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Identification of Shared Genes between Cerebral Small Vessel Disease and Alzheimer's Disease through Multi-Omics Analyses

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Abstract

Background: Cerebral small vessel disease (CSVD) and Alzheimer's disease are common causes of cognitive impairment and dementia. While previous studies have reported epidemiological associations between the two conditions, the molecular mechanisms underlying their connection remain unclear.

Methods: We conducted multi-omics analyses of gene expression profiles from CSVD and AD patients. Specifically, we performed differential expression analysis, functional enrichment analysis, and integrated analysis on four datasets comprising a total of 70 samples from GSE. Significant findings were validated in an independent dataset. Protein sequence conservation was also analyzed.

Results: A set of 16 genes exhibited consistent expression changes across the datasets, including upregulation of SMAD2, CCNA2, BAG2 and downregulation of GRM5, SLC8A3. These genes are involved in pathways such as protein degradation, tau protein binding, and calcium signaling, suggesting shared pathological processes. Validation in an independent cohort confirmed differential expression of 11 out of the 16 genes. Further, protein sequence alignment showed high conservation.

Conclusion: By leveraging multi-omics techniques, we have uncovered 16 candidate genes that may underlie both CSVD and AD. Our findings provide new insights into the molecular connection between the two conditions and pave the way for follow-up studies exploring the pathogenic roles and therapeutic potential of these shared targets.

Keywords: Cerebral Small Vessel Disease, Alzheimer's Disease, Gene expression analyses, Multi-Omics Analyse, Identification of Shared Genes

Introduction

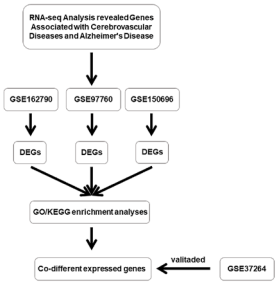
Cerebral Small Vessel Disease (CSVD) is a common cause of stroke and cognitive impairment. Epidemiological data shows that typical MRI features of CSVD, such as white matter hyperintensities and lacunar infarcts, are highly prevalent in community-based studies, and recent data suggests it's also a significant health burden in low and middle-income countries(1). CSVD primarily results from two common pathological mechanisms: arterial sclerosis and cerebral amyloid angiopathy. CSVD not only leads to strokes and cognitive decline but also affects motor function, balance, emotions, and behavior(2). Despite its public health importance, there are currently few confirmed effective treatments. Recent research has emphasized that blood pressure reduction can slow the progression of white matter hyperintensities and delay the onset of cognitive decline(3). However, there is relatively limited research on therapeutic approaches for secondary prevention of CSVD. Recent studies have also proposed various molecular processes related to endothelial dysfunction, nitric oxide synthesis, blood-brain

barrier integrity, extracellular matrix maintenance and repair, and inflammation as potential targets for understanding CSVD pathogenesis and treatment(4). These new findings provide clues for the mechanisms of CSVD and potential therapeutic targets.

Alzheimer's Disease is the most common cause of dementia, accounting for 50-70% of dementia cases (5). With increasing life expectancy and an aging population, the prevalence of AD is expected to continue rising, posing a significant burden on national healthcare systems(6). AD is a complex multifactorial disease, with genetic factors explaining 60-80% of the risk, and more than 40 genetic risk loci associated with AD have been identified, with the APOE gene variant being the strongest risk factor(7). Besides genetic factors, acquired factors such as cerebrovascular diseases, diabetes, hypertension, obesity, and lipid abnormalities also increase the risk of AD(8). There are currently no fully successful treatments for AD, but early detection and management, combined with appropriate drug therapy, are crucial for improving outcomes(9). Cognitive training, physical activity, and a healthy diet can reduce the risk of AD (10, 11). Further research into the molecular mechanisms of AD is needed to identify more precise screening, diagnostic, preventive, and therapeutic targets.

Previous research has shown a close connection between CSVD and AD. In cognitively intact elderly individuals, neuroimaging features of CSVD such as white matter hyperintensities, lacunar infarcts, and microbleeds are associated with an increased risk of AD(12, 13). Additionally, pathological changes of CSVD are often found in post-mortem brain tissue of AD patients(14). While there is some controversy regarding whether CSVD biomarkers precede typical AD biomarkers, it suggests that CSVD may be an important contributing factor to AD. Current research on the relationship between CSVD and AD is mainly limited to epidemiological associations, and our understanding of the common mechanisms underlying both diseases is still insufficient. Particularly, there is a lack of systematic research utilizing high-throughput sequencing technologies to study the commonalities between the two diseases. A deeper understanding of the relationship between CSVD and AD, especially at the molecular level, is crucial for the development of prevention and treatment strategies.

