Coordination of Gene Expression of Arachidonic and Docosahexaenoic Acid Cascade Enzymes during Human Brain Development and Aging

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Abstract

Background: The polyunsaturated arachidonic and docosahexaenoic acids (AA and DHA) participate in cell membrane synthesis during neurodevelopment, neuroplasticity, and neurotransmission throughout life. Each is metabolized via coupled enzymatic reactions within separate but interacting metabolic cascades.

Hypothesis: AA and DHA pathway genes are coordinately expressed and underlie cascade interactions during human brain development and aging.

Methods: The BrainCloud database for human non-pathological prefrontal cortex gene expression was used to quantify postnatal age changes in mRNA expression of 34 genes involved in AA and DHA metabolism.

Results: Expression patterns were split into Development (0 to 20 years) and Aging (21 to 78 years) intervals. Expression of genes for cytosolic phospholipases A2 (cPLA2), cyclooxygenases (COX)-1 and -2, and other AA cascade enzymes, correlated closely with age during Development, less so during Aging. Expression of DHA cascade enzymes was less inter-correlated in each period, but often changed in the opposite direction to expression of AA cascade genes. Except for the PLA2G4A (cPLA2 IVA) and PTGS2 (COX-2) genes at 1q25, highly inter-correlated genes were at distant chromosomal loci.

Conclusions: Coordinated age-related gene expression during the brain Development and Aging intervals likely underlies coupled changes in enzymes of the AA and DHA cascades and largely occur through distant transcriptional regulation. Healthy brain aging does not show upregulation of PLA2G4 or PTGS2 expression, which was found in Alzheimer’s disease.

Citation: 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(6): e100858. doi:10.1371/journal.pone.0100858

Editor: Walter Lukiv, Louisiana State University Health Sciences Center, United States of America

Received January 7, 2014; Accepted May 30, 2014; Published June 25, 2014

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Funding: This work was supported by the Intramural Research Programs of the National Institute on Aging at the National Institutes of Health. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

The human brain undergoes marked structural and functional changes after birth, such as synaptic growth followed by synaptic pruning, progressive myelination, neuroplasticity, and changes in energy metabolism, which likely underlie maturation and maintenance of cognitive and behavioral abilities [1–4]. Programmed changes are largely completed by 21 years of age, although myelination continues through 40 years in regions such as the prefrontal association neocortex [5–7]. After about 21 years, homeostatic mechanisms are important for maintaining brain integrity, but even with optimal health, neuropathological age changes are reported [3–9]. Furthermore, aging is a risk factor for Alzheimer’s and Parkinson’s diseases as well as other neurodegenerative diseases and contributes to worsening symptoms of schizophrenia and bipolar disorder [10,11].

In a genome-wide aging study of brain gene expression in humans and rhesus macaques, Somel et al found that expression variations of energy metabolism, synaptic plasticity, vesicular transport, and mitochondrial functions in the prefrontal cortex translated to related biological functions of the gene products [12]. DNA damage is increased in promoters of genes whose expression decreases with age, which may reduce the expression of selectively vulnerable genes involved in learning, memory and neuronal survival [13]. Epigenetic modifications also occur, as human brain aging is accompanied by a global promoter hypomethylation and hypermethylation of certain promoters, including those for brain derived neurotrophic factor (BDNF) and synaptophysin [14].

Lipids are constituents of brain cell membranes; their metabolism consumes approximately 25% of the brain’s ATP, and contribute to neurotransmission and gene transcription [15–18]. Furthermore, neurodevelopmental and neurodegenerative diseases have been associated with disturbances in brain lipid composition and related enzymes [19–23]. Therefore, we thought it of interest to examine the expression during brain development...
and aging of a limited number of genes involved in lipid metabolism. We focused on the pathways of two polyunsaturated fatty acids (PUFAs), arachidonic acid (AA, 20:6n-6) and docosahexaenoic acid (DHA, 22:6n-3), within their respective coupled metabolic cascades.

In the brain, AA and DHA are mainly esterified in the stereospecifically numbered (sn)-2 position of phospholipids, and in triacylglycerols and cholesterol esters to a lesser extent [19,24]. During neurotransmission, AA and DHA may be hydrolyzed from phospholipids by receptor-mediated activation of specific phospholipases A2 (PLA2). For example, Ca2+-dependent cytosolic cPLA2 and Ca2+-independent iPLA2 selectively release AA and DHA, respectively [25,26]. These PLA2s belong to large families and are found in the brain within neurons and astrocytes [27–29]. At synapses, cPLA2 co-localizes with cyclooxygenase (COX)-2, which converts the AA to eicosanoids including prostaglandin E2 (PGE2) [30–32].

