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Article

Upregulation of the AP-1 complex as a potential biomarker for Alzheimer's disease (AD)

Joy Zhu[1], Addy Veerendra[2], Shruthi Shankar[3], Ananya Cheri[4], Tammy Khong[5], Inhan Lee[6]

[1] BASIS Independent Fremont, Fremont, California
[2] Academy for Science and Design, Nashua, New Hampshire
[3] Lambert High School, Suwanee, Georgia
[4] Bridgeland High School, Cypress, Texas
[5] University of California: San Diego, La Jolla, California
[6] miRcore, Ann Arbor, Michigan

SUMMARY

Alzheimer's disease (AD) is a major neurodegenerative disorder caused by the structural protein buildup of neuritic plaques and tangles, which lead to neuronal death. AD symptoms may be exacerbated by inflammation, an immune response which can accelerate plaque formation. This plaque formation, also known as atherogenesis, reduces blood flow throughout the body and to neurons, which can worsen AD significantly. Because of the association between inflammation, atherogenesis, and AD, we hypothesized that genetic biomarkers present in inflammation and atherogenesis pathways could also be used for AD diagnosis. To test our hypothesis, we analyzed hippocampal expression data from AD and control patients to determine the most statistically significant differentially expressed genes. We analyzed the top 250 differentially expressed genes by significance to create a gene product interaction network graph, and we performed enrichment testing, which allowed us to identify enriched biological pathways and processes. Two enriched pathways were the inflammatory IL-17 signaling pathway and the fluid shear stress and atherosclerosis pathway, which shared enriched expression of genes whose protein products compose the Activator Protein 1 (AP-1) transcription complex. The increased activity of the AP-1 transcription complex, as well as the upregulation of inflammation and atherosclerosis, supports our hypothesis that biomarkers involved in inflammatory and atherosclerotic pathways can be used to predict AD in asymptomatic individuals. These results can be further investigated in future studies to determine whether the AP-1 complex can be targeted for therapies to reduce or reverse the symptoms of AD.

INTRODUCTION

Neurodegenerative disorders arise from a variety of different causes and involve complex mechanisms, many of which are not fully understood. Alzheimer's disease (AD) is a common neurodegenerative disorder, impacting 6.7 million people worldwide with the hallmark symptom of memory loss (1). AD is diagnosed by the presence of neuritic plaques and neurofibrillary tangles; the former referring to the accumulation of amyloid-beta (Aβ) protein, and the latter the accumulation of tau protein (2). Aβ comes from amyloid precursor protein (APP), a transmembrane protein that enhances cell growth. APP is cleaved by secretases to yield either the normal p3 fragment or the toxic Aβ fragment (3). These misfolded Aβ fragments then form extracellular clusters that accumulate with other fragments until their toxicity causes neuronal death (3). Neurofibrillary plaques, on the other hand, arise from hyperphosphorylation of tau, a microtubule binding protein, causing it to form intracellular tangles that have similar effects on neurons as Aβ plaques (2). Despite advances in understanding the mechanisms of AD, diagnosis and treatment remain challenging (2), warranting further biological studies.

Genetics are thought to play a crucial role in AD risk. For example, the Apolipoprotein E (*APOE*) gene, which encodes a lipid transport protein, appears to be a major genetic determinant in AD onset and progression (3). The expression of the allele *APOE4* is found to increase the risk of developing AD through lipid dysregulation (4). Additionally, other abnormalities in gene regulation may also affect the risk of developing AD, such as increased expression of genes involved in APP processing (3). Mutations in *PSEN1* and *PSEN2,* whose gene products compose the γ-secretase protein complex that cleaves APP, lead to the early onset of AD (3).