For example, genetic studies have identified some common genetic loci associated with CSVD and AD, suggesting shared pathways in the pathogenesis of both diseases(15, 16). However, these genetic association studies have limitations, including small sample sizes that need validation in larger cohorts. Furthermore, there is relatively limited transcriptomic and metabolomic research on CSVD and AD, and these omics data can help uncover the molecular mechanisms of these diseases. Therefore, future multi-omics studies of CSVD and AD patients using high-throughput sequencing technologies, systematically analyzing commonalities between the two diseases, will help identify potential therapeutic targets. This holds significant importance for the development of new drugs and treatment strategies for CSVD and AD(17).



Scheme. Identification of genes associated with both cerebral small vessel disease and Alzheimer's disease through RNA-sequencing

Methods

Data source

We initially searched in the GEO database using the keywords ‘cerebral small vessel disease’ or ‘Alzheimer's’, then screened the abstracts of the search results and performed PCA quality control

analysis on different datasets. Ultimately, we selected four datasets: GSE162790, containing gene expression data from 4 progressive SVD patients and 4 non-progressive SVD patients (Illumina NextSeq 500 platform); GSE97760, containing gene expression data from 9 groups of AD patients and 10 control subjects (Agilent Microarray); GSE150696, containing gene expression data from 9 AD patients and 9 elderly control subjects (Affymetrix Human Transcriptome Array); and GSE37264, containing gene expression data from 8 AD patients and 8 elderly control subjects (Affymetrix Human Exon Array).

Differential expression analyses

Based on the gene expression data from various sample groups, PCA analysis was performed to remove samples with abnormal gene expression. Subsequently, the limma package(18) was used for differential expression analysis. R language (factoextra v1.0.6, pheatmap v1.0.12) was used for visualization of the results. The criteria for selecting were Log2Fold change > 1 or < -1, and p-value < 0.05.

Functional enrichment analyses

Functional enrichment analyses on the significantly differentially expressed genes from the three datasets were conducted using clusterProfiler(19) , obtaining genes and biological processes associated with cerebral small vessel disease or Alzheimer's disease.

Combined analysis

Combining differential expression analysis and functional analysis of three datasets, genes that were correlated with the occurrence of both diseases were identified through Venn analysis(20), and the expression of these genes in each dataset was visualized. Additionally, for common genes, validation was performed using the GSE37264 dataset. Finally, based on the results of functional analysis, we explored the commonalities and shared genes between the two diseases at the molecular level.

Conservation analyses

Conservation analyses of shared genes were conducted by downloading the protein sequence information encoded by the respective genes from the UniProtKB database. Inter-species conservation analysis was performed using msa package (https://bioconductor.org/packages/release/bioc/html/msa.html, version 3.17).

Results

Differential expression analyses

We initially conducted PCA analysis to perform quality control on the gene expression data of 3 datasets. No obvious abnormal samples were observed in the GSE162790 and GSE97760 datasets (Fig. S1A and S1B). In the GSE150696 dataset, there were 3 samples with abnormal gene expression. After excluding these 3 cases, PCA analysis revealed no apparent abnormal samples (Fig. S1C and S1D). After quality control, these data were used for differential expression analysis (Log2Fold change > 1 or < -1, and p value < 0.05).

In the Progressive SVD group (GSE162790), there were 138 significantly upregulated genes and 183 significantly downregulated genes (Figure 1A). In the AD group (GSE97760 and GSE150696), there were 3812/651 significantly upregulated genes and 2236/1046 significantly downregulated genes (Figure 1B and 1C).

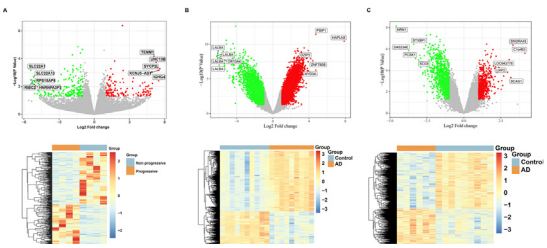


Figure 1. Differential expressed genes in (A) GSE162790, (Clancy et al) GSE97760 and (Clancy et al) GSE150696, along with their bidirectional clustering heatmaps.