Once released by a selective PLA2, unesterified AA and DHA may be recycled into phospholipid by an acyltransferase following its activation by an acyl-CoA synthetase (ACSL) to acyl-CoA (Figure S1) [33–35]. ACSLs and acyltransferases also belong to enzyme families with varying specificities to AA compared with DHA. ACSL4 is more selective for AA, while ACSL6 is more selective for DHA [36,37]. The lysophosphatidylcholine acyltransferase LPCAT3 is more selective for AA, LPCAT4 for DHA [38]. Another fraction of unesterified AA and DHA in brain undergoes enzymatic oxidation within distinct metabolic cascades [25,39,40], or non-enzymatic loss to reactive oxygen species and other bioactive products. COXs, lipoxigenases (LOXs), and cytochrome P450 epoxygenases (CYP450s) convert AA to eicosanoids such as prostaglandins or leukotrienes, involved in inflammatory responses, and DHA to neuroprotectins and resolvins, which show neuroprotective properties (Figure S1).

In the present study, we focused on transcriptional regulation of PUFAs metabolizing enzymes during human development and aging. We used the BrainCloud database, which contains mRNA expression levels of 30,176 gene expression probes [41]. This database was constructed from brains of 269 subjects without a neuropathological or a neuropsychiatric diagnosis, with ages ranging from the fetal period to 78 years [41].

We examined age-related expression of 34 genes largely involved in deacylation-recylation and enzymatic oxidation of AA and DHA. Based on the literature, we hypothesized that expression of genes for enzymes involved in direct synthesis of prostaglandins and leukotrienes from AA (e.g. COX, CYP450, PTGES) would increase with aging, while expression of genes involved with neuroprotectin and resolvin synthesis from DHA (e.g. PTGES3) would increase with aging, while expression of genes involved in deacylation-reacylation and enzymatic oxidation of AA and DHA. Based on the literature, we hypothesized that expression levels of 30,176 gene expression probes [41]. This database, which can be accessed and downloaded from http://braincloud.jhmi.edu/. The database contains gene expression data from postmortem prefrontal cortex from healthy individuals ranging from fetal ages to 78 years [41]. We studied the postnatal brain in two groups of subjects, a Development group aged 0.00348 to 20.95 years (87 subjects) and an Aging group aged 21.02 to 78.23 years (144 subjects). Fetal data was excluded. The brains were collected from the Office of the Chief Medical Examiner in the District of Columbia and Virginia, Northern District, as well as from the National Institute of Child Health and Development Brain and Tissue Bank for Developmental Disorders [41]. Subjects’ deaths were classified as natural causes, accident, or homicide [41]. The population of individuals in the Development interval consists of 26 females and 61 males, 32 of whom are African-American, 32 of whom are Caucasian, and 3 of whom are Hispanic [41]. The population of individuals in the Aging interval consists of 47 females and 97 males, 80 of whom are African-American, 4 of whom are Asian, 57 of whom are Caucasian, and 3 of whom are Hispanic [41]. See Supplemental Table 7 of Colantuoni et al for more information about the postmortem interval, pH, and RNA integrity of each sample [41]. The intervals were chosen from evidence that most brain development, including development of the prefrontal cortex, is largely completed by 20 years of age [49,50]. Henceforth, when referring to the intervals, they will be capitalized (e.g. Development and Aging) to distinguish from the processes (e.g. development and aging).

Some genes involved in AA or DHA metabolism were not found in the database and thus were excluded from the analysis (e.g. ALOX15). Some were detected by more than one probe (e.g. PTGES3). Pearson’s correlation coefficients were calculated to compare expression data from the probes. If the Pearson’s r-value was ≤0.5, as for TXES7 (r = 0.311 p = 0.0001 Development; r = 0.334 p < 0.0001 Aging) and PTGES3 (r = 0.910 p < 0.0001 Development; r = 0.856 p < 0.0001 Aging), then the average of the expression data for the probes was used and the gene was identified as GENENAME_avg. If not, the probe with the highest intensity was used and labeled GENENAME_hi. Expression data exported from BrainCloud are already linearly corrected for background and log2 ratios of the sample signal to the reference signal (reference is pooled RNA from all subjects) and normalized using loess correction as described in Colantuoni et al 2011[41,51].

The resulting expression data in the Development and Aging periods were analyzed with Cluster 3.0 software [52], with no filtering or adjustment. Distance between probes was calculated using the Euclidean distance calculation and clustered using the centroid linkage method. The Euclidean distance calculation takes the difference between two gene expression levels directly while taking into account the magnitude of changes in gene expression [53]. Euclidean distance also eliminates possible errors in distance measurements when using the centroid linkage clustering method [53]. Distance is defined as $d(x,y) = \frac{1}{n} \sum_{i=1}^{n} (x_i - y_i)^2$, where x and y are each two series of numbers, in this case the age-sorted gene expression values for any two given genes [53]. The output.cdt file was loaded into the TreeView program [54] to generate figures showing correlations between the genes of interest. Pearson’s correlations were performed for each gene to determine correlation with age and statistical significance. A t-test was performed using Partek Genomics Suite (Version 6.6 Copyright 2012, Partek Inc., St. Louis, MO, USA) to determine if expression levels in the Development and Aging intervals were significantly different for each gene. A similarity matrix was created for both Development and Aging, comparing expression data between genes using Partek Genomics Suite. This matrix then was clustered using Euclidean distance and centroid linkage clustering to generate a heat map of genes with correlated expression changes for the Development and Aging intervals.

