Alzheimer's Disease Disrupts Age-Related Senescence Gene Expression in the Human Fusiform Gyrus

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Abstract

Cellular senescence and mitochondrial dysfunction are hallmarks of aging, and interaction between senescence and mitochondrial dynamics affects aging trajectories. This study used secondary analysis of three bulk RNA-seq datasets of postmortem human brain tissue to test how senescence-associated and mitochondrial respiratory chain gene expression change together with age. It also tested how this relationship differs between patients with Alzheimer's disease and neurologically normal controls. In healthy brain tissue, neither senescence-associated nor mitochondrial respiratory chain gene expression showed a significant association with age. In two independent fusiform gyrus datasets , mitochondrial complexes I, III, IV, and V were significantly upregulated with age in Alzheimer's disease brains, while SenMayo genes were significantly downregulated with age in healthy controls but not in Alzheimer's disease brains. These findings replicated in a larger, partially overlapping cohort from the same research group and suggest that Alzheimer's disease disrupts the normal age-related trajectory of senescence-associated gene expression. This work identifies a transcriptional signature of disrupted senescence regulation in Alzheimer's disease and gives future single-cell studies a specific hypothesis to test about which cell types make these changes.

Introduction

Senescent cells accumulate in the brain with age and have been linked to neurodegenerative diseases (Melo Dos Santos et al.). However, most research on brain senescence focuses on diseases like Alzheimer's disease (AD), rather than on normal, healthy aging (Melo Dos Santos et al.). This means we do not actually know whether senescence is a normal part of brain aging on its own. In terms of human health, cellular senescence has many effects, mainly later in life. While senescence can be helpful early in life, for example, by stopping damaged cells from dividing and potentially becoming cancerous, its accumulation over time becomes dangerous (Melo Dos Santos et al.). When people age, the body becomes less efficient at clearing senescent cells, allowing them to build up in tissues and release constant inflammatory signals (Melo Dos Santos et al.). This increase in inflammation over time has been connected to many age-related health conditions, such as heart disease and diabetes (Childs et al.). In the brain specifically, the buildup of senescent cells is thought to contribute to the gradual decline in cognitive function seen in older adults, and in more severe cases, to diseases like Alzheimer's and Parkinson's (Melo Dos Santos et al.).

Cells can go through many changes in response to damage and stress. Two of the most known are cellular senescence and mitochondrial dysfunction. Cellular senescence is when a cell permanently stops dividing after being damaged or a problem occurs. These cells do not die, but instead release inflammatory signals that can affect the surrounding tissue (Melo Dos Santos et al.). Mitochondrial dysfunction is when the parts of the cell that are used for making energy start working less efficiently, which reduces energy availability and increases cellular stress as time progresses.

There is also evidence that mitochondrial gene expression declines as the brain ages. Hong et al. profiled mRNA expression in postmortem brain tissue from 191 donors aged 65 to 100, and they also profiled lymphocytes from 1,240 individuals aged 15 to 94 (Hong et al.). In both tissues, mitochondrial genes were overrepresented among the genes whose expression decreased with age, meaning the mRNA levels of those genes were lower in older individuals (Hong et al.). Expression of genes involved in oxidative phosphorylation showed this pattern in the brain but not in lymphocytes, which suggested that the mitochondrial changes of aging differ by tissue (Hong et al.). The study had several limitations. It used microarray technology, which detected only about 14,000 of the roughly 24,000 transcripts probed, and it measured expression across whole tissue instead of in individual cell types (Hong et al.). It also did not look for the mode of death, which can change mitochondrial gene expression in postmortem brain (Hong et al.). In addition, it could not determine whether declining mitochondrial gene expression causes aging or only reflects a general drop in metabolism in older people (Hong et al.). Although the paper used the term senescence, it used it to describe aging broadly and did not measure markers of cellular senescence, and it included no donors with Alzheimer's disease. To our knowledge, no study has directly compared senescence-associated and mitochondrial gene expression in the same human brain dataset, either in healthy aging or in Alzheimer's disease.