Functional analyses

To explore potential shared biological mechanisms between the two diseases, we conducted Gene Ontology (16) functional enrichment analysis on the differentially expressed genes. We found that upregulated genes were mainly enriched in functions related to proteoglycan binding, lipoprotein particle binding, ubiquitin-like protein activity, and tau protein binding, which are associated with protein and lipid binding degradation processes. Conversely, significantly downregulated genes were primarily enriched in activities related to calcium ion transmembrane transporter activity and calmodulin binding, which are involved in calcium ion transmembrane transport and signaling pathways (Figure 2A and 2B).

Furthermore, through the analysis of GO terms that were shared across all three datasets, we observed that the significantly highly expressed genes SMAD2 and BAG2 were involved in ubiquitin protein ligase binding and tau protein binding processes in all three datasets. NRGN and SLC8A3 participated in calmodulin binding (Figure 2C). In the case of significantly down-regulated genes, GRM5 was associated with neurotransmitter receptor activity, while genes like CACNA2D1 and CACNG3 were involved in calcium ion transmembrane transporter activity (Figure 2D).

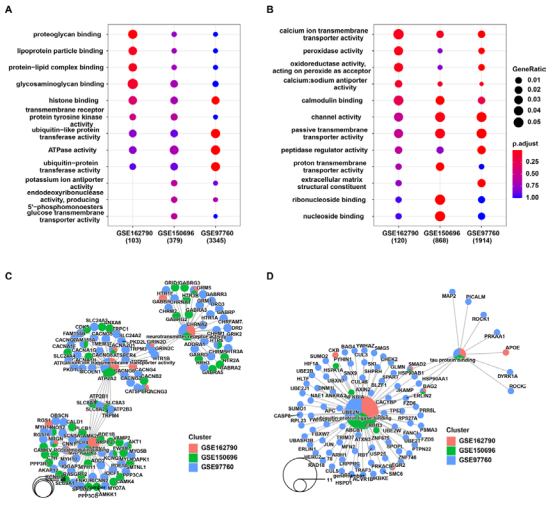


Figure 2. GO enrichment analyses bubble plot of (A) up-regulated and (Clancy et al) down-regulated genes. GO enrichment analysis network graph of (Clancy et al) up-regulated and (D) down-regulated genes.

In KEGG functional analysis, the three datasets exhibited similar pathway enrichment results. Downregulated genes were primarily enriched in pathways such as the calcium signaling pathway, neuroactive ligand-receptor interaction, and pathways of neurodegeneration (Figure 3A). Upregulated genes, on the other hand, were enriched in pathways including the PPAR signaling pathway, cholesterol metabolism, and cell senescence (Figure 3B).

Further analysis of shared KEGG pathways revealed that significantly downregulated genes like GRM5, SLC8A1/3 were enriched in pathways such as Alzheimer's disease, calcium signaling pathway, and neuroactive ligand-receptor interaction across all three datasets (Figure 3C). Significantly highly expressed genes like SMAD2, CCNA2, GH2, and LPAR6 were enriched in pathways such as cellular senescence, PI3K-Akt signaling pathway, and neuroactive ligand-receptor interaction (Figure 3D). In summary, these findings suggested that the occurrence of AD or cerebral SVD may be associated with changes in protein degradation, tau protein binding, calcium ion transmembrane transport, and neurotransmitter activity within the body.

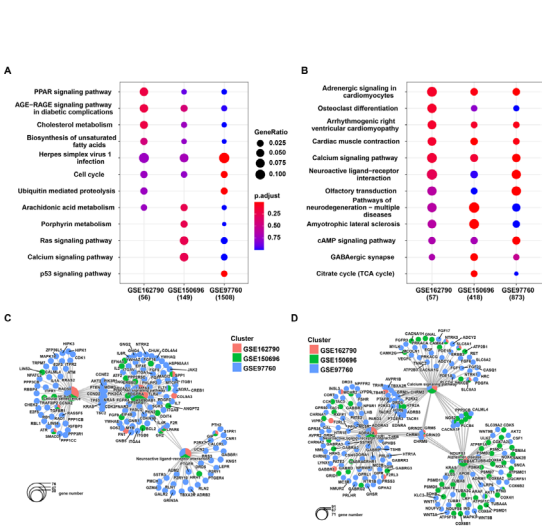


Figure 3. KEGG enrichment analyses bubble plot of (A) up-regulated and (Clancy et al) down-regulated genes. KEGG enrichment analysis network graph of (Clancy et al) up-regulated and (D) down-regulated genes.