**Methods**

The 34 genes included in this study are listed in Table 1. Expression data for these genes was exported from the BrainCloud database, which can be accessed and downloaded from http://braincloud.jhmi.edu/. The database contains gene expression data from postmortem prefrontal cortex from healthy individuals ranging from fetal ages to 78 years [41]. We studied the postnatal brain in two groups of subjects, a Development group aged 0.00348 to 20.95 years (87 subjects) and an Aging group aged 21.02 to 78.23 years (144 subjects). Fetal data was excluded. The brains were collected from the Office of the Chief Medical
Table 1. Correlation of mRNA expression with age over Development and Aging intervals and significance of difference between intervals.

| Family | Gene          | PUFA Preference | Expression pattern | Development | Pearson's r | p-value | Aging      | Pearson's r | p value | Development and Aging Difference | p-value |
|--------|---------------|-----------------|--------------------|-------------|-------------|---------|------------|-------------|---------|---------------------------------|---------|
| PLA₂   | PLA2G4A       | AA              | 6                  | 0.546       | <0.0001     | −0.125 | 0.1342     | 0.3201      |         |                                 |         |
|        | PLA2G4B       | AA              | 3                  | −0.480      | <0.0001     | 0.315  | 0.0001     | 0.4026      |         |                                 |         |
|        | PLA2G4C       | AA              | 2                  | 0.678       | <0.0001     | 0.111  | 0.1850     | 0.3201      |         |                                 |         |
|        | PLA2G4F       | AA              | 3                  | −0.248      | 0.0207      | 0.115  | 0.1685     | 0.4291      |         |                                 |         |
|        | PLA2G2D       | AA              | 9                  | 0.109       | 0.3144      | 0.069  | 0.4125     | 0.0597      |         |                                 |         |
|        | PLA2G10       | AA              | 1                  | 0.056       | 0.6075      | 0.111  | 0.1846     | 0.0071      |         |                                 |         |
|        | PLA2G2F_hi    | AA              | 9                  | −0.125      | 0.2498      | 0.032  | 0.7070     | 0.2576      |         |                                 |         |
|        | PLA2G6_hi     | DHA             | 3                  | 0.078       | 0.4745      | 0.041  | 0.6217     | 0.5958      |         |                                 |         |
|        | PNPLA8        | DHA             | 9                  | 0.226       | 0.0351      | −0.120 | 0.1532     | 0.0001      |         |                                 |         |
|        | PNPLA7_hi     | DHA             | 9                  | −0.206      | 0.0553      | −0.142 | 0.0897     | 0.0146      |         |                                 |         |
|        | PNPLA6        | DHA             | 1                  | −0.211      | 0.0501      | 0.233  | 0.0050     | 0.0509      |         |                                 |         |
| COX    | PTGS1_hi      | AA              | 6                  | 0.497       | <0.0001     | −0.101 | 0.2292     | 0.6592      |         |                                 |         |
|        | PTGS2_hi      | AA              | 6                  | 0.177       | 0.1020      | −0.270 | 0.0011     | 0.0029      |         |                                 |         |
| PGES   | PTGES         | AA              | 6                  | 0.648       | <0.0001     | −0.346 | 0.0001     | 0.0479      |         |                                 |         |
|        | PTGES2        | AA              | 9                  | 0.000       | 0.9990      | −0.014 | 0.8711     | 0.9506      |         |                                 |         |
|        | PTGES3_avg    | AA              | 6                  | 0.455       | <0.0001     | −0.072 | 0.3893     | 0.5366      |         |                                 |         |
| LOX    | ALOX5         | None            | 9                  | 0.259       | 0.0155      | −0.091 | 0.2795     | 0.0775      |         |                                 |         |
|        | ALOX12B       | None            | 1                  | 0.113       | 0.2979      | 0.265  | 0.0013     | <0.0001     |         |                                 |         |
|        | ALOX15B       | None            | 4                  | −0.280      | 0.0086      | 0.274  | 0.0009     | 0.0001      |         |                                 |         |
| Fatty Acid Binding Protein | FABP7       | None            | 5                  | −0.690      | <0.0001     | −0.149 | 0.0746     | <0.0001     |         |                                 |         |
| Acyl-CoA Synthetase | ACS4_4_h | AA              | 6                  | −0.238      | 0.0267      | −0.123 | 0.1433     | 0.0005      |         |                                 |         |
|        | ACS6          | DHA             | 7                  | −0.039      | 0.7208      | 0.032  | 0.6992     | 0.0235      |         |                                 |         |
|        | ACS3_3_hi     | None            | 9                  | 0.192       | 0.0742      | −0.098 | 0.2408     | 0.0015      |         |                                 |         |
| Acyltransferase | LPCAT3      | AA              | 8                  | −0.748      | <0.0001     | −0.075 | 0.3722     | <0.0001     |         |                                 |         |
|        | LPCAT4_hi     | DHA             | 1                  | 0.204       | 0.0590      | 0.037  | 0.6590     | 0.0001      |         |                                 |         |
| TXS    | TGBS1_avg     | None            | 1                  | 0.220       | 0.0407      | −0.163 | 0.0504     | <0.0001     |         |                                 |         |
| Cytochrome p450 | CYP4F3       | None            | 9                  | 0.018       | 0.8704      | 0.269  | 0.0011     | <0.0001     |         |                                 |         |
|        | CYP4F11       | None            | 9                  | −0.195      | 0.0708      | 0.150  | 0.0734     | 0.0803      |         |                                 |         |
|        | CYP4F22       | None            | 6                  | −0.098      | 0.3653      | 0.059  | 0.4833     | 0.0097      |         |                                 |         |
|        | CYP4F22_hi    | None            | 1                  | 0.173       | 0.1088      | 0.092  | 0.2750     | 0.0604      |         |                                 |         |
|        | CYP2C8        | AA              | 9                  | 0.054       | 0.6199      | 0.023  | 0.7862     | 0.0407      |         |                                 |         |
|        | CYP2J2        | AA              | 9                  | 0.190       | 0.0776      | 0.061  | 0.4707     | 0.7175      |         |                                 |         |
Results