To address this gap, this study analyzed publicly available bulk RNA-seq data from postmortem human brain tissue using two gene sets: SenMayo, a senescence gene set (Saul et al.), and mitochondrial respiratory chain genes from MitoCarta3.0 (Rath et al.). The SenMayo gene set was validated across multiple tissues and species as a marker of senescent cell burden, and has been shown to decrease following senolytic treatment, confirming its specificity to senescent cells rather than general aging processes. In the context of this study, SenMayo was used to assess whether senescence-associated transcriptional activity changes with age in human brain tissue. Using GSEA (Subramanian et al.), genes were ranked by how much their expression changed with age in each brain region, and both gene sets were then tested to see if their expression changed together.

This study tested how the expression of senescence-associated genes and mitochondrial respiratory chain genes changed with age in bulk RNA-seq data from postmortem human brain tissue, first in healthy donors across several brain regions and then in Alzheimer’s disease patients and neurologically normal controls. We hypothesized that senescence-associated and mitochondrial respiratory chain gene expression would change together with age in healthy brains, and that Alzheimer's disease would alter this relationship. Comparing these two gene sets side by side in healthy and Alzheimer’s disease brains separates the transcriptional changes of normal aging from those specific to neurodegeneration. A stronger idea of how senescence and mitochondrial gene expression change in the healthy aging brain also gives future work a structure for recognizing what goes wrong in disease and for deciding which cell types and pathways to study next.

Methods

Secondary analysis was used on three publicly available bulk RNA-seq datasets of postmortem human brain tissue. The datasets were retrieved from the Gene Expression Omnibus (GEO) using GEOquery (Davis and Meltzer). The primary dataset, GSE80655,

contained 281 psychiatrically normal control donors across three brain regions, the dorsolateral prefrontal cortex, anterior cingulate, and nucleus accumbens, and spanned a wide adult age range (Ramaker et al.). Two additional fusiform gyrus datasets were used to examine AD: GSE95587, which contained 117 samples (Friedman et al.), and GSE125583, which contained 289 samples (Srinivasan et al.). Differential expression with age was tested using DESeq2 (Love et al.). For the combined GSE80655 dataset, the design formula included brain region and age (region + age), and for region specific subsets, age alone was used. Significance was defined as an adjusted p-value (padj) < 0.05. Senescence associated expression was assessed using the SenMayo gene set (Saul et al.), and mitochondrial respiratory chain expression was tested using gene sets from MitoCarta3.0 (Rath et al.). Gene Set Enrichment Analysis was performed using clusterProfiler (Wu et al.), with genes ranked by the DESeq2 Wald statistic. Gene symbols were mapped using org.Hs.eg.db (Carlson). Results were visualized as dot plots using ggplot2 (Wickham). To test whether the findings replicated in Alzheimer's disease, the same pipeline was applied to the two fusiform gyrus datasets. For each dataset, DESeq2 was first run on the full sample set with the design ~diagnosis to identify differences in gene expression between AD and control groups overall. Samples were then subset by diagnosis, and DESeq2 was run separately within each group with the design ~age to test the effect of age on gene expression independently in AD and control brains.

Results

	GSE80655	**GSE95587**	**GSE125583**
Tissue	DLPFC, AnCg, nAcc	Fusiform gyrus	Fusiform gyrus
Total samples	281	117	289
Diagnosis	Healthy controls	84 AD, 33 control	219 AD, 70 control
Mean age	46.74	85.56	84.26
Median age	48	86	85
Age range	19-72	60-103	60-103
Female	59	52	130
Male	222	65	158
Platform	Illumina HiSeq 2000	Illumina HiSeq 2000	Illumina HiSeq 2000

Table 1: Summary of datasets included in this study. GSE80655 contains healthy control donors across three brain regions. GSE95587 and GSE125583 contain postmortem fusiform gyrus tissue from Alzheimer's disease patients and neurologically normal controls. Age statistics for GSE125583 exclude one sample with missing age metadata. Sex counts for GSE125583 exclude one sample with missing sex metadata. AD = Alzheimer's disease; DLPFC = dorsolateral prefrontal cortex; AnCg = anterior cingulate cortex; nAcc = nucleus accumbens.

