Dissociable Cellular and Genetic Mechanisms of Cortical Thinning at Different Life Stages

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Abstract

Mechanisms underpinning age-related variations in cortical thickness in the human brain remain poorly understood. We investigated whether inter-regional age-related variations in cortical thinning (in a multicohort neuroimaging dataset from the ENIGMA Lifespan Working Group totalling 14,248 individuals, aged 4-89 years) depended on cell-specific marker gene expression levels. We found differences amidst early-life (<20 years), mid-life (20-60 years), and late-life (>60 years) in the patterns of association between inter-regional profiles of cortical thickness and expression profiles of marker genes for CA1 and S1 pyramidal cells, astrocytes, and microglia. Gene ontology and enrichment analyses indicated that each of the three life-stages was associated with different biological processes and cellular components: synaptic modeling in early life, neurotransmission in mid-life, and neurodegeneration in late-life. These findings provide mechanistic insights into age-related cortical thinning during typical development and aging.

Keywords: Cortical Thickness, Gene Enrichment, Gene Ontology, Magnetic Resonance Imaging, Virtual Histology

Introduction

The human cerebral cortex undergoes substantial thinning, beginning in late childhood and extending throughout the lifespan[1,2]. These macro-scale changes are underpinned by cellular and molecular processes that vary across life-stages. Cortical thinning during development is likely attributable to adaptive synaptic and dendrite elimination [3-5] combined with increased intracortical myelin [6,7]. In late adulthood it has been attributed to deterioration in vasculature, shrinkage of large neurons [8], loss of myelinated axonal fibers [9], and regressive synaptic density reduction [10]. Elucidating the cellular and genetic underpinning of the typical trajectory of cortical thinning provides the foundation for understanding how deviations may occur in development and ageing.

To address this challenge, our group developed a virtual-histology approach that is based on the spatial correlation – across the human cerebral cortex – of gene-expression and magnetic resonance imaging (MRI)-derived data [11]. In our prior studies using this approach, we found that higher expression of marker genes of CA1 pyramidal cells, astrocytes, and microglia was associated with less cortical thinning in childhood and adolescence [12,13], and with accelerated cortical thinning in late-life[13]. These findings suggest that biological mechanisms supported by the same cellular populations may differ according to life-stage. Higher expression of marker genes for CA1 pyramidal cell may contribute to neurite sprouting during development [14] and shrinkage during ageing [15], while higher expression of marker genes for glial cells may support the consolidation of brain organization in early life [16], but facilitate a heightened proinflammatory profile in the ageing brain [17].

In the present study, we extended these investigations in two distinct directions. First, we used one of the largest available samples of neuroimaging data from healthy participants (N=14,248) covering the human lifespan from the Enhancing Neuroimaging Genetics through Meta-analysis (ENIGMA) consortium [18]. Leveraging these data, our first aim was to test the reproducibility of

the prior findings regarding the association of cell-specific gene expression and cortical thinning, which have relied on smaller samples (<4,000). Our second aim was to use gene co-expression and over-representation analyses to test whether the cell-specific gene sets associated with cortical thinning implicate differing biological processes and cellular components that support either developmental or regressive events at different life-stages.

Materials and Methods

Neuroimaging Dataset

De-identified cross-sectional demographic data and MRI-derived estimates of cortical thickness from 35 cohorts participating in the ENIGMA Lifespan Working Group were pooled to create the neuroimaging dataset. The pooled sample comprised 14,248 individuals (52.7% female) aged 4-89 years. Ethical approval and informed consent were overseen by the investigators of each cohort, and data were shared in accordance with the ENIGMA data-use agreements and local policies. Participants in each cohort had been screened to exclude psychiatric disorders, medical and neurological morbidity, and cognitive impairment. Details of demographic characteristics and screening procedures are presented in Supplementary Tables 1 and 2 and Supplementary Figure 1.

Estimation of age-related cortical thinning

Standardized ENIGMA analysis and quality assurance protocols were applied to T_1-weighted whole-brain MRI scans to extract estimates of thickness from cortical regions defined by the Desikan-Killiany Atlas [19] based on the FreeSurfer pipelines (http://surfer.nmr.mgh.harvard.edu). Details of the neuroimaging analyses and quality assurance protocols are presented in supplemental data and Supplemental Table 2. Only the left-hemisphere measures (N=34) were used in subsequent analyses to align with the availability of the gene-expression data. Separate generalized additive mixed models (GAMM; implemented with the *mgcv* R-package) [20] were used to model the association between age and each of the 34 regional measures of cortical thickness, adjusted for sex, as fixed effects, and scanner identifiers as random intercepts. The GAMM does not assume linearity in the relationship between outcome and predictors and uses flexible functions (i.e., splines) to model nonlinear relationships. Splines behave as polynomial functions, each covering a small range of the data, which converge on each other at different points (i.e., knots). For each region, we specified cubic splines as functional approximators and

