.16E-15 | |
| TSPO | SNCA | -0.60 | 1.41E-15 | | |

In p-values, term E-number = $10^{-\text{number}}$. doi:10.1371/journal.pone.0110972.t002
Since the subjects were considered to be healthy, some presumably deleterious expression changes in the Aging interval had not progressed sufficiently to produce noticeable functional deficits, although they may have increased vulnerability to stress and other disease factors. Upregulated translator protein (TSPO) has been imaged using positron emission tomography in patients with mild cognitive impairment (MCI) and Alzheimer’s disease [79,93,94]. Thus, the higher TSPO expression in Aging than Development is consistent with late-stage “inflamm-aging” in presumably healthy subjects (Table 1) [26]. In support, expression levels of TRAF6, NFKB1, TLR4, and IL1R1, important in the initial inflammatory response involving NF-κB (Figure 1), and of GFAP, were upregulated in the Aging interval. Nevertheless, not all gene markers of inflammation or microglial/astrocytic activation were upregulated. Expression levels of inflammation-related genes CASP1, PTGS2, MYD88, and IL1B, and of microglia genes CD68 and AIF1 (also known as IBA1), were reduced in the Aging group.

Our age correlations identified unexpected relations between different genes. Highly significant pairwise correlations were found between expression levels of APP and MAP2, of AIF1 (IBA1) and CD68, and of IL1R1 and TNFRSF1A. APP, a component in Alzheimer’s disease pathogenesis, normally helps to maintain functional synapses [95]. Microtubule-associated protein 2 (MAP2) is found in post-synaptic dendrites and is functionally similar to tau protein, whose abnormal phosphorylation is another key component in Alzheimer’s disease. AIF1 (IBA1), expressed in macrophages and microglia, and CD68, expressed in macrophages and monocytes, contribute to the inflammatory response in brain. Also, IL1R1 and the TNFα receptor (TNFRSF1A) are important in initial signaling in inflammation (Figure 1). Among the other highly correlated genes, CASP1 is involved in inflammosomes formation [96], and TSPO is upregulated during neuroinflammation. Disturbed α-synuclein (SNCA) and APP occurs in Parkinson’s disease and Alzheimer’s disease respectively, and expression of SNCA and APP was very highly correlated in Development \( r = 0.67 \) and in Aging \( r = 0.56 \) (Figure S1). The Aging interval had a greater number of significant correlations between genes, and many significant correlations occurred consistently in Development and Aging. The correlated expression of genes in the canonical pathway of NF-κB (NFKB1, MYD88, TLR4, IL1R1, TRAF6) also showed a highly integrated network of genes with similar expression patterns with age [25].

Significant correlations in gene expression that corresponded to chromosome proximity (Table S1) for PLA2G4A (cPLA2 IVA) and PTGS2 (COX-2) at locus 1q25, and for IL1R1, IL1B, and IL1RN at locus 2q14 indicate robust co-regulatory elements and chromosome proximity (Table S1) for TRAF6, NFKB1, TLR4, and IL1R1, important in the initial inflammatory response involving NF-κB (Figure 1), and of GFAP, were upregulated in the Aging interval. Nevertheless, not all gene markers of inflammation or microglial/astrocytic activation were upregulated. Expression levels of inflammation-related genes CASP1, PTGS2, MYD88, and IL1B, and of microglia genes CD68 and AIF1 (also known as IBA1), were reduced in the Aging group.

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Some of the expression changes with senescence in this study correspond to changes reported in postmortem frontal cortex from Alzheimer’s disease, bipolar disorder, and schizophrenia patients, compared to age-matched non-pathological cortex. Alzheimer’s disease cortex showed significantly increased mRNA and protein levels of IL-1β, TNFα, GFAP, CD11b, cPLA2, IVA sPLA2, IIA COX-1 and COX-2 [13,103,104], but decreased levels of pre-synaptic synaptophysin (SYT) and post-synaptic drubrin (DBN1) [9,13]. In bipolar disorder, protein and mRNA levels of neuroinflammatory markers (IL-1B, IL-1R, MYD88, NF-κB1) and of activated microglia and astroglial markers (GFAP, NOS2, c-Fos, and CD11b) also were significantly higher in control cortex [69,105]. These changes were accompanied by reduced expression of anti-apoptotic factors (B cell lymphoma (Bcl)-2, BDNF, SYN, and DBN1), but increased expression of pro-apoptotic Bax, BAD, and active caspase (CASP-3 and CASP-9 [15]. Ca²⁺-dependent cPLA2 IVA, secretory sPLA2 IIA and COX-2 also were overexpressed [70]. Similar changes were noted in schizophrenic frontal cortex [71].