We first examined how senescence-associated and mitochondrial respiratory chain gene expression changed with age in healthy human brain tissue. For this analysis, we used GSE80655, a bulk RNA-seq dataset of 281 postmortem human brain samples from three brain regions. Differential expression analysis was then run with DESeq2 using the design formula ~region + age, which estimated the effect of age on gene expression while controlling for brain region. In total, 11,862 genes had significantly higher expression with age, and 4,725 genes had significantly lower expression (padj < 0.05) [Figure 1]. The log2 fold changes were small (approximately -0.05 to 0.10) because age was a continuous variable in the design formula. Each fold change therefore reflected the difference in expression per year of age and not a comparison between two discrete groups.

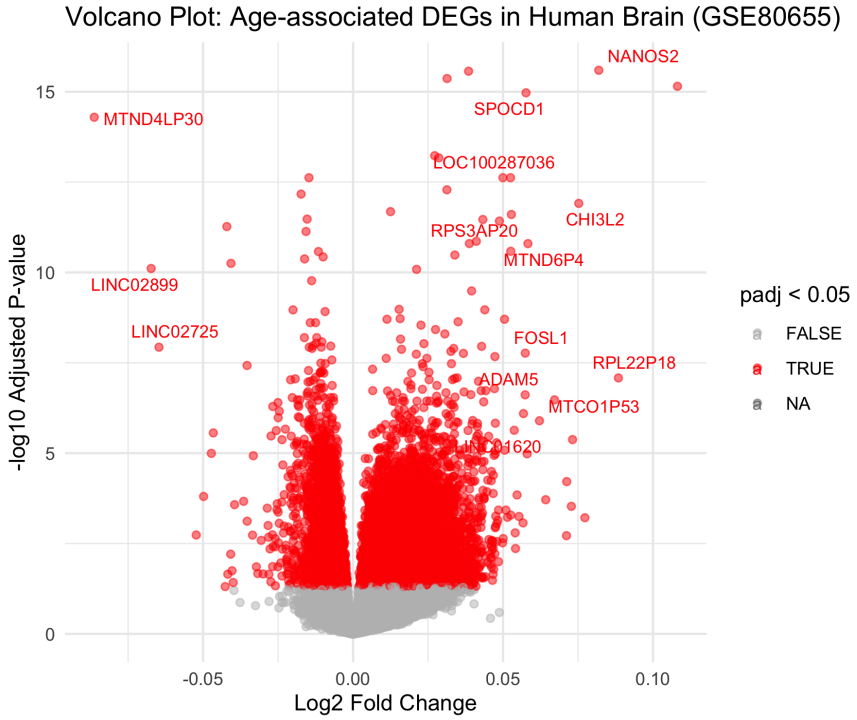

Figure 1: Volcano plot showing age-associated differentially expressed genes in postmortem human brain tissue from GSE80655. These were from 281 samples across DLPFC, anterior cingulate, and nucleus accumbens. Log2 fold change magnitudes are small (range approximately -0.05 to 0.10), reflecting per-year expression differences from the continuous age design rather than a group comparison. Red dots indicate genes with padj < 0.05. X-axis shows log2 fold change with age, y-axis shows -log10 adjusted p-value.

GSEA using the SenMayo senescence gene set and MitoCarta3.0 mitochondrial complex gene sets showed no statistically significant enrichment for either gene set (SenMayo NES = 1.08, padj = 0.95). These results show that senescence-associated and mitochondrial gene expression do not have detectable coordinated changes with age in bulk brain tissue from healthy controls.