Figure 1 illustrates nine representative graphs of expression data produced by the BrainCloud program, which represent characteristic trends seen in the two age intervals. The values 1 and −1 on the y-axes represent a two-fold change in gene expression in the positive or negative direction, respectively [51,55]. As noted, some probes change at a fairly steady rate throughout life, either increasing (Fig 1.1, ALOX12B) or decreasing (Fig 1.7, ACSL4hi) continuously throughout both the Development and Aging periods. Some probes change at different rates, increasing (Fig. 1.2, PLA2G4C) or decreasing (Fig. 1.5, FABP7), but usually changing more quickly during Development than Aging. Others decrease during Development and increase during Aging (Fig. 1.3, PLA2G4B), or increase during Development and decrease during Aging (Fig. 1.6, CYP4F2hi). Other genes do not have significant changes in expression levels during life (Fig. 1.9, PTGES2).

Referring to the patterns in Figure 1, Table 1 lists pattern classification for each gene. There was not a distinct trend of either up- or down-regulation with age for either AA or DHA metabolism genes.

Table 1 also indicates correlations between gene expression and age over the Development and Aging intervals, and whether the correlation with age differed significantly during those two intervals. Gene expression of the AA-selective cPLA2 enzymes (PLA2G4A, PLA2G4B, and PLA2G4C) was correlated with age during Development, whereas only PLA2G4B (cPLA2 IVB) expression correlated with age during the Aging interval \( r = 0.577, p<0.001 \). During Development, expression of PLA2G4A (cPLA2 IVA) and PLA2G4C (cPLA2 IVC) correlated positively with age, while that of PLA2G4B correlated negatively \( p<0.001 \). Only PLA2G4C showed a significant difference in correlation with age between Development and Aging.

PTGS1 (COX-1) and PTGES3 (prostaglandin (PG) E synthase 3, cPGES) correlated positively \( p<0.001 \) with age during Development, whereas PTGS2 (COX-2) \( p<0.01 \) and PTGES3 \( p<0.001 \) correlated negatively with age during the Aging interval. Age-correlations differed significantly for PTGS2, suggesting different roles in neurodevelopment and cell maintenance in the conversion of AA to PGE2.

FABP7 (fatty acid binding protein 7, which has a high affinity for brain DHA [56]) and LPCAT4 correlated significantly with age during the Development but not the Aging interval. Like PLA2G4B, PTGES (PGE synthase 1, mPGES1) and ALOX15B (15-LOX-B), which selectively converts AA to 5S-HETE, were significantly correlated with age during both intervals, but in opposite directions, showing a switch in gene expression pattern. During the Aging interval, PNP46 (an iPLA2 selective for DHA), ALOX12B (12-LOX-B), and CYP4F3 (cytochrome P450 family 4, subfamily F, polypeptide 3) expression levels were correlated positively with age, whereas PTGS2hi (COX-2) had a negative correlation with age. Other genes in the list were not, or were weakly, correlated with age and often displayed variable expression patterns (Figure 1, Table 1).

A t-test was also used to compare gene expression patterns during Development and Aging. More than half of the comparisons were statistically significant, which confirms that these selected intervals are relevant to analyze variation of gene expression throughout the life span (Table 1).

Correlations in expression levels between the genes are illustrated in correlation trees for the two age intervals, Development (Figure 2A) and Aging (Figure 2B). Some gene groups are closely correlated in both of the two intervals, such as PLA2G4F (cPLA2 IVF) and CYP4F22 (cytochrome P450 family 4 subfamily F...
polypeptide 22), or PTGS1 (COX-1) and ALOX5 (5-LOX). In the Aging tree, FABP7 (fatty acid binding protein) expression was not associated with any other genes because it was very downregulated as compared to the other genes (Figure 2B).