Other conditions and cellular functions can promote the progression of AD or indicate increased disease severity. Research has shown that susceptibility to vascular conditions could also contribute to increased risk for AD. Atherosclerosis, the buildup of plaques in the walls of arteries, is a specific vascular condition that has significant association with AD, as intracranial atherosclerosis greatly increases the risk of dementia in patients (5, 6). The reduced blood and oxygen circulation in the brain due to atherosclerosis has been found to promote Aβ accumulation, which in turn creates a positive feedback loop by negatively impacting cerebral blood flow and contributing to cognitive decline (6). Atherosclerosis is exacerbated by the fluid shear stress pathway, since disturbed blood flow is pro-atherogenic and increases inflammation in walls of blood vessels (7). Inflammation is another condition that is associated with AD. Studies indicate that inflammation of neurons and blood vessels in the brain are heightened in Alzheimer's patients, with Aβ buildup enhancing the expression of pro-inflammatory genes (8). Furthermore, the presence of pro-inflammatory microglia in the brain has been found to correlate with the buildup of tau proteins that is characteristic of AD (9). A common factor in both inflammation and atherosclerosis is Activator Protein 1 (AP-1), a protein complex consisting of a dimer of Jun and Fos, which each consist of multiple smaller proteins (8). Notably

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Jun was determined to be activated by Aβ — specifically, both Jun expression levels and activation levels were increased in the presence of Aβ (8).

As of now, there are no known treatments for AD (1). Thus, early detection is valuable for determining appropriate treatment plans to stifle its progression. Current practices for identifying AD include brain imaging techniques and biomarker testing from cerebrospinal fluid or blood (2). However, there are still limitations in grasping the pathogenesis of AD and the conditions that colocalize with it. For example, although brain inflammation has been found to be an influential factor in AD, a comprehensive understanding of its role has yet to be achieved (9). Overall, it would be beneficial to find more robust biomarkers in the earlier stages of disease development in order to improve symptom management and enhance treatment effectiveness.

Our research aimed to explore the connections between Alzheimer's, atherosclerosis, and inflammation through computational research on gene expression and interactions. Based on prior studies indicating a positive correlation between intracranial atherosclerosis and the onset of Alzheimer's symptoms, we hypothesized that upregulation of biological pathways involving atherogenesis and inflammation may play a significant role in the development of AD and that the components of such pathways could potentially be explored further as therapeutic targets to reduce or even reverse the progression of AD (5, 6). To test this prediction, we performed differential expression analysis on microarray data collected from AD patients. We then explored enriched protein-protein interactions and enriched biological pathways among differentially expressed genes in order to determine possible biomarkers for AD. Our study revealed that the fluid shear stress and atherosclerosis pathway and the inflammatory IL-17 pathway were upregulated in AD patients. Both pathways shared increased expression of the AP-1 transcription factor complex. The enrichment of the AP-1 complex and its roles in major enriched biological pathways in AD points to the entire complex's potential as a viable biomarker that can be targeted for future disease risk assessments and treatments.

RESULTS

Analyzing differential gene expression

To explore the transcriptional differences between individuals with AD and unaffected individuals, we analyzed the publicly available Gene Expression Omnibus dataset GSE29378, which includes gene expression data from microarrays prepared from the hippocampal regions of 31 AD human patients and 32 control human patients. (11, 12, **Table 1**). 51.61% of the AD group patients were male and 48.39% of the AD group patients were female, while 68.75% of the AD group patients were male and 31.25% of the AD group patients were female. The average age of the AD group was 76.55 years, and the average age of the control group was 81.63 years. We generated a heatmap to visualize and compare gene expression levels of each of the patient samples and found that the control and disease samples had different patterns of gene regulation (13, **Figure 1**). The samples were clustered based on similarities in gene expression of the top 100 most differentially expressed genes. Most of the samples tended to cluster together with other samples that belonged to the same disease state, indicating that samples belonging to the same disease state tended to be more similar to one another compared to samples from the other disease state. Although the samples are generally clustered together by disease state, the separation is incomplete and could be attributed to the variation of gene expression between samples in the same disease state. The general separation of the control and disease samples, combined with the clustering of samples of the same disease group together, demonstrates the distinguishable difference in gene regulation between patients with AD and individuals without AD (**Figure 1**). Gene expression was generally elevated in the AD samples compared to the controls (**Figure 1**).