Next, we examined how the relationship between senescence-associated and mitochondrial gene expression differed between patients with Alzheimer's disease and neurologically normal

controls. For this analysis, we used the first of two fusiform gyrus datasets, which contained postmortem samples from 84 Alzheimer's disease patients and 33 controls. We first compared gene expression between all Alzheimer's disease samples and all control samples, using a model with diagnosis as the only predictor. Expression of 5,572 genes was significantly higher in Alzheimer's disease samples, and expression of 4,838 genes was significantly lower (padj < 0.05) [Figure 2A]. We then split the samples by diagnosis and tested the effect of age on gene expression separately in the Alzheimer's disease group and in the control group, using a model with age as the only predictor. This approach let us compare how age affected gene expression in each group.

To confirm these findings in a larger sample, we repeated the same analysis in a second, larger fusiform gyrus dataset from the same research group, which shared a subset of samples with the first dataset. DESeq2 identified 7,494 genes upregulated and 6,788 genes downregulated between AD and control samples (padj < 0.05). Within AD samples, 393 genes were upregulated and 471 downregulated with age. Within control samples, almost no genes changed significantly with age (0 up, 2 down), consistent with GSE95587 [Figure 2B].

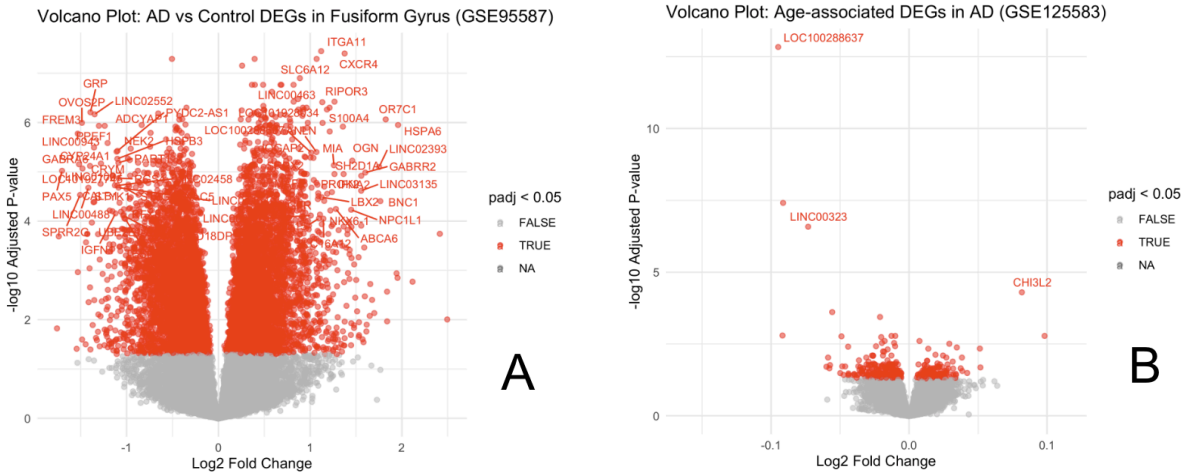

Figure 2: (A) Volcano plot showing differentially expressed genes between AD and neurologically normal control samples in postmortem fusiform gyrus tissue from GSE95587 (117 samples). (B) Volcano plot showing age-associated differentially expressed genes within AD samples from GSE125583 (218 AD samples; one additional AD sample was excluded due to missing age data). Red dots indicate genes with padj < 0.05.

Within the AD group, 124 genes were upregulated and 102 downregulated with age. Within the control group, only 3 genes were upregulated and 12 downregulated with age, suggesting that age has a much stronger transcriptional effect within AD brains than in healthy brains.
GSEA revealed important differences between the two groups [Figure 3]. In AD samples, mitochondrial complexes I, III, IV, and V were all significantly upregulated with age (ComplexI NES = 2.73, padj = 6.0e-10; ComplexV NES = 2.26, padj = 3.0e-05; ComplexIV NES = 2.15, padj = 4.6e-06; ComplexIII NES = 1.97, padj = 1.4e-03), while SenMayo was not significant (NES = -1.44, padj = 0.22). In contrast, in control samples, SenMayo was significantly downregulated with age (NES = -2.00, padj = 2.12e-06), while mitochondrial complexes showed weaker or non-significant enrichment.