Combined analyses

After gaining a preliminary understanding of the functional aspects of differentially expressed genes, we conducted combined analyses to identify shared genes. There was a total of 10 genes significantly downregulated in all three datasets and 6 genes significantly upregulated (Figure 4A). By visualizing the expression of these 16 genes in each dataset, we observed that genes such as BAG2, CCNA2, and GH2 exhibited significantly increased expression in patients with AD or cerebral small vessel disease, while genes like GRM5 and SLC8A3 showed significant downregulation in these patient groups (Figure 4B).

Furthermore, we validated the expression of these genes in the GSE37264 dataset, as shown in Figure 4C and 4D. Apart from GIP2 and SMAD2, CCNA2, BAG2, and GH2 were significantly upregulated in patients with AD, with statistical significance ($p < 0.05$). Except for RIN2, all genes were significantly downregulated in AD patients, with statistical significance ($p < 0.05$). In order to analyze the conservation of the aforementioned genes, we obtained protein information encoded by them through NCBI and UniprotKB and conducted conservation analyses. Their conservation was relatively high in both humans and mice (Fig. S2-6). These genes may play roles in both cerebral small vessel disease and Alzheimer's disease, and further research into the functions of these proteins will help us gain a deeper understanding of the mechanisms underlying the development of cerebral small vessel disease and Alzheimer's disease, as well as uncover potential connections between these two conditions.

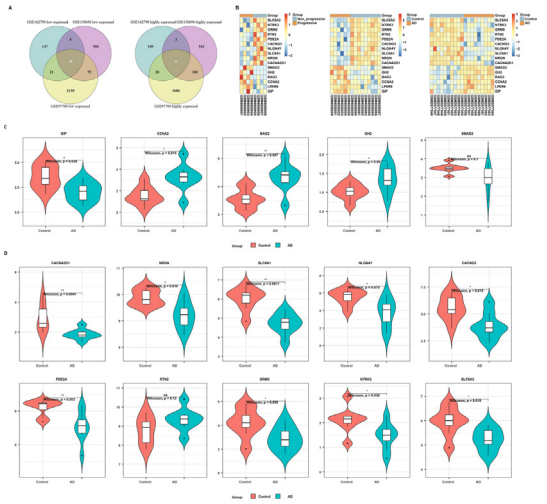


Figure 4. (A) Venn diagram; (Clancy et al) The expression levels of 16 common genes in three datasets; The expression levels of (Clancy et al) 6 up-regulated and (D) 10 down-regulated gene in GSE37264.

Discussion

This study conducted differential gene expression analysis, functional enrichment analysis, and integrated analysis of gene expression profile chip data from patients with Cerebral Small Vessel Disease (CSVD) and Alzheimer's Disease. It identified 16 genes with consistent expression changes in both diseases, including significantly upregulated genes like SMAD2, CCNA2, BAG2, and significantly downregulated genes like GRM5, SLC8A3, among others(15, 21, 16). These genes are involved in biological processes such as protein degradation(22), tau protein binding(16), and calcium ion transmembrane transport (21), suggesting shared pathological mechanisms such as protein metabolism abnormalities and disruptions in cellular signal transduction in both diseases. However, it's important to note that the expression changes of these genes were not entirely consistent across different datasets, indicating that individual variations among samples may influence the results. For instance, CCNA2 did not show significant upregulation in the GSE162790 dataset, and BAG2 was not significantly upregulated in the GSE150696 dataset. Furthermore, although these genes are involved in known SVD and AD-

related pathways such as calcium signaling, their exact roles in disease development need further validation, possibly through functional studies in animal models.