Similarity matrices were calculated showing correlations between each pair of genes for the Development (Figure 3A) and Aging intervals (Figure 3B). From each of these matrices, groups of genes appeared highly correlated, either positively (red), or negatively (blue). During Development, two groups of highly correlated genes were PNPLA7_hi (patatin-like phospholipase domain-containing protein 7, an iPLA2), PTGES2 (mPGES-2), PNPLA6 (neuropathy target esterase, iPLA 2 delta), CYP4F22 (CYP450 family 4, subfamily F, polypeptide 22), PLA2G2F_hi (sPLA2 IIF), PLA2G4F (cPLA2 IVF) (Group 1) and PTGES (mPGES1), PLA2G4C (cPLA2 IVC), PTGES3_avg (cPGES), PTGS1_hi (COX-1), ALOX5 (5-LOX), TXAS1_avg (thromboxane-A synthase 1, TXS), PLA2G4A (cPLA2 IVA) (Group 2). Another group made of ACSL3_hi (ACSL3), ACSL4_hi (ACSL4), and CYP2C8 (CYP450 family 2, subfamily C, polypeptide 8) (Group 3) showed a strong negative correlation with Group 1. Yet another group including FABP7 (fatty acid binding protein), ALOX15B (15-LOX-B), PLA2G4B (cPLA2 IVB), and LPCAT3 (LPCAT3) (Group 4) correlated negatively with Group 2.

The Aging matrix (Figure 3B) showed only two groups of high correlation. The first group was PLA2G4B (cPLA2 IVB), CYP4F22 (CYP450 family 4, subfamily F, polypeptide 22), PLA2G2F_hi (sPLA2 IIF), PLA2G6_hi (iPLA2 VI), CYP2J2 (CYP450 family 2, subfamily J, polypeptide 2), LPCAT3 (LPCAT3), CYP4F11 (CYP450 family 4, subfamily F, polypeptide 11), and PLA2G4C (cPLA2 IVC) (Group 1); these genes were highly positively correlated with each other. The second highly positively correlated group was PTGES3_avg (cPGES), PTGS1_hi (COX-1), PLA2G4A (cPLA2 IVA), ACSL3_hi (ACSL3), ACSL4_hi (ACSL4), and PTGS2_hi (COX-2) (Group 2). The blue area on the heat map indicates a strong negative correlation between Group 1 and Group 2.

Pearson’s correlation coefficients were calculated for each pair of genes located on the same chromosome and only significantly
correlated (p<0.01) gene pairs are presented in Table 2. Among pairs correlated at p<0.0001, PLA2G4A (cPLA2 IVA) and PTGS2 (COX-2) were positively correlated for both the Development and Aging intervals, while PTGES3 (cPGES) and LPCAT3 (LPCAT3) were inversely correlated for both intervals. PLA2G4A and PTGS2 are close to each other on chromosome 1 [31,32]. However, PTGES3 and LPCAT3 are not located on the same arm of chromosome 12. Furthermore, the loci of many genes on chromosome 19 are very close to each other without being significantly correlated. Thus it appears that proximity in locus is not associated with correlation in expression for the AA and DHA metabolism genes.

Pearson’s correlation values were also calculated for genes in the same family (e.g. the LOX or PGES family), for genes known to be coupled (e.g. PLA2G4A and PTGS2), or between transcription factors and their associated genes (Table 3). The correlations between PLA2G4A/PTGS2 (cPLA2 IVA/COX-2) and PTGES2/PTGES3 (mPGES-2/cPGES) were the only significant correlations in both the Development and Aging intervals (PLA2G4A/PTGS2 p<0.0001; PTGES2/PTGES3 p=0.0009 Development, p<0.0001 Aging). Furthermore, the transcription factors NFKB1 (nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, NF-kB) and TFAP2D (transcription factor AP-2 delta, AP-2) were not correlated (r<0.2, p>0.05) with their associated genes, PTGS2 (COX-2) and PLA2G4A (cPLA2 IVA), respectively. Therefore, gene expression within functional families did not follow the same pattern throughout life and there was no correlated expression between transcription factors and the genes they regulate. However, lack of correlation between transcription factors and genes they regulate would be expected since these transcription factors regulate multiple genes, and the genes studied are regulated by multiple transcription factors.

We performed Pearson’s r correlations for functionally similar pairs of genes that are selective for one PUFA over the other: PLA2G4A/PLA2G6 (cPLA2 IVA/iPLA2 VI), ACLS4/ACLS6 (ACSL4/ACSL6), and LPCAT3/LPCAT4 (LPCAT3/LPCAT4) (Table 4). Within the PLA2 family, there was a significant negative correlation during Development (r = -0.274, p = 0.0102) and Aging (r = -0.476, p<0.0001) between the AA-selective PLA2G4A (cPLA2 IVA) and the DHA-selective PLA2G6 (cPLA2 VI). During Aging, there was no significant correlation between the acyl-CoA synthetase genes, but the acyltransferase genes were negatively correlated (r = -0.240, p = 0.003).

Discussion

We examined age variations throughout life span in human brain expression levels of a limited set of genes involved in PUFA metabolism. We chose AA and DHA metabolism because these PUFAs and their metabolites influence multiple brain processes, including neurotransmission, synaptic growth, gene transcription, membrane fluidity, and the pathological processes of apoptosis, neuroinflammation and excitotoxicity [57–61].