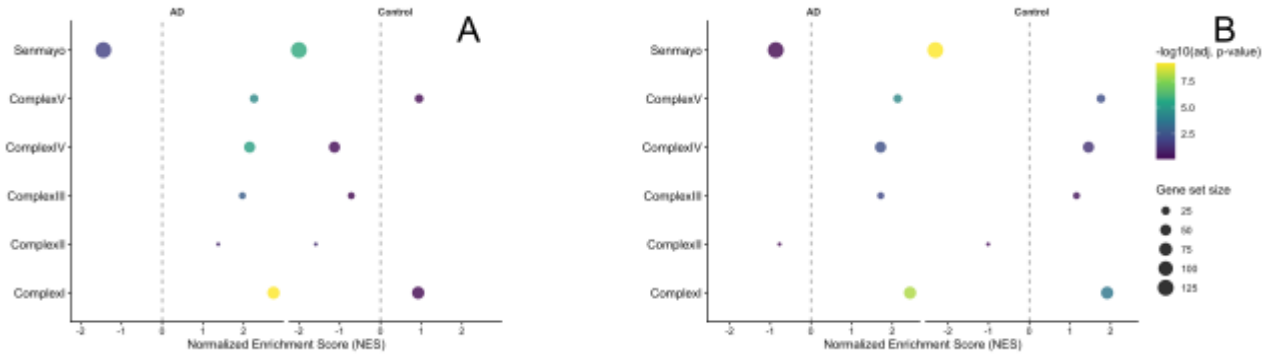

Figure 3: Dot plots showing GSEA results for SenMayo and MitoCarta3.0 mitochondrial complex gene sets in AD and control samples from (A) GSE95587 and (B) GSE125583. Point color indicates -log10 adjusted p-value (brighter = more significant) and point size indicates gene set size. Dashed line marks NES = 0; points to the right are enriched among genes increasing with age, points to the left among genes decreasing with age.

GSEA results closely replicated those from GSE95587 [Figure 3B]. In AD samples, mitochondrial complexes I, III, IV, and V were significantly upregulated with age (ComplexI NES = 2.44, padj = 8.47e-09; ComplexV NES = 2.13, padj = 3.40e-05; ComplexIV NES = 1.71, padj = 4.40e-03; ComplexIII NES = 1.72, padj = 8.10e-03), while SenMayo remained non-significant (NES = -0.87, padj = 0.79). In control samples, SenMayo was significantly downregulated with age (NES = -2.31, padj = 6.0e-10), while mitochondrial complexes showed weaker enrichment. The consistent replication of these findings across both fusiform gyrus datasets strengthens confidence in these results, although the two cohorts are not fully independent.

Discussion

This study used secondary analysis of three publicly available bulk RNA-seq datasets to examine how senescence associated and mitochondrial respiratory chain gene expression relate to each other during human brain aging. The primary dataset, GSE80655, was used to test this directly in healthy brain tissue, while two additional datasets, GSE95587 and GSE125583, were used to examine how these patterns change in the context of Alzheimer's disease.

In GSE80655, GSEA using the SenMayo senescence gene set and MitoCarta3.0 mitochondrial complex gene sets showed no statistically significant enrichment in healthy brain tissue across age. This negative result suggests that in brains from psychiatrically normal individuals, senescence associated and mitochondrial respiratory chain gene expression did not show a detectable coordinated change with age in bulk tissue. However, bulk RNA-seq averages expression across all cell types in the tissue, which may dilute cell-type-specific senescence signals that would only be detectable at the single-cell level. Single nucleus and spatial transcriptomic work in the human dorsolateral prefrontal cortex, one of the three regions in this dataset, suggests that senescence related changes do occur in this region. Oligodendrocytes in the human dorsolateral prefrontal cortex, one of the three regions sampled in GSE80655, show multiple hallmarks of senescence, including a linear increase in CDKN2A expression with age,

alongside declining oxidative phosphorylation-related gene expression across dlPFC cell types (Sloan et al.). Because these effects were detected at single-nucleus resolution and concentrated in specific cell populations rather than distributed evenly across the tissue, they would likely fall below the threshold of detection in a bulk RNA-seq approach like the one used here. This evidence supports the interpretation that the null GSEA result shows the limited resolution of bulk tissue and not an absence of senescence associated expression changes in these brain regions.