Regarding SMAD2, it participates in the TGF-β signaling pathway(23), exhibits abnormal cytoplasmic localization in brain tissues of AD patients(24), and is associated with tau protein hyperphosphorylation(25). As for BAG2, it is involved in tau protein degradation(26), and literature reports its downregulation in AD patients and animal models(27, 28), while this study found it to be upregulated in both AD and CSVD, which requires further confirmation. CCNA2's role in AD or CSVD is currently underreported. Given the relatively small sample size in this study, the consistency and stability of differential expression need validation in larger cohorts. In comparison to previous research, this study employed multi-omics and bioinformatics analysis methods to uncover potential molecular associations between SVD and AD without delving into mechanisms, providing clues for further mechanistic investigations. Therefore, future research could consider using cerebrospinal fluid (CSF) samples to reflect central changes, expanding sample sizes to enhance result reliability, conducting longitudinal studies to reveal the dynamic relationship between gene expression and disease progression, and performing animal experiments to elucidate the functional roles of these genes in disease development. A comprehensive and systematic understanding of the underlying connections between SVD and AD will aid in discovering new therapeutic targets and developing novel treatment strategies.

In summary, this study has preliminarily identified potential shared pathogenic mechanisms between SVD and AD through multi-omics analysis, providing valuable insights for future research. Subsequent investigations can further clarify the roles of these genes in the development of both diseases through functional studies, improve research quality through CSF samples and longitudinal study designs, and comprehensively and systematically uncover the intrinsic connections between the two diseases, which holds significant importance for discovering new therapeutic targets and developing novel treatment strategies.

Author contributions

CW and HY: designed the project, interpreted results, provided approval of the final version for publication. JL, JY and YL: analyzed the data. JY: provided study supervision. QZ, XZ: drafted the initial version of the manuscript. All authors edited and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Reference:

[1] Lam, BYK, Cai, Y, Akinyemi, R, et al. The global burden of cerebral small vessel disease in low- and middle-income countries: A systematic review and meta-analysis. INT J STROKE. 2022; 18 (1): 15-27. doi: 10.1177/17474930221137019

[2] Clancy, U, Gilmartin, D, Jochems, ACC, et al. Neuropsychiatric symptoms associated with cerebral small vessel disease: a systematic review and meta-analysis. Lancet Psychiatry. 2021; 8 (3): 225-236. doi: 10.1016/S2215-0366(20)30431-4

[3] Siedlinski, M, Carnevale, L, Xu, X, et al. Genetic analyses identify brain structures related to cognitive impairment associated with elevated blood pressure. EUR HEART J. 2023; 44 (23): 2114-2125. doi: 10.1093/eurheartj/ehad101

[4] Markus, HS, de Leeuw, FE. Cerebral small vessel disease: Recent advances and future directions. INT J STROKE. 2023; 18 (1): 4-14. doi: 10.1177/17474930221144911

[5] Serrano-Pozo, A, Growdon, JH. Is Alzheimer's Disease Risk Modifiable? J ALZHEIMERS DIS. 2019; 67 (3): 795-819. doi: 10.3233/JAD181028

[6] 2021 Alzheimer's disease facts and figures. ALZHEIMERS DEMENT. 2021; 17 (3): 327-406. doi: 10.1002/alz.12328

[7] Belloy, ME, Napolioni, V, Greicius, MD. A Quarter Century of APOE and Alzheimer's Disease: Progress to Date and the Path Forward. NEURON. 2019; 101 (5): 820-838. doi: 10.1016/j.neuron.2019.01.056

[8] Silva, MVF, Loures, CMG, Alves, LCV, et al. Alzheimer's disease: risk factors and potentially protective measures. J BIOMED SCI. 2019; 26 (1): 33. doi: 10.1186/s12929-019-0524-y

[9] Ogbodo, JO, Agbo, CP, Njoku, UO, et al. Alzheimer's Disease: Pathogenesis and Therapeutic Interventions. Curr Aging Sci. 2022; 15 (1): 2-25. doi: 10.2174/1874609814666210302085232

[10] Jia, RX, Liang, JH, Xu, Y, et al. Effects of physical activity and exercise on the cognitive function of patients with Alzheimer disease: a meta-analysis. BMC Geriatr. 2019; 19 (1): 181. doi: 10.1186/s12877-019-1175-2

[11] Stefaniak, O, Dobrzyńska, M, Drzymała-Czyż, S, et al. Diet in the Prevention of Alzheimer's Disease: Current Knowledge and Future Research Requirements. Nutrients. 2022; 14 (21): doi: 10.3390/nu14214564