This study has several limitations. Selection criteria for the microarray gene probes were a way to biologically standardize all probes by their protein-coding regions. There are other criteria of selection; however, we found that taking mean expression data of all probes for a given gene was not a good representation of gene’s expression (data not shown), as expression levels differed between probes of the same gene.

BrainCloud contains expression data selective to the prefrontal cortex [48]. The prefrontal cortex shows prolonged development and preliminary degradation associated with aging earlier than other brain regions [5,106,107]. Other studies have found differential gene expressed based on cell type, such as the Allen Brain Atlases and the Loerch study; however, BrainCloud did not differentiate between cell types [108,109,110]. Favorably, BrainCloud has a large number of samples \( n = 269 \) compared with other aging databases with time points through a lifespan. For further discussion on BrainCloud and its application, see publications [47,48].

In the future, it would be of interest to consider mechanisms underlying the age-related expression changes. These may involve histone acetylation and methylation, transcription factors, miRNAs, DNA sequences of cis-elements (transcription factor binding sites), all of which can influence mRNA expression [57,111,112,113]. In this regard, many genes whose expression decreases with age appear to have higher promoter GC content than other genes [49], suggesting differences in methylation state, and human brain aging is associated with changes in global methylation [34,57]. As we considered only two transcription factors in this study, TFAP2A (AP-2) and NFKB1 (NF-κB), future aging studies may consider more.

**Supporting Information**

**Figure S1** Matrices showing Pearson correlation coefficients between expression levels of individual gene pairs during Development (A) and Aging (B). Green highlights coefficients that are significant at \( p < 0.0001 \). Hierarchy in gene order corresponds to hierarchy in Figures 3A and 3B. (XLSX)

**Table S1** Selected genes, chromosomal locations, protein description, and major reported functions. Based on the literature, genes whose protein products participate in major functions may be categorized as follows: (1) Synaptic function, SYT, DBN1, SNCA; (2) Growth and maintenance, BDNF, NGF, GAP43, PDGF-A; (3) Myelin integrity, MOBP; (4) Neuroinflammation: (a) Microglial activation, CD68, TSPO, TLR4, TLR2, NOS2, AIF1; (b) Cytokine and chemokine processes, CX3CR1, CX3CL1, IFI16, IL1B, IL1R1, IL1RN, IL1RAP, IL6, TNFRSF1A, TRAF6, TRAP1, MAPK14, MYD88; (c) Glial activation, GFAP; (d) Apoptosis, CASP1; (e) Arachidonic acid cascade, PLA2G4A, PLA2G10, PTGS1, PTGS2; (f) Amyloid
membrane processing, APP, BACE; (6) Microtubules, MAP2; (7) Transcription factors, NFKB1, TFAP2A
(58x724)