In both GSE95587 and GSE125583, age had almost no transcriptional effect in healthy control brains (3 up/12 down in GSE95587; 0 up/2 down in GSE125583), while hundreds of genes changed significantly with age within AD brains (124 up/102 down in GSE95587; 393 up/471 down in GSE125583). This difference between groups indicates that AD alters how the brain responds to aging at the transcriptional level, amplifying age-related changes that are otherwise transcriptionally silent in healthy tissue.

Within AD brains, mitochondrial complexes I, III, IV, and V were significantly upregulated with age in both datasets. Mitochondrial dysfunction is a well-established hallmark of AD, so a decline in mitochondrial gene expression might be expected (Wang et al.). One possible interpretation of the observed upregulation is a compensatory transcriptional response, in which cells increase expression of mitochondrial respiratory chain genes in an attempt to offset declining mitochondrial function. However, because this study measured only transcript-level changes, it cannot distinguish a true compensatory mechanism from other explanations, such as stress-induced transcriptional activation that does not correspond to increased functional mitochondrial activity, or shifts in cellular composition with age. This pattern replicated across four complexes and both datasets, making the transcriptional finding itself robust, but the biological mechanism is still uncertain.

SenMayo genes were significantly downregulated with age in healthy control brains in both AD datasets, while showing no significant age-related trend in AD brains. This decline was consistent across both control cohorts, suggesting it reflects a genuine, reproducible pattern in healthy fusiform gyrus tissue rather than a dataset-specific artifact, and the biological process cannot be determined from only transcriptomic data. The AD disease process disrupts this normal trajectory by eliminating the decline seen in healthy aging rather than driving senescence genes up. This indicates that AD interferes with whatever regulatory process underlies senescence-associated gene expression in normal aging, which may distinguish pathological neurodegeneration from normal brain aging.

Several limitations should be noted. Bulk RNA-seq averages expression across all cell types, which may obscure cell-type-specific signals. Single-cell or single-nucleus RNA-seq would provide greater resolution for future studies. GSE80655 uses psychiatrically normal donors rather than neurologically normal donors; the original study screened donors via toxicology and medical record review as part of diagnosis, but detailed medication history was not available in the GEO metadata used for this analysis, so residual confounding from medication use cannot be fully excluded at the level of this secondary analysis. The fusiform gyrus datasets were generated by the same research group and sequencing platform, and approximately 89 of the 289 samples in GSE125583 overlap with subjects previously profiled in GSE95587, meaning

these two datasets are not fully independent cohorts. This may limit how much the GSE125583 analysis represents a truly independent confirmation of the GSE95587 findings, though the additional roughly 200 new samples still meaningfully expand statistical power. Finally, the age ranges of the three datasets differ substantially, with GSE80655 spanning ages 19 to 72 while the AD datasets span 60 to 103, making direct cross-dataset comparisons difficult.

Conclusion

This project used secondary analysis of bulk RNA-seq data from three postmortem human brain datasets to investigate the transcriptional relationship between senescence and mitochondrial gene expression during brain aging. While no coordinated signal was detected in healthy brain tissue, analysis of Alzheimer's disease datasets revealed two consistent and replicable findings: mitochondrial respiratory chain genes are upregulated with age specifically within AD brains, and senescence-associated genes decline with age in healthy brains but lose this trajectory in AD. These findings suggest that AD fundamentally alters how the brain ages at the transcriptional level, disrupting normal senescence regulation and amplifying mitochondrial gene expression in an age-dependent manner. Future work using single-cell sequencing and additional brain regions could help identify the specific cell types driving these patterns and clarify whether mitochondrial upregulation represents a compensatory or pathological response.

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