We generated a volcano plot with GEO2R to visualize the number of differentially expressed genes and the extents of their differential expression, which were computed with a two-sample *t*-test. Through our GEO2R analysis, we noticed many differentially expressed genes as well as three particular genes significant to our research (**Figure 2**). 5281 genes had a statistically significant level of differential gene expression in AD samples compared to controls ($p < 0.05$). Of those genes, 107 were downregulated in the AD group (log2FC < -0.5), while 164 genes were upregulated in the AD group (log2FC > 0.5). The most statistically significant downregulated genes in the AD group were *LZTS1*, *NCALD*, and *CA10*, and the most statistically significant upregulated genes in the AD group were *RGS1*, *CD163*, and *FOS* (**Figure 2**).

GEO Accession	Disease State	Hippocampus Region	Sex	Age	GEO Accession	Disease State	Hippocampus Region	Sex	Age
GSM726118	AD	CA1	Male	68	GSM726117	Control	CA1	Male	90
GSM726096	AD	CA3	Male	68	GSM726088	Control	CA3	Male	90
GSM726107	AD	CA1	Female	90	GSM726100	Control	CA1	Female	83
GSM726098	AD	CA3	Female	90	GSM726140	Control	CA3	Female	83
GSM726111	AD	CA1	Female	84	GSM726109	Control	CA1	Male	85
GSM726135	AD	CA3	Female	84	GSM726125	Control	CA3	Male	85
GSM726094	AD	CA1	Male	67	GSM726134	Control	CA1	Male	81
GSM726123	AD	CA3	Male	67	GSM726084	Control	CA3	Male	81
GSM726138	AD	CA1	Male	72	GSM726139	Control	CA1	Male	90
GSM726092	AD	CA3	Male	72	GSM726090	Control	CA3	Male	90
GSM726095	AD	CA3	Male	90	GSM726102	Control	CA1	Male	73
GSM726115	AD	CA1	Male	81	GSM726103	Control	CA3	Male	73
GSM726114	AD	CA3	Male	81	GSM726099	Control	CA1	Female	74
GSM726145	AD	CA1	Female	68	GSM726144	Control	CA3	Female	74
GSM726142	AD	CA3	Female	68	GSM726086	Control	CA1	Male	86
GSM726091	AD	CA1	Female	61	GSM726131	Control	CA3	Male	86
GSM726143	AD	CA3	Female	61	GSM726089	Control	CA1	Female	88
GSM726093	AD	CA1	Male	66	GSM726136	Control	CA3	Female	88
GSM726083	AD	CA3	Male	66	GSM726121	Control	CA1	Male	84
GSM726129	AD	CA1	Female	74	GSM726133	Control	CA3	Male	84
GSM726141	AD	CA3	Female	74	GSM726085	Control	CA1	Female	72
GSM726120	AD	CA1	Female	79	GSM726113	Control	CA3	Female	72
GSM726106	AD	CA3	Female	79	GSM726130	Control	CA1	Male	80
GSM726108	AD	CA1	Female	80	GSM726137	Control	CA3	Male	80
GSM726087	AD	CA1	Male	81	GSM726105	Control	CA1	Male	85
GSM726119	AD	CA3	Male	81	GSM726132	Control	CA3	Male	85
GSM726128	AD	CA1	Male	90	GSM726124	Control	CA1	Male	75
GSM726122	AD	CA3	Male	90	GSM726110	Control	CA3	Male	75
GSM726127	AD	CA1	Female	79	GSM726097	Control	CA1	Male	90
GSM726126	AD	CA3	Female	79	GSM726112	Control	CA3	Male	90
GSM726101	AD	CA1	Male	83	GSM726116	Control	CA1	Female	70
					GSM726104	Control	CA3	Female	70

Table 1: Samples in the GSE29378 dataset sorted by disease state. The 63 samples from the GSE29378 dataset (12). The "GEO Accession" columns contain the unique identification codes for each sample. The "Disease State" columns identify if a sample comes from an Alzheimer's disease (AD) patient or a control patient without AD. Additional information, such as hippocampus region, gender, and age, is also listed. This data was obtained from Gene Expression Omnibus, an online database of array and sequence-based data (11).