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References

1. de Graaf-Peters VB, Hadders-Algra M (2006) Ontogeny of the human central nervous system: what is happening when? Early human development 82: 257–266.
2. Hedden T, Gabriel JDE (2004) Insights into the ageing mind: a view from cognitive neuroscience. Nature reviews Neurosciences 5: 87–96.
3. Hunterlocher PR (1984) Synapse elimination and plasticity in developing human cerebral cortex. American journal of mental deficiency 89: 408–496.
4. Hunterlocher PR (1990) Morphometric study of human cerebral cortex development. Neuropsychology 20: 517–527.
5. Yakovelj P, Lecoury AR (1967) The myelogenetic cycles of regional maturation of the brain. In: Minkowski A, editor. Regional Development of the Brain in Early Life. Philadelphia: F. A. Davis. pp. 3–70.
6. Chugani HT, Hovda DA, Villablanca JR, Phelps ME, Xu WF (1991) Metabolic maturation of the brain: a study of local cerebral glucose utilization in the developing cat. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism 11: 35–47.
7. Winell ER, Thompson PM, Toga AW (2004) Mapping changes in the human cortex throughout the span of life. The Neuroscientist: a review journal bringing neurobiology, neurology and psychiatry 10: 372–392.
8. Rapoport SI (1990) Integrated phylogeny of the primate brain, with special reference to humans and their diseases. Brain research Brain research reviews 15: 267–294.
9. Hatanpää K, Hatanpää K, Isaacs KR, Isaacs KR, Shirao T, et al. (1999) Loss of proteins regulating synaptic plasticity in normal aging of the human brain and in Alzheimer disease. Journal of neuropathology and experimental neurology 58: 637–643.
10. Aarabi NP, Rapoport SI, Saleterno JA, Grady CL, Gonzalez-Aviles A, et al. (1992) Interregional correlations of resting cerebral glucose metabolism in old and young women. Brain research 589: 279–290.
11. Creney H, Rapoport SI (1985) The aging human brain. Annual of neurology 17: 2–10.
12. Koss E, Haxby JV, DeCarli CS, Schapiro MB, Friedland RP, et al. (1991) Patterns of performance preservation and loss in healthy aging. Dev Neuropsychol 7: 99–123.
13. Rao JS, Rapoport SI, Kim H-W (2011) Altered neuroinflammatory, arachidonic acid cascade and synaptic markers in postmortem Alzheimer’s disease brain tissue. Translational psychiatry 1: e31.
14. Rao JS, Kellom M, Kim H-W, Rapoport SI, Reese EA (2012) Neuroinflammation and synaptic loss. Neurochemical research 37: 903–910.
15. Kim H-W, Rapoport SI, Rao JS (2010) Altered expression of apoptotic factors and synaptic markers in postmortem brain from bipolar disorder patients. Neurobiology of disease 37: 596–603.
16. Craggo AP, Harvey PD, Baldessarini RJ (2009) Neurocognitive impairment in bipolar disorder patients: functional implications. Bipolar disorders 11: 133–125.
17. McGeer PL, McGeer EG (2004) Inflammation and the Degenerative Diseases of Aging. Annals of the New York Academy of Sciences 1031: 104–116.
18. Perry VH, Bolton SJ, Anthony DC, Betrouni S (1996) The contribution of inflammation to acute and chronic neurodegeneration. Research in immunol 149: 721–725.
19. Kraft AD, Kraft AD, Harry GJ, Harry GJ (2011) Features of microglia and neuromicroglia relevant to environmental exposure and neurotoxicity. International Journal of environmental research and public health 8: 2980–3018.
20. Kumar A, Loane DJ (2012) Neuroinflammation after traumatic brain injury: opportunities for therapeutic intervention. Brain, behavior, and immunity 26: 1191–1201.
21. Chin SH, Aid S, Bossert F (2009) The distinct roles of cyclooxygenase-1 and -2 in neuroinflammation: implications for translational research. Trends in pharmacological sciences 30: 174–181.
22. Hock WG, Hock WG, Ramesha CS, Ramesha CS, Chang DJ, et al. (1993) Cytoplasmic phospholipase A2 activity and gene expression are stimulated by tumor necrosis factor: dexamethasone blocks the induced synthesis. Proceedings of the National Academy of Sciences of the United States of America 90: 4473–4479.
23. Spriggs DR, Sherman ML, Imanura K, Mohr M, Rodriguez C, et al. (1990) Phospholipase A2 activation and autoinduction of tumor necrosis factor gene expression by tumor necrosis factor. Cancer research 50: 7101–7107.