evaluated models with an increasing number of knots beginning at 5 (see also sensitivity analyses in Supplementary Table 3 and Supplementary Figure 3). The model that used one degree of freedom less than what was allowed was considered optimal, allowing us to tailor the model parameters to each region while minimizing overfitting. We then employed the *gratia* R-package [21], which uses a finite differences approach, to compute the GAMM derivatives as an estimate of cortical thinning of each region for every 1-year shift in chronological age. As the age range of the ENIGMA Lifespan dataset is 4-89 years, these analyses resulted in 86 age-specific inter-regional profiles of cortical thinning across the 34 cortical regions (Supplementary Figure 2). Each age-specific inter-regional profile represents the spatial variation in cortical thinning across the 34 cortical regions, at that specific age.

Cell-specific gene expression profiling

Cell-specific gene expression profiling followed our previously developed methods [11-13,22] using the Allen Human Brain Atlas (AHBA) [23] as the source of gene expression data (details in the Supplement section A4). Subsequent analyses were restricted to a panel of 2,511 genes, henceforth referred to as the *consistent genes panel*, identified by the consistency of their inter-regional expression in the 34 left-hemisphere cortical regions in the AHBA (across donors) and between AHBA and the BrainSpan dataset. [12]. The interregional profile of gene expression represents the spatial variation in the expression of a given gene across these cortical regions. Cell-specific gene labels provided by Zeisel and colleagues (2015) were updated as per Mancarci and French [24] and clustered into 9 cell-specific gene panels: S1 pyramidal neurons (n□=□73 human gene symbols), CA1 pyramidal neurons (n□=□103), interneurons (n□=□100), astrocytes (n□=□54), microglia (n□=□48), oligodendrocytes (n□=□60), ependymal (n□=□84), endothelial (n□=□57), and mural (pericytes and vascular smooth muscle cells; n□=□25).

Study-Specific Gene Expression Database and Co-Expression Networks

We created a study-specific gene-expression database based on information from post-mortem cortical tissue from 572 unique donors, aged 0 to 102 years at the time of death, by harmonizing data from five databases: the AHBA, the BrainCloud [25,26], the Brain eQTL Almanac project [27], the BrainSpan (http://brainspan.org), and the Genotype-Tissue Expression Project [28]. The study-specific database included expression levels for 15,380 genes that were available in all five databases, 2,321 of which were also part of the consistent genes panel. The co-expression matrix of each of these 2,321 genes was determined using linear mixed models and ranked according to effect size (details in Supplement Section 5A).

Statistical Analyses

Statistical procedures throughout the study were implemented in R (www.r-project.org, v. 3.6.1) (Team RC, 2013). The threshold for statistical significance was adjusted for multiple testing using false discovery rate (FDR) correction.

Correlation between lifespan cortical thinning and cell-specific gene expression profiles: We quantified associations between each inter-regional profile of cortical thinning (n=86; obtained from the GAMM derivative sampled across the entire age-range of the sample) and interregional expression of each cell-specific gene (N=604) using the Pearson's correlation coefficient as per our previous work [12,13]. We computed the Pearson's correlation between the thinning and the gene expression profiles, for each cortical profile (n = 86, one profile for each age from 4 to 89 years) and consistent cell-specific gene panels (n =9). The statistical significance of these associations was established through resampling and permutation testing against a null distribution generated for each actual cell-specific gene panel, by assessing the correlation between cortical-thinning profiles and pseudo-panels of randomly selected cell-specific genes of equal number to each of the actual panels. Statistical significance was established by combining Bonferroni-like correction for the number of inter-regional cortical thinning profiles (n=86) and FDR correction for the number of cell-specific panels (details in supplementary data).

Gene Co-Expression and Enrichment: Gene co-expression networks were generated by considering all the consistent genes for which information was available in the study-specific database (N=2,311), irrespective of their cell-specific assignment, as several of these genes are likely to influence biological pathways across multiple cell types. The co-expression matrix of these 2,321 genes was determined using linear mixed models and ranked according to effect size (details in supplemental data). Guided by the preceding analyses, we selected those inter-regional cortical thinning profiles that had significant correlations with inter-regional cell-specific gene expression profiles; these inter-regional cortical-thinning profiles were then averaged to generate a single life-stage inter-regional cortical-thinning profile for three paradigmatic age-groups consistent with early life (below 20 years of age), mid-life (20-60 years of age) and late-life (over 60 years of age). Quantification of associations between each life-stage cortical thinning profile and gene co-expression networks proceeded as per Sliz and colleagues [29]. Accordingly, we indexed those cell-specific genes showing high fidelity, i.e., genes that were in the top 5% among the genes consistently associated with the cortical-thinning profile. Based on our previous work[22], we selected the top 0.1% of the genes in the study-specific co-expression database that were co-expressed with the high-fidelity subset of the consistent genes panel. We then conducted gene ontology (GO) enrichment analysis using the *clusterProfiler* package [30]. GO provides a unified vocabulary to describe gene functions and their inter-relations in terms corresponding to biological processes (BP) and cellular components (CC). BP and CC gene sets between 10 and 500 genes were considered. The threshold of statistical significance was set at P_{FDR}<0.01 to account for the three life-stages**.** The enrichment analysis was generally invariant to the number of genes most co-expressed with the high-fidelity genes, as well as cell-type and age-range-specific analyses (details in Supplement Section 6A).