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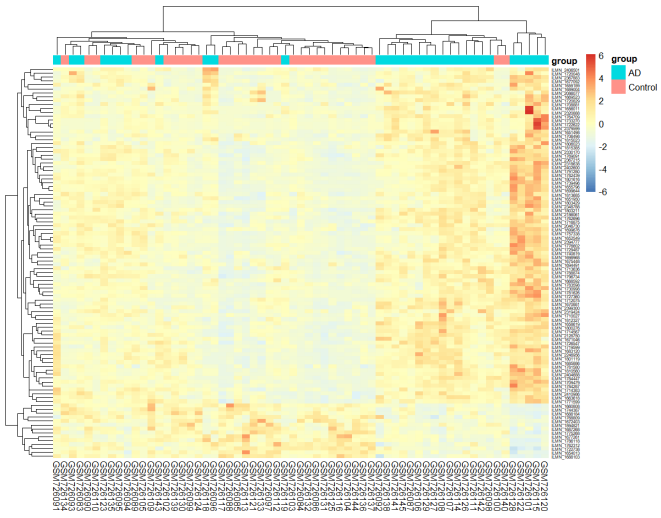

Figure 1: Differential gene expression in Alzheimer's disease (AD) and control samples. Heatmap of the top 100 differentially expressed genes with the lowest *p*-values. Each column represents a sample from the GSE29378 dataset, and each row represents a gene (12). AD samples are labeled in teal, while control samples are labeled in salmon. The samples and genes were grouped based on similarity with a complete linkage hierarchical clustering method. Expression values are color-coded by intensity, with warmer colors indicating higher gene expression, and colder colors indicating lower gene expression (13).

Analyzing enriched pathways and genes

We then explored connections between the top 250 differentially expressed genes with STRING-db, which identified various enriched Gene Ontology (GO) cellular components (14, **Table 2**). The transcription factor AP-1 complex (GO ID 0035976) was the most highly enriched cellular component by strength. The AP-1 complex is a transcription factor that regulates the expression of multiple genes, including neuroinflammatory genes involved in AD pathology (8). It is composed of the proteins FOS, JUN, JUNB, JUND, and NFATC2 (14, **Figure 3**). The genes encoding three of the proteins in this complex, *JUN* ($p = 0.0016$, log2FC = 0.548), *JUNB* ($p = 0.00124$, log2FC = 0.618), and *FOS* ($p = 0.000428$, log2FC = 1.285), were significantly upregulated in our GEO2R dataset (**Figure 4**). Upregulation is shown as positive log2FC values in this two-sample *t*-test, with larger numbers signifying a larger difference in gene expression between the two groups.

Analyzing the impact of gene upregulation on various pathways

Our STRING-db analysis also revealed seven statistically significant enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) biological pathways (15, **Table 2**). These pathways are the pertussis, colorectal cancer, rheumatoid arthritis, IL-17 signaling, fluid shear stress and atherosclerosis, salmonella infection, and MAPK signaling pathways. Out of all the enriched pathways, we observed two pathways that involve the AP-1 complex: the IL-17 signaling pathway (KEGG ID 04657) and the fluid shear stress and atherosclerosis pathway (KEGG ID 05418).

DISCUSSION

The goal of our study was to explore the association between AD, atherosclerosis, and inflammation, as these conditions may be comorbid with one another (6, 9). We hypothesized that genes involved in atherogenesis and inflammatory pathways were also involved in AD pathogenesis and performed differential expression analysis on an Alzheimer's dataset to discern possible biomarkers for AD. By using STRING-db and GO, we identified three significantly enriched genes in AD samples relative to controls: *JUN*, *JUNB*, and *FOS*, which are key components of the AP-1 transcription complex (8). We also found two significantly enriched pathways through KEGG that involved these gene products: the IL-17 signaling pathway and fluid shear stress and atherosclerosis pathway. Within the IL-17 signaling pathway, the AP-1 complex serves to activate inflammatory gene transcription (16). In the fluid shear stress and atherosclerosis pathway, the AP-1 complex plays a similar pro-inflammatory role by activating genes involved in immune functions and atherogenesis (7, 17). We conclude that there is increased mRNA expression of the AP-1 complex in AD patients, which may point to increased activity in inflammation and atherosclerosis pathways.