24. Bauer MK, Bauer MK, Lieb K, Lieb K, Schulze-Osthoff K, et al. (1997) Expression and regulation of cyclooxygenase-2 in rat microglia. European journal of biochemistry/FEBS 248: 726–731.
25. Sahimen A, Huissoon J, Ojala J, Kauppinnen A, Kaarminanta K, et al. (2008) Activation of innate immunity system during aging: NFkB signaling is the molecular culprit of inflamming-aging. Aging research reviews 7: 93–103.
26. Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, et al. (2007) Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mechanisms of aging and development 128: 92–105.
27. VanGuilder HD, Bidler GV, Brucklacher RM (2011) Concurrent hippocampal induction of MHC II pathway components and glial activation with advanced aging is not correlated with cognitive impairment. J Alzheimers Dis 23(3): 369–378.
28. Dilger RN, Johnson RW (2008) Aging, microglial cell priming, and the discordant central inflammatory response to signals from the peripheral immune system. Journal of leucocyte biology 84: 932–939.
29. Letiembre M, Hao W, Liu Y, Walter S, Milkulevic JI (2007) Innate immune receptor expression in normal brain aging. Neuroscience.
30. Griffin R, Nally R, Nolan Y, McCartney Y, Linden J, et al. (2006) The age-related attenuation in long-term potentiation is associated with microglial activation. Journal of neurochemistry 99: 1293–1272.
31. Goldstein BI, Kemp DE, Soczynska JK, McIntyre RS (2009) Inflammation and the phenomenon, pathophysiology, comorbidity, and treatment of bipolar disorder: a systematic review of the literature. The Journal of clinical psychiatry 70: 1079–1080.
32. Frank MG, Barrientos RM, Birdenkapp JC, Ruby JW (2006) mRNA up-regulation of MHC II and pivotal pro-inflammatory genes in normal brain aging. Neurobiology of aging 27(7): 1255–1263.
33. Sheffield LG, Berman N (1999) Microglial expression of MHC; class II increases in normal aging of nonhuman primates. Neurobiology of Aging.
34. Kelesiani VL, Modi HR, Rapoport SI, Rao JS (2013) Aging is associated with altered inflammatory, arachidonic acid cascade, and synaptic markers, influenced by epigenetic modifications, in the human frontal cortex. Journal of Neurochemistry 125: 63–73.
35. Liu T, Pan Y, Kao S-Y, Li C, Kojane I, et al. (2004) Gene regulation and DNA damage in the aging human brain. Nature 429: 893–891.
36. Dinarello CA (2002) The IL-1 family and inflammatory diseases. Clin Exp Rheumatol 20: S1–S13.
37. Buka SL, Tsuang MT, Torrey EF, Kleinman MA, Wagner RL, et al. (2001) Maternal cytokine levels during pregnancy and adult psychosis. Brain, behavior, and immunity 15: 415–420.
38. Garden GA, Moller T (2006) Microglia biology in health and disease. Journal of neuroimmunology: the official journal of the Society on Neuroimmunology 125: 127–137.
39. Kim SM, de Vellis J (2005) Microglia in health and disease. Journal of neuroscience research 81: 302–313.
40. Deverman BE (2009) Inflammation and the CNS. Clinics in developmental medicine. New York 64: 61–78.
41. Kaneko M, Stellwagen D, Malekta RC, Snyder MP (2008) Tumor necrosis factor-alpha mediates one component of competitive, experience-dependent plasticity in developing visual cortex. Neuron 50: 673–680.
42. Johnstone DM, Graham RM, Trinder D, Riveros C, Olynik JK, et al. (2012) Changes in brain transcripts related to Alzheimer’s disease in a model of HFE hemochromatosis are not consistent with increased Alzheimer’s disease risk. Journal of Alzheimer’s disease: JAD 30: 791–803.
43. Groth CC, Elowitz MB, Hasty P, Swat S (2002) Synthetic network dynamics and genetic control of transcription in vitro approaches. Transcription 5.
44. Hashimshony T, Yanai I (2010) Revealing developmental networks by comparative transcriptomics. Transcription 1: 154–158.
45. Ryan VH, Primiani CT, Rao JS, Ahn K, Rapoport SI, et al. (2014) Coordination of Gene Expression of Arachidonic and Docosahexaenoic Acid Cascade Enzymes during Human Brain Development and Aging. PLoS ONE 9(10): e100854. doi:10.1371/journal.pone.0100854
46. Colantuoni C, Lipska BK, Ye T, Hyde TM, Tao R, et al. (2011) Temporal dynamics and genetic control of transcription in the human prefrontal cortex. Nature 478: 319–325.
76. Shankar SK (2010) Biology of aging brain. Indian journal of pathology & microbiology 53: 595–604.