We analyzed two postnatal age intervals, Development (0–20 years), and Aging (21 years and older), chosen on the basis of known functional and structural brain changes [1–3,12]. Confirming these intervals as separate time periods involving distinct aspects of brain function and structure, we showed that expression patterns of most genes were statistically different between...
Development and Aging. Correlations between gene expression level and age were generally lower in the Aging interval than the Development interval, suggesting that with aging, gene expression regulation is less connected to programmed brain changes. Thus as an individual ages, gene expression likely depends more on individual factors, such as health status, environmental stress, nutrition, and other factors influencing lipid metabolism [4, 18, 62–64].

Generally, significant correlations between genes were not related to chromosomal location. However, we did find strong positive correlations between expression of PLA2G4A (cPLA2 IVA, locus 1q25) and PTGS2 (COX-2, locus 1q25.2-q25.3) in both the Development and Aging intervals. The coding regions for PLA2G4A and PTGS2 are separated by only about 149 kb of DNA along the long arm of chromosome 1 (1q) [65]. cPLA2 IVA (PLA2G4A) selectively releases AA from the sn-2 position of phospholipids, while COX-2 (PTGS2) catalyzes the rate-limiting step of released AA’s conversion to PGF2α [36, 65]. Their highly correlated expression supports the functional coupling between

![Figure 3. Similarity matrices showing correlations between genes in the Development (A) and Aging (B) intervals.](image)

Development: n = 87, Aging: n = 144.

doi:10.1371/journal.pone.0100858.g003

Table 2. Correlations between pairs of genes on the same chromosome.

| Chromosome | Gene | Locus | Interval | Gene Pair | Pearson’s r | p-value |
|------------|------|-------|----------|-----------|-------------|---------|
| 1          | PLA2G4A | 1q25 | Development | PLA2G4A and PTGS2 | 0.577 | <0.0001 |
|            | PLA2G2D | 1p36.12 | Aging | PLA2G4A and PTGS2 | 0.541 | <0.0001 |
|            | PLA2G2F | 1p35 | Aging | PLA2G4A and PLA2G2F | -0.420 | <0.0001 |
|            | PTGS2   | 1q25.2-q25.3 | Aging | PLA2G4A and CYP2J2 | -0.425 | <0.0001 |
|            |         |       |         | PTGS2 and PLA2G2F | -0.266 | 0.0091 |
|            |         |       |         | PTGS2 and CYP2J2 | -0.227 | 0.0013 |
|            |         |       |         | PLA2G2F and CYP2J2 | 0.169 | 0.0027 |
| 6          | FABP7   | 6q22-q23 |       |           |       |         |
|            | TFA2P2D | 6p12.3 |       |           |       |         |
| 7          | PNPLA8  | 7q31 | Aging | PNPLA8 and TBXAS | 0.262 | 0.0015 |
|            | TBXAS1  | 7q34-q35 |       |           |       |         |
| 9          | PNPLA7  | 9q34.3 | Development | PNPLA7 and PTGS2 | 0.474 | 0.0004 |
|            | PTGS1   | 9q32-q33.3 | Development | PTGES and PTGS1 | 0.282 | 0.0031 |
|            | PTGES   | 9q34.3 | Aging | PTGES and PTGS2 | 0.310 | 0.0002 |
|            | PTGES2  | 9q34.12 | Aging | PNPLA7 and PTGS2 | 0.487 | 0.0002 |
|            |         |       |         | PTGES2 and PTGS1 | -0.189 | 0.0032 |
| 10         | ALOX5   | 10q11.2 |       |           |       |         |
|            | CYP2C8  | 10q24.1 |       |           |       |         |
| 12         | PTGES3  | 12q13.13 | Development | PTGES3 and LPCAT3 | -0.589 | <0.0001 |
|            | LPCAT3  | 12p13.13 | Aging | PTGES3 and LPCAT3 | -0.526 | <0.0001 |
| 15         | PLA2G4B | 15q11.2-q21.3 |       |           |       |         |
|            | PLA2G4F | 15q15.1 |       |           |       |         |
|            | LPCAT4  | 15q14 |       |           |       |         |
| 17         | ALOX12B | 17p13.1 |       |           |       |         |
|            | ALOX15B | 17p13.1 |       |           |       |         |
| 19         | PLA2G4C | 19q13.3 | Development | CYP4F22 and PNPLA6 | 0.514 | <0.0001 |
|            | PNPLA6  | 19p13.2 | Development | CYP4F11 and CYP4F22 | 0.294 | 0.0057 |
|            | CYP4F3  | 19p13.12 | Aging | CYP4F3 and CYP4F11 | 0.432 | <0.0001 |
|            | CYP4F11 | 19p13.1 | Aging | CYP4F3 and PLA2G4C | 0.333 | <0.0001 |
|            | CYP4F22 | 19p13.12 | Aging | CYP4F11 and CYP4F22 | 0.393 | <0.0001 |
|            | CYP4F2  | 19p13.12 | Aging | CYP4F22 and PNPLA6 | 0.301 | 0.0002 |
|            |         |       |         | CYP4F11 and PLA2G4C | 0.222 | 0.0074 |

All chromosomes that contain multiple AA or DHA metabolism gene loci are listed. Loci were found using the HUGO Gene Nomenclature Committee database (genenames.org). Correlations between genes are split into Development and Aging intervals. Only correlations where p<0.01 are shown. Development: n=87, Aging: n=144.
doi:10.1371/journal.pone.0100858.t002
these two AA-selective enzymes that has been reported in cell cultures and in the brain in vivo [25,43,44]. Functionally, inducible COX-2 can only convert AA-released by cPLA2 and is not active on exogenous AA [47,66]. The co-localization and high correlation of expression levels of PLA2G4A (cPLA2 IVA) and PTGS2 (COX-2) in Development and Aging also indicate tight transcriptional co-regulation and co-evolution.