The results from our study suggest that the AP-1 transcription factor complex is involved in the pathophysiology of AD and can potentially be targeted by therapies for the disease. The upregulation of genes encoding AP-1 complex components in AD patients demonstrates that increased

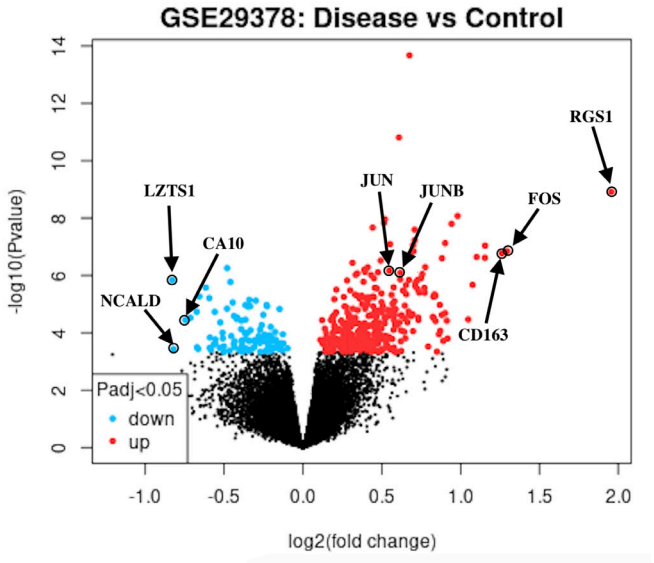

Figure 2: Comparing differential expression between AD and control samples. Volcano plot of GEO2R analysis of dataset GSE29378 (11, 12). Each dot represents a gene, and genes with statistically significant differential expression are highlighted in either blue or red. Blue genes have lower expression in the AD group relative to the control group, while red genes have higher expression in the AD group. Black dots represent statistically insignificant genes with p-values above the significance threshold ($p > 0.05$). Three genes of interest that encode members of the AP-1 complex, JUN, JUNB, and FOS, are labeled in addition to the three most statistically significant upregulated (RGS1, CD163, FOS) and downregulated (LZTS1, NCALD, CA10) genes.

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Enriched Cellular Components (GO)			
GO ID	Cellular Component Description	Strength	False Discovery Rate
GO:0035976	Transcription factor AP-1 complex	1.7	0.0070
GO:0043034	Costamere	1.32	0.0066
GO:0005879	Axonemal microtubule	1.07	0.0089
GO:0072562	Blood microparticle	0.81	0.0022
GO:0042383	Sarcolemma	0.8	0.00096
GO:0062023	Collagen-containing extracellular matrix	0.7	5.15e-07
GO:0031012	Extracellular matrix	0.63	5.15e-07
Enriched Pathways (KEGG)			
KEGG ID	Pathway Description	Strength	False Discovery Rate
hsa05133	Pertussis	0.84	0.0280
hsa05210	Colorectal cancer	0.79	0.0401
hsa05323	Rheumatoid arthritis	0.78	0.0401
hsa04657	IL-17 signaling pathway	0.74	0.0478
hsa05418	Fluid shear stress and atherosclerosis	0.72	0.0244
hsa05132	Salmonella infection	0.65	0.0103
hsa04010	MAPK signaling pathway	0.61	0.0045

Table 2: Most highly enriched GO Cellular Component terms and KEGG pathways in AD patients. The "GO ID" column lists the identifier for the Gene Ontology (GO) Cellular Components. The "KEGG ID" lists the identifier for the Kyoto Encyclopedia of Genes and Genomes" pathways. The "Strength" column quantifies how highly enriched the cellular component or pathway was within the network generated by STRING-db. The "False Discovery Rate" is a measure of adjusted *p*-value and determines how statistically significant the component or pathway's enrichment was in the dataset. This data was obtained from our analysis in STRING-db finding interactions between the top 250 differentially expressed genes from our GEO2R analysis (14).

expression of AP-1 components could be linked to a higher risk and occurrence of AD by triggering atherosclerosis and inflammation.