Results

Correlation between lifespan cortical thinning and cell-specific gene expression profiles

The MRI-based lifespan changes in regional cortical thickness displayed the prototypic pattern of decline (Supplementary Figure 2). Several inter-regional cell-specific gene profiles showed significant correlations with the inter-regional cortical thinning profiles (Figure 1 and 2) that were robust to the GAMM parameters for cortical thickness derivative estimates (Supplementary Figure 3). Positive correlation coefficients indicate less cortical thinning with higher expression levels of a cell-specific gene profile and the opposite is the case for negative coefficients. Positive correlations between inter-regional cortical thinning profiles were observed with the inter-regional expression profiles of astrocyte-specific genes both in early-life (below 16 years of age) and in mid-life (between the ages of 45-60 years). Negative correlations between inter-regional cortical thinning and interregional expression levels of S1-pyramidal-specific genes were found both in early-life (below 23 years of age) and in mid-life (between the ages of 36-64 years). Correlations between inter-regional cortical thinning profiles with inter-regional expression levels of microglia- and CA1 pyramidal-specific genes were positive in early-life (below 18 years of age) but negative in late-life (after the age of 67 years). Inter-regional expression levels of ependymal-specific and oligodendrocyte-specific genes respectively showed positive and negative correlations with inter-regional cortical thinning profiles in mid-life (between 45-60 years of age).

Association of life-stage inter-cortical thinning profiles with gene co-expression and enrichment

Significant associations of the early-life inter-regional cortical thinning profile with GO:BP terms related to the regulation of trans-synaptic signaling, synaptic pruning, and cognition (Figure 3, Supplementary Figure 4) while associations with GO:CC terms related to synaptic and post-synaptic structures, glutamatergic transmission, and secretory granules membrane, a

component of microglial cells (Figure 3, Supplementary Figure 5). Significant associations of the mid-life-stage inter-regional cortical thinning profile with GO:BP terms related to neurotransmitter, amino-acid, and synaptic vesicle transport (Figure 3, Supplementary Figure 6) while associations with GO:CC terms related to synaptic and vesicular membrane components and glutamatergic synapses (Figure 3, Supplementary Figure 7). Significant associations of the late-life-stage inter-cortical thinning profile with GO:BP terms related to the Ras protein signal transduction and acute inflammatory responses (Figure 3, Supplementary Figure 8), while GO:CC terms related to exocytic and synaptic vesicles and components of synapses involved in γ-aminobutyric acid (GABA) and glutamate function (Figure 3, Supplementary Figure 9). These results were robust to selecting genes related to a specific cell-type, selecting the exact age-range where the gene-expression cortical thinning association was significant for a specific cell-type, and different thresholding parameters for the most significant genes and co-expression patterns (Online Figures- see Supplement section B4)

Discussion

Using MRI-based estimates of cortical thickness obtained from a multicohort sample of 14,248 healthy individuals, aged 4-89 years, we identified distinct spatial patterns of cell-specific gene expression, biological processes and cellular components underlying cortical thinning at different life-stages across the human cerebral cortex.

Higher expression levels of marker genes for microglia and CA1 pyramidal cells were associated with less cortical thinning in early life (<20 years) but with accelerated cortical thinning in late life (>60 years). These results largely replicate our previous findings in an adolescent sample [12], and in a smaller neuroimaging sample (N=4,004) with lifespan coverage [13]. The opposing associations between cortical thinning and expression levels of marker genes for CA1 pyramidal cells in early and late-life are also aligned with our previous findings [12,13], as well as with histological findings of neurite sprouting during development and shrinkage during ageing [14,15]. The same pattern was also noted for the expression of marker genes for microglia; this is consistent with their role in adaptive elimination of neurites and synapses during development and in facilitating pro-inflammatory responses in ageing [16,17]. The current study also confirmed the association between the level of expression of marker genes for S1 pyramidal cells and accelerated cortical thinning in early-life [12], and suggests that this association persists in mid-life. Marker genes for the S1 pyramidal cells are known to include those that regulate potassium signaling [12], which has been linked to cortical thinning during the consolidation of brain organization in development [31] and through apoptosis later in life [30,32]. We did not detect significant associations between cortical thinning and expression levels of gene markers for oligodendrocytes during early life, which is partly consistent with prior studies [12,13]. Nonetheless, accelerated cortical thinning has been associated with higher oligodendrocyte-specific genes in male adolescents [12], and with higher expression levels of a myelin-specific gene panel in children and adolescents regardless of sex [33].