60. Johnson WE, Li C, Rabinovic A (2007) Adjusting batch effects in microarray with empirical Bayes methods. Bioinformatics (Oxford, England) 23: 118–127.

49. Somel M, Guo S, Fu N, Yan Z, Hu HY, et al. (2010) MicroRNA, mRNA, and protein expression link development and aging in human and macaque brain. Genome research 20: 1207–1218.

50. Rodwell GF, Sonn R, Zahn JM, Lund J, Wilhelmy J, et al. (2004) A transcriptional profile of aging in the human kidney. PLoS biology 2: e427.

51. Noorden DM, Godbout JP (2015) Review: Microglia of the aged brain: primed to be activated and resistant to regulation. Neuropathology and Applied Neurobiology 39: 19–34.

52. Troutman TD, Bazan JF, Pasare C (2012) Toll-like receptors, signaling enzymes in postmortem brain from bipolar disorder patients. Molecular psychiatry 15: 384–392.

53. Hussain MO, Sert RJ, Svedlov HF, Verbong-van Kempen RB (2004) The molecular evolution of the interleukin-1 family of cytokines. IL-18 in teleost fish. Developmental and comparative immunology 28: 395–413.

54. Jo YH, Chua S Jr (2009) Transcription factors in the development of medial neuroinflammation. Journal of neuroscience research 97: 138–149.

55. Kumar A, Muzik O, Shandal V, Chugani D, Chakraborty P, et al. (2012) Increased binding of Cytosolic phospholipase A2 to and cyclooxygenases in immediate and delayed prostanoid biosynthetic pathways. J Biol Chem 287: 3103–3105.

56. Goyal MS, Raichle ME (2013) Gene expression-based modeling of human cortical microstructure in Alzheimer’s disease. Neuroimage 34: 115–131.

57. Norden DM, Godbout JP, Consiglio M, Godbout RM, et al. (2016) Global epigenomic reconfiguration during mammalian brain development. Science 341: 1237905.

58. Roca J, Chiappelli J, Kochunov P, Rapoport SI, Hong LE (In preparation) Age Changes in Brain Derived Neurotrophic Factor in Postmortem Gray and White Matter from Schizophrenia and Control Subjects.

59. Terry AV Jr, Kuitiyanavalla A, Pillai A (2011) Age-dependent alterations in nerve growth factor (NGF)-related proteins, sortilin, and learning and memory consolidation. Physiological & biochemical biology = Biochimie et biologie cellulaire 84: 477–489.

61. Pruitt KD, Tatusova T, Maglott DR (2012) NCBI Reference Sequence (RefSeq): current status, new features and genome annotation policy. Nucleic Acids Res 40: D130–D135.

62. Pruitt KD, Tatusova T, Klimke W, Maglott DR (2009) NCBI Reference Sequences: current status, policy and new initiatives. Nucleic Acids Res 37: D211–D221.

63. Rebhan M, Chalifa-Caspi V, Prilusky J, Lancet D (1997) GeneCards: a comprehensive gene information resource at the genome scale. Genome research 7: 33–41.

64. Rebhan M, Chalifa-Caspi V, Prilusky J, Lancet D (1997) GeneCards: a comprehensive gene information resource at the genome scale. Genome research 7: 33–41.

65. Tatemichi TK, Maeshima K, Courchesne E, Tomblin J, Mink J, et al. (2004) Extraordinary neoteny of synaptic spines in the human prefrontal cortex. Proceedings of the National Academy of Sciences of the United States of America 101: 13281–13286.

66. Troutman TD, Bazan JF, Pasare C (2012) Toll-like receptors, signaling enzymes in postmortem brain from bipolar disorder patients. Molecular psychiatry 15: 384–392.

67. Kim HW, Rapoport SI, Rao JS (2011) Altered arachidonic acid cascade markers, and reduced neuroinflammatory markers in postmortem frontal cortex from bipolar disorder patients. Journal of neuroscience research 93: 151–156.