Our conclusions regarding the relationship between AD and atherosclerosis are supported by other studies determining that AD prevalence significantly increases with atherosclerosis, which is caused by increased levels of atherogenesis (5, 6). While the exact role of atherosclerosis in AD has yet to be agreed upon, there is evidence that carotid intima–media thickness (CIMT), a key biomarker for atherosclerosis, can be monitored to estimate AD risk due to its increased levels in AD patients (6).

Our findings on the connections between AD, AP-1, and inflammation also parallel existing research. AP-1 is also linked to inflammation via the IL-17 signaling pathway, where it activates transcription of genes that promote inflammation and regulate immune cell responses (18). Studies using western blot analyses have shown that phosphorylation of c-Jun in AP-1 resulted in the upregulation of the expression of MCP-1, a cytokine that escalates immune response (8). At the same time, c-Jun is activated by Aβ, so inhibition of c-Jun phosphorylation results in less neuroinflammation (8). AP-1 also activates IL-17 signaling pathway inflammatory genes *IL-1β* and *TNFα*, which each factor into AD development by disrupting neuronal metabolism, causing apoptosis and hindering the ability of microglia to clear away Aβ plaques in the brain (16, 19). Therefore, regulating the transcription of these genes upstream by targeting AP-1 in treatments could potentially mitigate onset of AD more broadly.

Our results present a potential biomarker, the AP-1 complex, as well as other notable related biological processes involving atherosclerosis and inflammation that could be studied further to help with the diagnosis of AD. Using biomarkers for atherosclerosis and inflammation diagnoses as identifiers for AD development could help individuals make more informed decisions to prevent arterial plaque buildup and slow the onset of AD. In the future, we would explore how targeting the AP-1 complex could present a viable diagnostic marker for the treatment of AD. This question could be addressed by performing a longitudinal study specifically focused on measuring the expression of the AP-1 complex in AD and non-AD patients. Additionally, the strong connection between processes regulated by AP-1 and AD could hint at other highly impactful gene products involved in the neurodegenerative processes of AD.

It is important to address certain limitations of our experiments and findings. Our findings related to gene regulation and significance were all collected from samples belonging to a singular public GEO dataset, which may have prevented us from identifying other vital enriched biological processes due to the small sample size. Additionally, the data we used was pooled across different hippocampal regions and Braak stages, so our findings on the relationship between AD, atherosclerosis, and inflammation may not necessarily be relevant for certain areas of the hippocampus or across all stages of disease. Furthermore, while we found that there were significant relationships between AD and the atherosclerosis and inflammation pathways, the AP-1 complex genes showed somewhat minor upregulation in AD patients, which could potentially undermine the efficacy of the AP-1 complex as a diagnostic target. Lastly, the prevalence of the AP-1 complex as transcription factors across various biological pathways raises concerns about its potential to be explored as a possible marker for AD diagnosis. Some potential alternative targets for exploration include *IL17RB* and *IL17D*, which are both upregulated in the AD group in the dataset and could possibly offer more precision due to their specific pro-inflammatory roles in the IL-17 pathway. Other

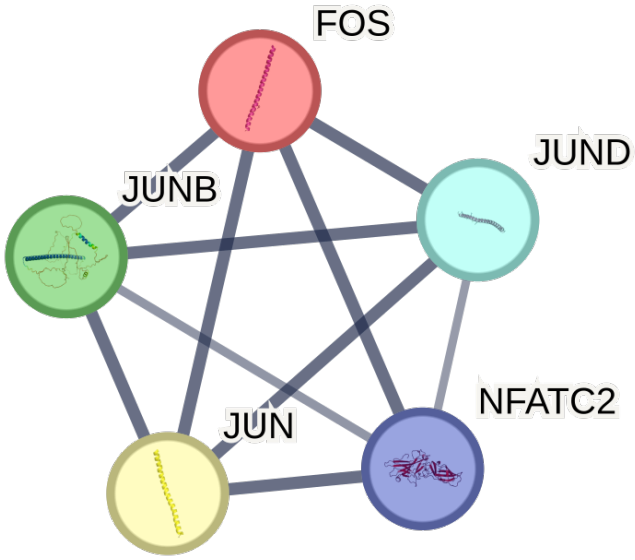

Figure 3: Graph of protein-protein interactions for the AP-1 complex. String map showing protein interactions within the AP-1 cellular component. Nodes include the gene products of *JUN*, *JUNB*, and *FOS*, from the top 250 differentially expressed genes from our GEO2R analysis. Each line represents a protein-protein interaction, and line thickness represents how strongly evidence supports the existence of the interaction (14).