[12] Bos, D, Wolters, FJ, Darweesh, SKL, et al. Cerebral small vessel disease and the risk of dementia: A systematic review and meta-analysis of population-based evidence. ALZHEIMERS DEMENT. 2018; 14 (11): 1482-1492. doi: 10.1016/j.jalz.2018.04.007

[13] Gertje, EC, van Westen, D, Panizo, C, et al. Association of Enlarged Perivascular Spaces and Measures of Small Vessel and Alzheimer Disease. NEUROLOGY. 2020; 96 (2): e193-e202. doi: 10.1212/WNL.0000000000011046

[14] Kalaria, RN, Sepulveda-Falla, D. Cerebral Small Vessel Disease in Sporadic and Familial Alzheimer Disease. AM J PATHOL. 2021; 191 (11): 1888-1905. doi: 10.1016/j.ajpath.2021.07.004

[15] Dunn, PJ, Lea, RA, Maksemous, N, et al. Investigating a Genetic Link Between Alzheimer's Disease and CADASIL-Related Cerebral Small Vessel Disease. MOL NEUROBIOL. 2022; 59 (12): 7293-7302. doi: 10.1007/s12035-022-03039-3

[16] Sargurupremraj, M, Suzuki, H, Jian, X, et al. Cerebral small vessel disease genomics and its implications across the lifespan. Nat Commun. 2020; 11 (1): 6285. doi: 10.1038/s41467-020-19111-2

[17] Klyucherev, TO, Olszewski, P, Shalimova, AA, et al. Advances in the development of new biomarkers for Alzheimer's disease. Transl Neurodegener. 2022; 11 (1): 25. doi: 10.1186/s40035-022-00296-z

[18] Jia, A, Xu, L, Wang, Y. Venn diagrams in bioinformatics. BRIEF BIOINFORM. 2021; 22 (5): doi: 10.1093/bib/bbab108

[19] Ritchie, ME, Phipson, B, Wu, D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. NUCLEIC ACIDS RES. 2015; 43 (7): e47. doi: 10.1093/nar/gkv007

[20] Yu, G, Wang, LG, Han, Y, et al. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012; 16 (5): 284-7. doi: 10.1089/omi.2011.0118

[21] Duperron, MG, Knol, MJ, Le Grand, Q, et al. Genomics of perivascular space burden unravels early mechanisms of cerebral small vessel disease. NAT MED. 2023; 29 (4): 950-962. doi: 10.1038/s41591-023-02268-w

[22] Hainsworth, AH, Oommen, AT, Bridges, LR. Endothelial cells and human cerebral small vessel disease. BRAIN PATHOL. 2015; 25 (1): 44-50. doi: 10.1111/bpa.12224

[23] Lee, MY, Park, BY, Kwon, OK, et al. Anti-inflammatory activity of (-)-aptosimon isolated from Daphne genkwa in RAW264.7 cells. INT IMMUNOPHARMACOL. 2009; 9 (7-8): 878-85. doi: 10.1016/j.intimp.2009.03.012

[24] Lee, HG, Ueda, M, Zhu, X, et al. Ectopic expression of phospho-Smad2 in Alzheimer's disease: uncoupling of the transforming growth factor-beta pathway? J NEUROSCI RES. 2006; 84 (8): 1856-61. doi: 10.1002/jnr.21072

[25] Baig, S, van Helmond, Z, Love, S. Tau hyperphosphorylation affects Smad 2/3 translocation. NEUROSCIENCE. 2009; 163 (2): 561-70. doi: 10.1016/j.neuroscience.2009.06.045

[26] Carrettiero, DC, Hernandez, I, Neveu, P, et al. The cochaperone BAG2 sweeps paired helical filament- insoluble tau from the microtubule. J NEUROSCI. 2009; 29 (7): 2151-61. doi: 10.1523/JNEUROSCI.4660-08.2009

[27] de Oliveira, AS, Santiago, FE, Balioni, LF, et al. BAG2 expression dictates a functional intracellular switch between the p38-dependent effects of nicotine on tau phosphorylation levels via the α7 nicotinic receptor. EXP NEUROL. 2015; 275 Pt 1 69-77. doi: 10.1016/j.expneurol.2015.10.005

[28] Patil, SP, Tran, N, Geekiyanage, H, et al. Curcumin-induced upregulation of the anti-tau cochaperone BAG2 in primary rat cortical neurons. NEUROSCI LETT. 2013; 554 121-5. doi: 10.1016/j.neulet.2013.09.008