We also identified a larger group of genes whose expression was inter-correlated during the Aging period. This group includes PTGS1_, hi (COX-1), PTGS2_, hi (COX-2), PLA2G4A (cPLA2 IVA), ACSL3_, hi (ACSL3), ACSL4_, hi (ACSL4), and PTGES3_avg (cPGES), all of which had a high positive correlation with each other. These genes operate together in a multi-enzymatic cascade catalyzing the conversion of AA to specific eicosanoids [25,44,67], and their high positive correlations indicate cooperative regulation during Aging.

mRNA and protein levels of cPLA2 IVA (PLA2G4A), sPLA2 IIA (PLA2G2A), COX-1 and -2 (PTGS1, PTGS2), mPGES1 (PTGES1), and LOX-12 and -15 (ALOX12B, ALOX15B), are increased in Alzheimer’s disease in the frontal cortex [68], hippocampus [69–72], and cerebellum [69]. In contrast to these reported increases, both PLA2G4A (cPLA2 IVA) and PTGS2 (COX-2) belong to expression pattern groups that decrease during healthy Aging and both genes correlate negatively with age. Genes whose mRNA levels decline with age have significantly greater promoter DNA damage [13], so some mechanism may prevent normal downregulation of PLA2G4A and PTGS2 in Alzheimer’s disease. Furthermore, the expression of DHA selective iPLA2 VIA (PLA2G6) is reduced in Alzheimer’s disease [68], but we found that its expression is increased in Aging, which shows another disconnection between healthy and pathological aging.

There is some evidence that the brain DHA concentration in brain is reduced with age, especially in patients who develop neurodegenerative disease [19,22]. DHA regulates membrane fluidity, gene transcription, and can be metabolized to anti-inflammatory neuroprotectins and resolvins [73–76]. However,

Table 3. Correlations between expression levels of functionally coupled enzymes.

| Genes         | Interval   | Pearson’s r | p-value   |
|---------------|------------|-------------|-----------|
| cPLA2 and COX-2 | PLA2G4A and PTGS2 | Development | 0.577     | <0.0001   |
| LOX           | ALOX12B and ALOX15B | Development | −0.006    | 0.9581     |
|               |            | Aging       | 0.156     | 0.0621     |
|               | ALOX12B and AOX5 | Development | −0.125    | 0.2502     |
|               |            | Aging       | −0.327    | <0.0001    |
|               | ALOX15B and AOX5 | Development | −0.233    | 0.0302     |
|               |            | Aging       | −0.013    | 0.8799     |
| PGES          | PTGES and PTGES2 | Development | 0.070     | 0.5217     |
|               |            | Aging       | 0.310     | 0.0002     |
|               | PTGES and PTGES3 | Development | 0.200     | 0.0633     |
|               |            | Aging       | −0.130    | 0.1209     |
|               | PTGES2 and PTGES3 | Development | −0.349    | 0.0009     |
|               |            | Aging       | −0.448    | <0.0001    |
| Transcription factor and gene it regulates | PLA2G4A and TFAP2D | Development | 0.191     | 0.0764     |
|               |            | Aging       | 0.121     | 0.1496     |
|               | PTGS2 and NFkB1 | Development | 0.140     | 0.1965     |
|               |            | Aging       | 0.072     | 0.3898     |

Gene pairs that are in the same family, function in sequential steps of a part of the pathways, or are transcription factors/target gene pairs. Significant (p < 0.05) p-values are bolded. Development: n = 87, Aging: n = 144.

doi:10.1371/journal.pone.0100858.t003

Table 4. Correlation between AA and DHA metabolism.

| Genes          | Interval   | Pearson’s r | p-value   |
|----------------|------------|-------------|-----------|
| cPLA2 IVA and iPLA2 VI | PLA2G4A and PLA2G6 | Development | −0.274    | 0.0102     |
| ACSL           | ACSL4 and ACSL6 | Development | −0.255    | 0.017      |
|                |            | Aging       | −0.089    | 0.287      |
| LPCAT          | LPCAT3 and LPCAT4 | Development | 0.055     | 0.6146     |
|                |            | Aging       | −0.240    | 0.0038     |

Functionally similar genes with specificity for either AA or DHA are correlated to show how the two pathways are associated during Development and Aging. Significant (p < 0.05) p-values are bolded. Development: n = 87, Aging: n = 144.