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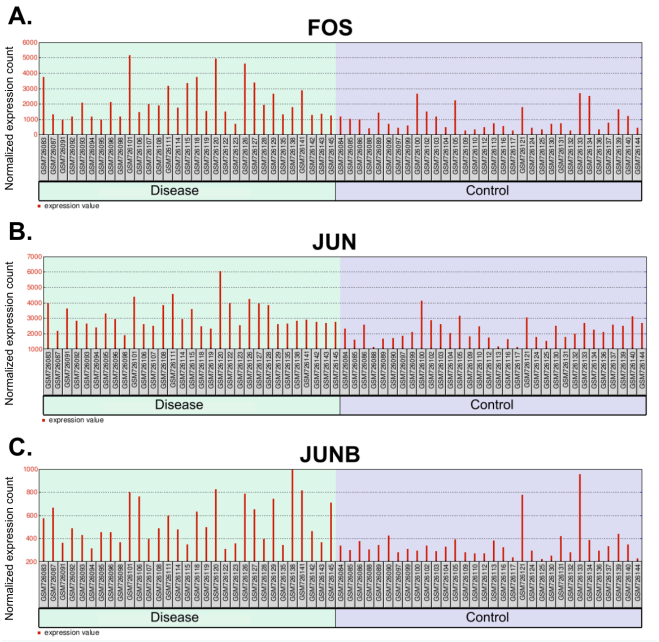

Figure 4: Differential expression of *FOS*, *JUN*, and *JUNB* between AD and control samples. Results were generated from GSE29378 data using GEO2R (11, 12). AD, or disease, samples are located on the left section of the diagram highlighted in green, and control samples are located on the right section highlighted purple. The red bars indicate the expression levels of the relevant gene for each of the samples collected in the dataset. A) The expression of *FOS* is generally higher in AD samples, so *FOS* is upregulated. B) *JUN* is less upregulated compared to *FOS*, since the AD group has slightly higher expression than the control group. C) *JUNB* is also upregulated and generally expressed more in AD samples, although there are a few outliers in the control group that have relatively high *JUNB* expression (GSM726121, GSM726133).

studies have demonstrated that these proteins can be used to characterize the pathology of AD and suggest that more research can be performed to determine if they are viable candidates for AD therapies (20, 21).

There are still unanswered questions regarding how genes and pathways relate to AD in specific groups of AD patients or components of the brain. In the future, we could look deeper into the specific factors (such as age, sex, hippocampal region, Braak stage) of AD instead of pooling the data to explore interactions between these factors, considering that different factors may affect the relationship between AD and other pathways differently. For example, there may be region-specific differences in gene expression between the CA1 and CA3 regions of the hippocampus due to their different functions, and perhaps there are stronger connections between specific genes and AD in each region. Similarly, there may also be differences in gene expression between each Braak stage of the disease, since different genes may be active during each Braak stage. Further investigations of these factors may also help to explain through differential gene expression why AD is more prevalent in females than males (1).

Overall, our findings provide strong support for our hypothesis that biomarkers involved in inflammatory and atherosclerotic pathways can be used for AD diagnosis. Our analysis of differential gene expression points to the AP-1 transcription complex as one such biomarker. This protein complex is significantly upregulated in AD patients, and its links to the fluid shear stress and atherosclerosis pathway suggest the possibility of a strong connection between atherosclerosis and AD. Additionally, our results support the theory that AD symptoms are self-sustaining due to the upregulation of processes related to neuroinflammation. These results may help us approach AD in a new way, allowing for the possibility of new therapeutics and biomarkers to benefit patients with AD.