The gene co-expression and enrichment supported and extended the virtual histology results. Across three paradigmatic age-groups, corresponding to early-, mid- and late-life, genes with the most substantial association with the corresponding life-stage cortical thinning profile and their co-expression networks were identified and tested for enrichment. In early life, enrichment identified genes involving GO:BP and GO:CC terms relating to cognition and the formation and maturation of synaptic circuits. In mid-life, enrichment involved GO:BP terms relating to neurotransmitter synthesis, transport and secretion and GO:CC terms, which support these processes across neurotransmitter systems in general, and for glutamatergic and GABAergic synapses in particular. Neurotransmission is the most important process of neuronal communication, and this pattern of enrichment highlights its importance for cortical organization throughout mid-life. In late life, GO:BP and GO:CC terms relating to Ras protein transduction and inflammation were most prominent. The human Ras superfamily of small guanosine triphosphatases (GTPases) has over 150 members implicated in multiple biological pathways [34]. Some of these RAS protein transduction genes, such as the CDC42SE2, the CDH13, and the NKAP1A have been implicated in the aetiology of neurodegenerative diseases [35-38]. Enrichment in GO:BP terms such as the cellular response to lipopolysaccharide in late, but not early life, supports the notion of a pro-inflammatory function for microglia in brain ageing [39].

An inherent limitation of the virtual histology approach is that the neuroimaging and the cell-specific gene expression data originate with different individuals. We have already demonstrated, however, the value of this approach in yielding mechanistic insights with regards to typical age-related cortical thinning [12,13,33,40], and cortical reductions associated with psychiatric disorders [13,22]. The current study benefits from a large MRI-based dataset of cortical thickness. Although the neuroimaging data are cross-sectional, there is overlap between contributing cohorts in their age-distribution. The overlap of the current virtual-histology results

with those from our previous longitudinal study [13](Vidal-Pineiro et al. 2020) strengthens confidence in these cross-sectional findings. Reliable mapping of gene-expression data to cortical regions was available only from left hemisphere, and derived from a small number of donors. Additionally, detailed gene expression levels mapped to cortical thickness are not currently available for different life-stages.

In summary, we show that neurotypical cortical thinning in early, mid-, and late life is associated with distinct biological pathways related, respectively, to synaptic modeling, neurotransmission, and neurodegeneration. This information provides useful insights into the potential mechanisms underlying age-related changes in cortical organization.

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Conflict of Interests

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. Any views expressed are those of the author(s) and not necessarily those of the funders.

Figure Legends

Figure 1. Virtual Histology of Cortical Thinning Profiles Across the Lifespan.

Significant correlations between inter-regional cortical thinning and cell-specific gene expression profiles across the lifespan. The horizontal axis represents age in years and the vertical axis the correlation coefficients. Positive correlation coefficients indicate less cortical thinning with higher expression levels of a cell-specific gene profile and the opposite is the case for negative coefficients. Color key: Astrocytes=Red; Mural=Brown; CA1 Pyramidal Cells=Green; Endolthelial=Yellow; Ependymal Cells=Purple; Interneuron=Orange; Microglia=Blue; Oligodendrocytes=Pink; S1 Pyramidal Cells=Gray.

Figure 2. Distribution of the Correlation Coefficients of Between Life-Stage Cortical Thickness Profiles and Inter-Regional Cell-Specific Gene Expression Levels in Early-, Mid- and Late-Life.

Each plot presents the distribution of the correlation coefficients of between life-stage cortical thickness profiles and the average inter-regional gene expression levels for each type of cells, in early-life (<20 years of age), mid-life (20-60 years of age), and late-life (>60 years of age). The horizontal axis represents the correlation coefficients between cortical thinning and expression profiles for each set of cell-specific marker genes and the vertical axis indicates the estimated probability density for the correlation coefficients. Each dot represents a correlation value between cortical thinning for a given age (in the corresponding age group) and cortical gene expression pattern related to any one of the cell-type specific genes (for the corresponding cell-type). In each age group, the widest age range with FDR-corrected significant results is chosen to generate the figure. The solid black line represents the mean correlation coefficient between the cortical thinning profile and gene-expression for that specific cell-type in that age-group. The dashed lines represent the mean and 95% confidence intervals for the null distribution.