doi:10.1371/journal.pone.0100858.t004
Unlike AA selective enzymes, expression of DHA selective enzymes (PLA2G6, ACSL6, LPCAT4) was not specifically correlated during Aging (Figure 3B). Although BrainCloud is a powerful database, there are some limitations to the program. First, its expression data are obtained only from postmortem prefrontal cortex gray matter [41]. This brain region has comparatively prolonged myelination and is reported to show disproportionate degeneration with aging as compared to other neocortical regions [2,6,49]. Expression patterns would be expected to differ between regions and many age related changes in brain occur in white matter, which is not analyzed in the BrainCloud project [49]. Finally, BrainCloud does not distinguish between cell types. The Allen Brain Atlases found that astrocytes, oligodendrocytes, and neurons exhibit different age-related changes in gene expression [77–79]. On the other hand, to-date BrainCloud has the largest number of samples of gene expression data in the prefrontal cortex. The Allen Human Brain Atlas contains data from only 3 individuals, all male, while the Loehr study contains data from 28 human samples [41,77,78]. As such, BrainCloud is an extremely powerful tool for studying age-related gene expression changes in a diverse sample population (including both sexes and four races).

In the future, it would be of interest to investigate possible mechanisms of the age-related changes in mRNA levels. Methylation of gene promoters, histone acetylation and methylation state, transcription factors, miRNAs, DNA sequences of cis-elements (transcription factor binding sites), and feedback regulation by AA and DHA and their metabolites likely play a role in changing mRNA expression levels [80]. Generally, gene groups whose expression decreases with age appear to have higher promoter GC content than other genes [12], suggesting differences in methylation state, and human brain aging is associated with a global hypomethylation [14]. Gene-specific promoter methylation can now be analyzed in BrainCloudMethyl, a database similar to BrainCloud that contains CpG methylation data [81]. Histone acetylation and methylation state have been shown to affect aging of cells [82,83] and miRNAs have been shown to influence aging in stem cells [84] and the brain [85]. Cis-element sequences affect binding of transcription factors and thus could affect levels of transcripts. Of the two transcription factors included in this study, TFAP2D (AP-2) and NFκB1 (NF-kB), only one (NFκB1) correlated significantly with age during both Development and Aging and had significantly different expression patterns between the two intervals. Transcription factors not included in this study also likely differ in expression during these intervals. These multiple factors likely cooperate to regulate gene expression during Development and Aging and contribute not only to the pattern switch from Development to Aging, but also to the switch from healthy to pathological aging. Their role in the regulation of the genes examined here warrants further investigation.

While there does not appear to be a trend of either upregulation or downregulation with age for AA or DHA related genes, the genes involved in the AA pathway were highly positively correlated with each other, indicating coordinated regulation during aging, which was not the case for the genes involved in DHA metabolism. Further, AA and DHA genes were negatively correlated with each other, indicating independent or inverse regulation, and competition between these two major pathways. These findings are consistent with previous studies in which reduced dietary n-3 PUFA content led to downregulation of DHA and upregulation of brain AA metabolic cascade enzymes in the rat brain, whereas the reverse was true when reducing dietary n-6 PUFA content [86]. Furthermore, mood stabilizers that are used to treat bipolar disorder, lithium, carbamazepine, and valproate, downregulated the AA but not the DHA cascade in rat brain [87].

In summary, we have demonstrated coupled and distinct patterns of changes in mRNA expression of two metabolic brain cascades, that not only suggest different roles of the individual cascades in healthy human brain Development and Aging, but also in underlying processes such as brain growth and neurotransmission during these life periods. The known inverse coupling of the AA and DHA cascades is underscored by profound and coordinated regulation of gene expression of their enzymes. The interaction between transcriptional and phenotypic mechanisms in the normal as well as pathological brain deserves further exploration.

Supporting Information
Figure S1  The brain arachidonic and docosahexaenoic acid cascades. After AA within phospholipid is released by cPLA2 or sPLA2, a portion is converted to prostaglandin H2 (PGH2) by COX-1 or COX-2, to hydroxyicosatetraenoic acid (20-HETE), to hydroperoxyeicosatetraenoic acids (HPETES) by lipooxygenase (LOX) subtypes 5, 12 or 15. PGH2 is converted to prostaglandin E2 (PGE2) by membrane prostaglandin synthase-1 and 2 (mPGES-1, 2) or cytosolic prostaglandin synthase (cPGES). PGE2 also can be converted to thromboxane A2 (TXA2) by thromboxane synthase (TXS). In brain, the COX-1 is constitutively expressed, whereas COX-2 is inducible. cPGES uses PGH2 produced by COX-1, whereas mPGES-1 uses COX-2-derived endoperoxide. Unconverted AA has a Co-A group added by ACSL4 and is re-incorporated into the membrane by LPCAT3.

Acknowledgments
We thank Drs. Barbara K. Lipska and Chuck C. Chen for their comments during manuscript preparation.

Author Contributions
Conceived and designed the experiments: HB SR VR CP. Performed the experiments: VR. Analyzed the data: VR CP. Contributed reagents/materials/analysis tools: KA JR. Wrote the paper: VR HB SR.

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