MATERIALS AND METHODS

We analyzed the gene expression data from a publicly available dataset, GSE29378, on the Gene Expression Omnibus platform (11). Each sample had data that displayed the hippocampal region associated with the sample (CA1 or CA3), the age of the donor (range of 60–90 years old), the postmortem interval, plaque scores, Braak stage, *APOE* allele presence, and disease duration. Expression profiling by array was collected using an Illumina HumanHT-12 V3.0 expression beadchip.

We used GEO2R to create a spreadsheet of the differentially expressed genes between AD and control samples, and to obtain their *p*-value and logFC (14). GEO2R was run using an alpha value cutoff of $p < 0.05$, a log2FC cutoff of 0.5, and a Benjamini and Hochberg *p*-value adjustment. The packages GEO2R, limma, GEOquery, and UMAP were used in the GEO2R analysis. All samples were included, and no forced normalization was used. Genes and differentiation results were sorted by lowest to highest adjusted *p*-value. Additionally, we used the R environment (1.0.13) to visually represent the relationship between gene expression and disease state by using the R package *pheatmap* to create a heatmap (13).

The top 250 differentially expressed genes we found from the GEO2R *t*-test analysis were analyzed with STRING-db in order to find enriched pathways that may play a significant role in the pathology of AD (14). We generated a protein interaction pathway enrichment analysis in STRING, which also returned information on pathways that were enriched within that dataset. We analyzed interactions of genes in pathways from databases like Gene Ontology and KEGG to further understand the mechanisms of how differentially expressed genes affect the onset and symptoms of AD. Gene Ontology and KEGG pathways illustrated the specific interactions between different proteins in pathways, helping us understand and hypothesize about the correlation between AD and various biological pathways.

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APPENDIX

R heatmap code:

```
# Parse matrix file and load data
data <- read.table(file="GSE29378_series_matrix.txt",header=T, row.names=1,
comment.char="!")
# Separate AD and control sample IDs
AD_ID <-
c("GSM726118","GSM726096","GSM726107","GSM726098","GSM726111","GSM726135","GS
M726094","GSM726123","GSM726138","GSM726092","GSM726095","GSM726115","GSM726
114","GSM726145","GSM726142","GSM726091","GSM726143","GSM726093","GSM726083","
GSM726129","GSM726141","GSM726120","GSM726106","GSM726108","GSM726087","GSM7
26119","GSM726128","GSM726122","GSM726127","GSM726126","GSM726101")
control_ID <-
c("GSM726117","GSM726088","GSM726100","GSM726140","GSM726109","GSM726125","GS
M726134","GSM726084","GSM726139","GSM726090","GSM726102","GSM726103","GSM726
099","GSM726144","GSM726086","GSM726131","GSM726089","GSM726136","GSM726121","
GSM726133","GSM726085","GSM726113","GSM726130","GSM726137","GSM726105","GSM7
26132","GSM726124","GSM726110","GSM726097","GSM726112","GSM726116","GSM726104
")
AD_data <- data[,AD_ID]
control_data <-data[,control_ID]
# Concatenate grouped sample data
dataframeOfSamples <- cbind(AD_data,control_data)
# Extract top 100 genes, sorted by p-value
GenesByPVal <-read.table(file="GSE29378.top.table.tsv",header=T,sep="\t", nrows=100)
head(GenesByPVal)
# Obtain gene expression of the top 100 genes
getGeneExpOfTop <- dataframeOfSamples [GenesByPVal$ID,]
# Generate heatmap, where columns are samples and rows are the top 100 genes
library(pheatmap)
group <-
c('AD','AD','AD','AD','AD','AD','AD','AD','AD','AD','AD','AD','AD','AD','AD','AD','AD','AD','AD','AD','AD','
AD','AD','AD','AD','AD','AD','AD','AD','AD','AD','AD','Control','Control','Control','Control','Control','
Control','Control','Control','Control','Control','Control','Control','Control','Control','Control','Control',
'Control','Control','Control','Control','Control','Control','Control','Control','Control','Control','Control'
,'Control','Control','Control','Control','Control')
annotation_col = as.data.frame(group)
rownames(annotation_col) <- colnames(getGeneExpOfTop)
pheatmap(getGeneExpOfTop,annotation_col = annotation_col,scale="row",border_color=NA,
show_rownames=T)
```