Figure 3. Gene Ontology and Enrichment Analysis for Biological Processes and Cellular Components for Early-, Mid- and Late-Life.

Each panel represents the number of genes corresponding to gene ontology terms for biological processes and cellular components that were significantly associated with cortical thinning in early-life (<20 years of age), mid-life (20-60 years of age), and late-life (>60 years of age). The size of each circle represents the gene count and its color the *P*-value of the enrichment analysis results.

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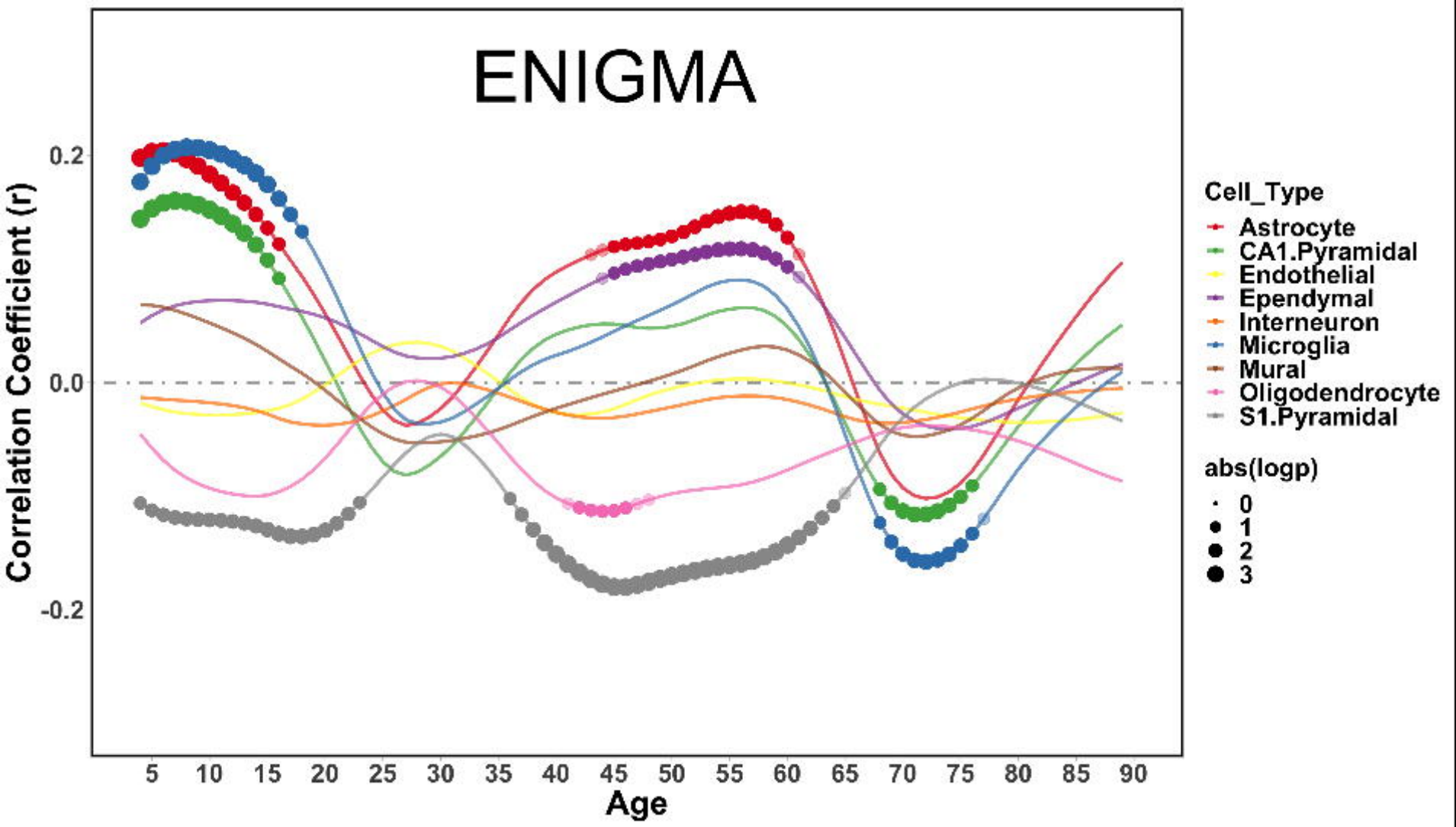
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-0.2
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85
90
Age
Cell_Type
Astrocyte
CA1.Pyramidal
Endothelial
Ependymal
Interneuron
Microglia
Mural
Oligodendrocyte
S1.Pyramidal