68. Lesch KP, Haber S, Drexler H, Riederer P, Heils A, et al. (2000) The COMT gene and risk of schizophrenia. Nature genetics 26: 7212–7221.

69. Marisathasan S, Newton K, Mouacik DM, Vucic D, French DM, et al. (2004) Differential activation of the inflammasome by caspase-1 adaptors ASC and NAIP. Nature 430: 2343–2344.

70. Kim HW, Rapoport SI, Rao JS (2011) Extracellular-derived calcium does not initiate in vivo neurotransmission involving docosahexaenoic acid. J Lipid Res 53: 2343–2344.

71. Youm Y-H, Adijiang A, Vandanmagsar B, Burk D, Ravussin A, et al. (2011) Age-related alterations in nerve growth factor (NGF)-related proteins, sortilin, and learning and memory consolidation. Physiological & biochemical biology = Biochimie et biologie cellulaire 84: 477–489.

72. Shankar SK (2010) Biology of aging brain. Indian journal of pathology & microbiology 53: 595–604.

73. Glass CK, Sajio K, Winner B, Winner B, Marchetto MC, et al. (2010) Mechanisms underlying inflammation in neurodegeneration. Cell 140: 918–934.

74. Allan SM, Rothwell NJ (2003) Inflammation in central nervous system injury. Philosophical transactions of the Royal Society of London Series B, Biological sciences 358: 1669–1677.

75. Lipska BK, Weinberger DR (1995) Genetic variation in vulnerability to the development of schizophrenia. Journal of affective disorders 34: 115–131.

76. Shankar SK (2010) Biology of aging brain. Indian journal of pathology & microbiology 53: 595–604.
104. Moses GSD, Jensen MD, Lue L-F, Walker DG, Sun AY, et al. (2006) Secretory PL2-IIA: a new inflammatory factor for Alzheimer’s disease. Journal of neuroinflammation 3: 28.
105. Rao JS, Harry GJ, Rapoport SI, Kim H-W (2009) Increased excitotoxicity and neuroinflammatory markers in postmortem frontal cortex from bipolar disorder patients. Molecular psychiatry.
106. Casey BJ, Giedd JN, Thomas KM (2000) Structural and functional brain development and its relation to cognitive development. Biological psychology 54: 241–257.
107. Morrison JH, Baxter MG (2012) The ageing cortical synapse: hallmarks and implications for cognitive decline. Nature reviews Neuroscience 13: 240–250.
108. Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, et al. (2007) Genome-wide atlas of gene expression in the adult mouse brain. Nature 445: 169–176.
109. Loerch PM, Lu T, Dakin KA, Vann JM, Isaacs A, et al. (2008) Evolution of the aging brain transcriptome and synaptic regulation. PloS one 3: e3329.
110. Hawrylycz MJ, Lein ES, Guillozet-Bongaarts AL, Shen EH, Ng L, et al. (2012) An anatomically comprehensive atlas of the adult human brain transcriptome. Nature 489: 391–399.
111. Somel M, Liu X, Khaitovich P (2013) Human brain evolution: transcripts, metabolites and their regulators. Nature reviews Neuroscience 14: 112–127.
112. Rhie SK, Coetzee SG, Noshmehr H, Yan C, Kim JM, et al. (2013) Comprehensive functional annotation of seventy-one breast cancer risk Loci. PloS one 8: e63925.
113. Persengiev, S, Kondova I, Bontrop R (2013) Insights on the functional interactions between miRNAs and copy number variations in the aging brain. Frontiers in molecular neuroscience 6: 32.
114. Vecuman L, Bode J, Gaimer M, Caballero B, Pe’er Y, et al. (2012) Effects of 18kDa translocator protein knockdown on gene expression of glutamate receptors, transporters, and metabolism, and on cell viability affected by glutamate. Pharmacogenetics and genomics 22: 606–619.
115. Medzhitov R, Preston-Hurlburt P, Janeway CA Jr (1997) A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature 388: 394–397.
116. Takeda K, Akira S (2005) Toll-like receptors in innate immunity. International immunology 17: 1–14.