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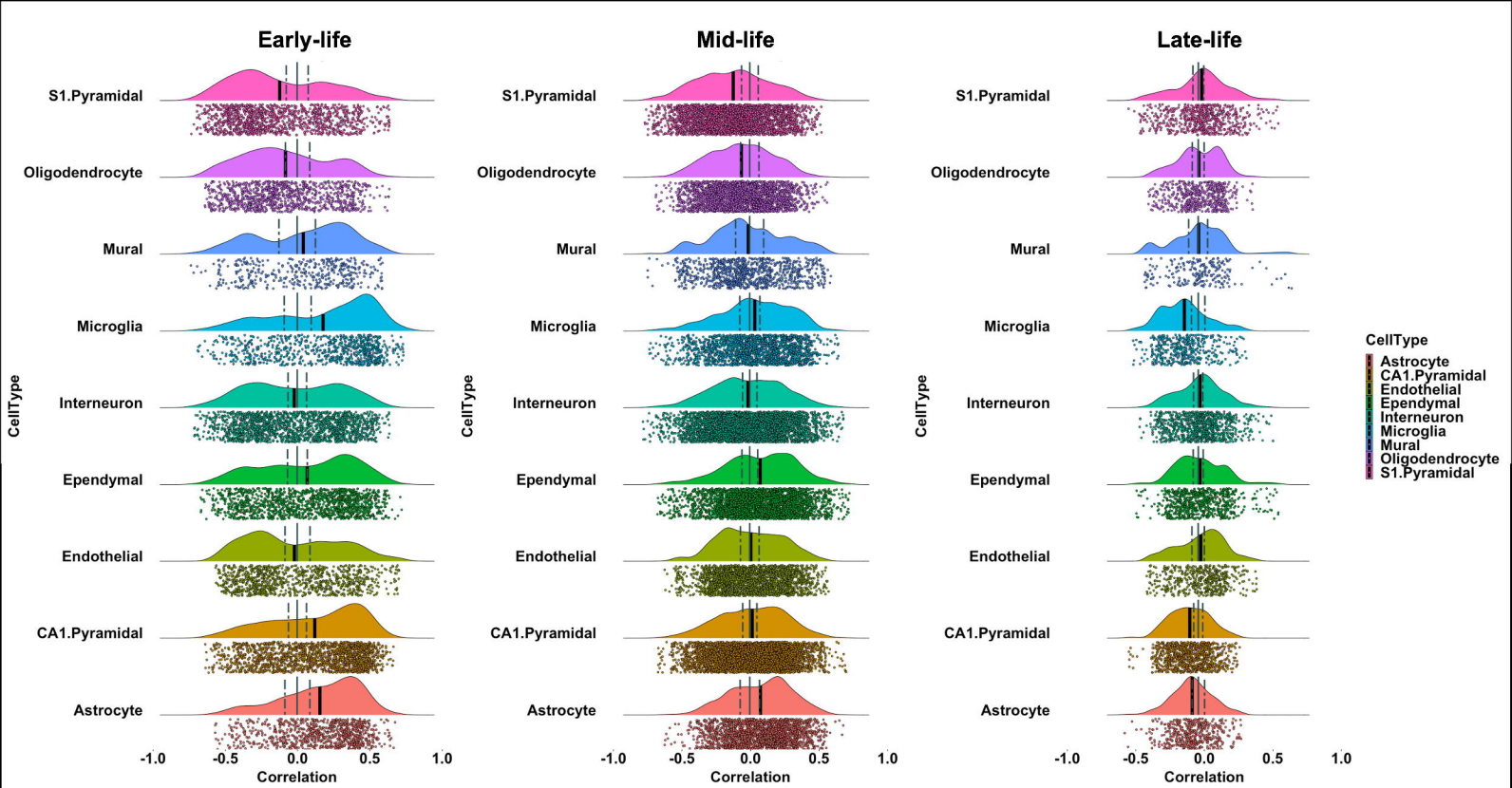
Early-life
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Late-life
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Interneuron
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CellType
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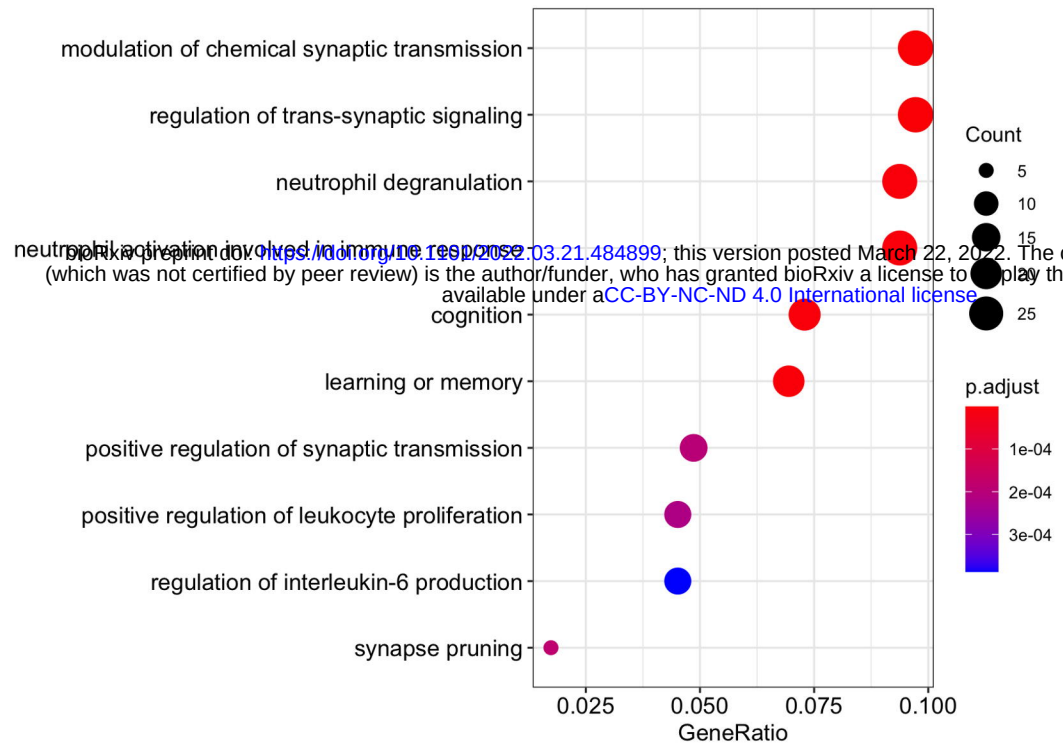
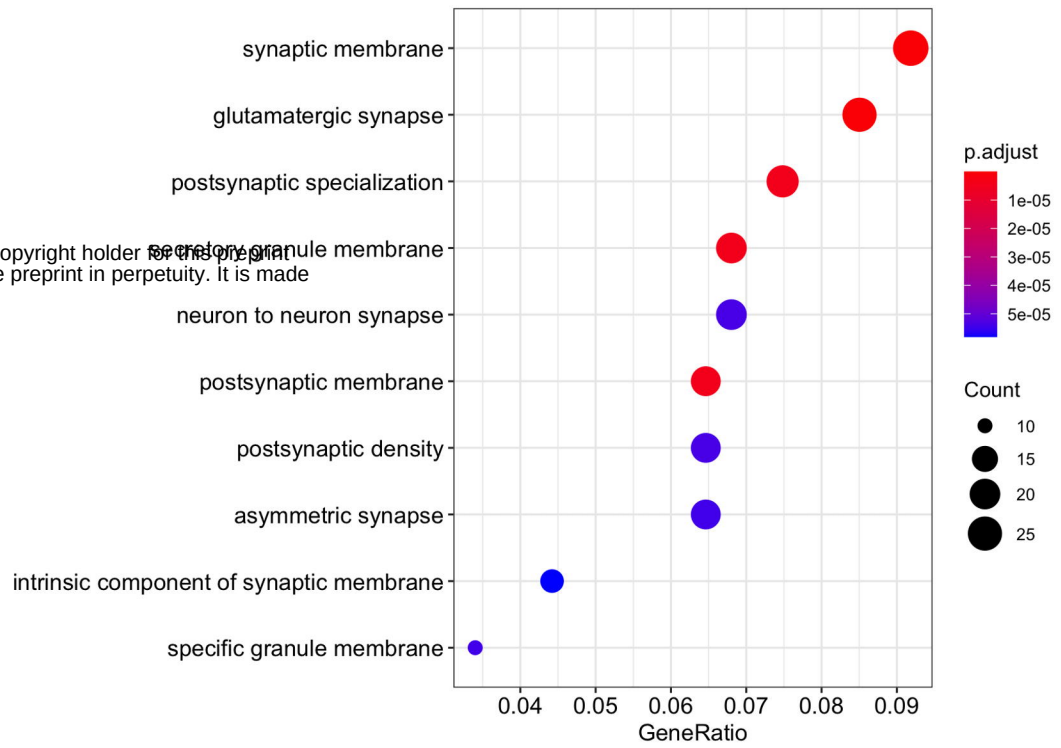
Biological Processes

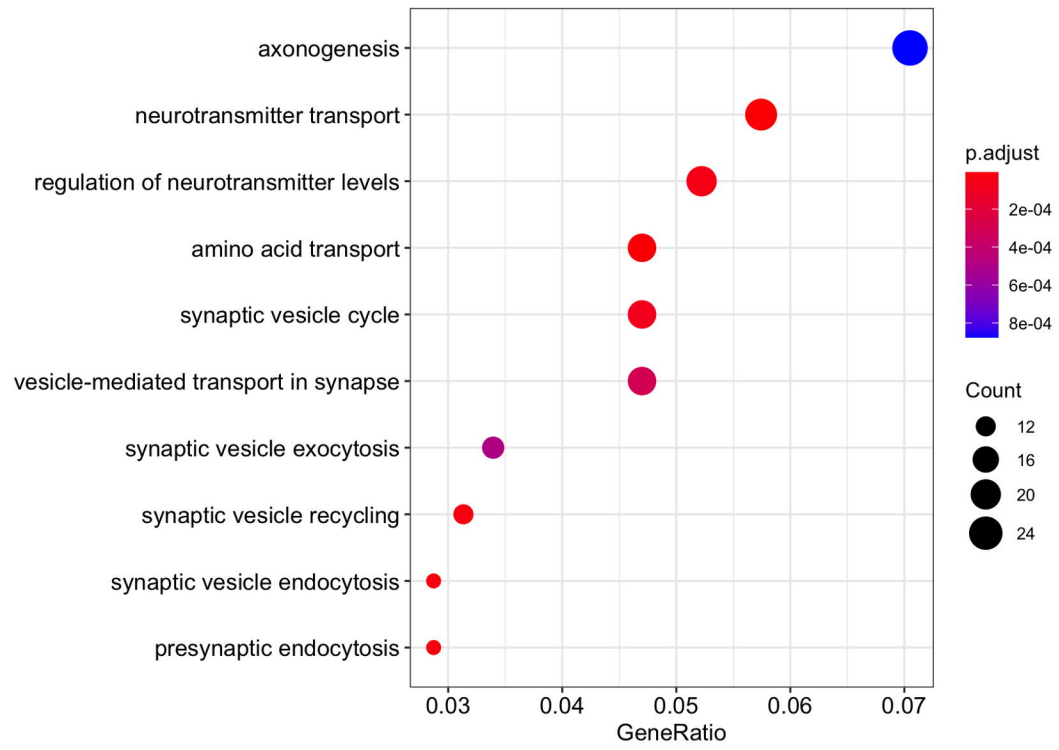


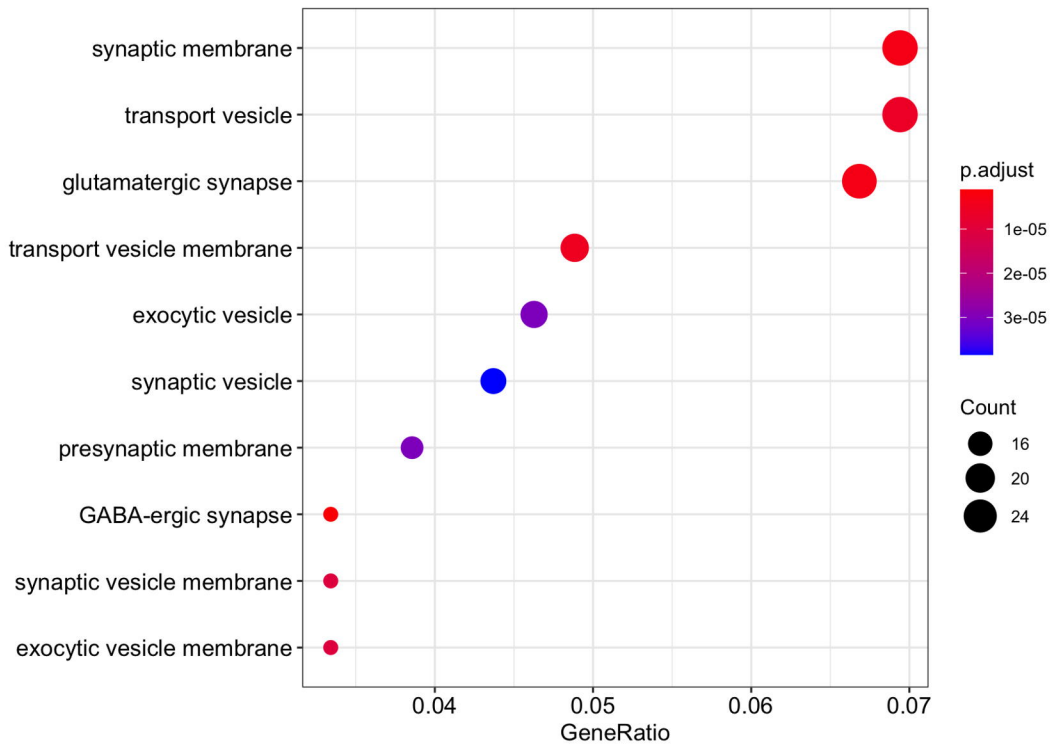
Cellular Components



Early-Life

Mid-Life





Late-Life