

A differential expression analysis of genes involved in the regulation of apoptosis in Alzheimer's and non-Alzheimer's patients

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder characterised by cognitive dysfunction. AD is associated with many genetic risk factors and biological pathways, this paper focusing on apoptosis as a potential biological pathway that impacts the progression of AD, looking into the different genes associated with apoptosis and whether they are differentially expressed in individuals with AD compared to control individuals.

All genes in the biological pathway, NRIF (nuclear receptor-interacting factor) Signals Cell Death from the Nucleus, were found to be upregulated in those with AD through the likelihood ratio test, the most significant of these being NGFR and PSEN1. This suggests future direction in the field of AD research targeted towards neuronal apoptosis. Hence, this secondary investigation furthers understanding relating to the development of AD and the associated genetic risk factors.

Introduction

AD is a chronic progressive neurodegenerative disorder and characterised by cognitive dysfunction, including loss of language capabilities, and loss of memory, praxis, judgement, and stability. These symptoms progress from more mild symptoms of memory loss to very severe dementia over time, having a significant effect on people's ability to carry out daily activities.

AD is due to large scale degeneration of many parts of the brain characterised by two abnormalities: accumulation of amyloid β in the form of plaques, and intracellular neurofibrillary tangles, the complex interplay between these two abnormalities and other factors possibly resulting in AD (Joe, E. and Ringman, J.M., 2019). The beta-amyloid ($A\beta$) protein involved in AD is formed from the breakdown of a larger protein called the amyloid precursor and tau is a brain-specific, axon-enriched microtubule-associated protein. There is evidence that indicates that soluble forms of $A\beta$ and tau work together, independently of their accumulation into plaques and tangles, to drive healthy neurons into the diseased state.

Apoptosis is an essential cellular process that regulates cell death, and is crucial for cell survival and homeostasis. Apoptosis is predetermined and is the programmed mechanism of cell death. Since it regulates several molecular events it may contribute to neurodegenerative manifestations and hence, disease. The dysregulation of apoptosis pathways could cause abnormal neuronal loss and since AD is characterized by the loss and damage of neurons, the process of apoptosis may provide valuable insight into the damage that occurs in the brain of an individual with AD.



(Technology Networks, 2020)

Methods and Materials

The study conducted was a secondary data analysis, analysing the expression of genes involved in the regulation of apoptosis in AD patients compared to control individuals. From the data in DisGeNet, there were 12 AD individuals, 10 old control individuals and 8 young control individuals. The expression of genes was defined as being either upregulated or downregulated in AD patients or having no significant difference compared to controls where up and down regulation referred to changes in gene expression due to the increase or decrease in the production of RNA and proteins from a particular gene. Upregulation referred to the gene expression being increased, enhancing cellular functions, while down regulation referred to gene expression being decreased, hence reducing cellular activity.

A likelihood ratio test was conducted in order to generate a table which displayed statistical values for the genes displayed in the box plots. This test compared the linear model of the disease samples to that of the healthy control samples with the alpha value set at 0.05. It generated the gene's log(cpm), likelihood ratio, and p-value which was then used to generate the FDR (false discovery rate) which was used along with the fold change to calculate if the threshold was true or false for each gene. The likelihood ratio was discounted as the p-value and FDR were better measures for determining statistical significance. A volcano plot function was created to visualise the significance of the genes.

Results

The genes included in figure 2 were used in a likelihood ratio test in order to see if there is a significant difference in gene regulation between AD patients for an alpha value of 0.05 compared to the controls for:
H₀: Genes involved in the regulation of apoptosis are not differentially expressed in individuals with AD.
H_a: Genes involved in the regulation of apoptosis are differentially expressed in individuals with AD.
It was found that all 8 genes included in the test had p-values below 0.05 and hence were considered statistically, leading to the rejection of the null hypothesis for this statistical test.

The genes from figures 2 are displayed in a volcano plot. All 8 genes were located on the true threshold for the plot which means the fold change was high enough and the FDR was low enough for the result to be considered significant. All 8 genes also had a positive fold change, which indicates that a certain gene is expressed at a higher level in the group of interest, in this case AD patients, compared to the control groups. This shows that all 8 genes were upregulated in AD individuals.

Statistical results for the Genes Involved in NRIF Signals Cell Death from the Nucleus:

Gene	log(FC)	log(cpm)	P-Value	FDR	Threshold
APH1A	0.537	4.888	5.076e-03	8.804e-03	TRUE
NCSTN	0.534	4.997	5.166e-03	8.921e-03	TRUE
UBC	0.563	9.759	2.946e-03	5.722e-03	TRUE
PSEN1	0.769	6.111	4.993e-05	2.131e-04	TRUE
APH1B	0.706	4.050	2.429e-04	7.748e-04	TRUE
UBB	0.506	8.231	7.587e-03	1.207e-02	TRUE
NGFR	1.286	3.297	4.131e-11	1.214e-09	TRUE
PSENEN	0.699	3.047	3.691e-04	1.088e-03	TRUE

Figure 2: Statistical results from likelihood ratio test, includes log(FC) (log fold change), logCPM, P-value, FDR, and threshold.

Significance of Genes Involved in NRIF Signals Cell Death from the Nucleus:

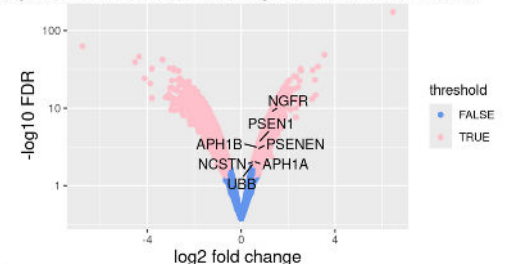


Figure 1: Volcano plot for $-\log_{10}FDR$ (negative log of false discovery rate) vs $\log_2$ fold change which shows the significance of genes involved in NRIF signals cell death based on whether threshold is true or false for the filter $FDR < 0.05$.

Discussion

The statistical values associated with the genes involved in the box plots which were generated from a likelihood ratio test. The alpha value was set at 0.05 and the FDR for all genes is < 0.05 , which is considered a significant value as an FDR of 0.05 means that 5% of significant DEGs are false positives, thus the smaller number shows confidence that the differential expression is significant and not up to chance. The threshold was also true for each gene which accounts for log fold change and FDR in order to determine significance, a true threshold meaning that the fold change is high enough, and the FDR is low enough for the differences that occurred in expression to be considered significant and unlikely to be up to chance. Hence all eight genes displayed in the box plots are considered differentially expressed in individuals with AD, allowing for a rejection of the null hypothesis stated in the results.

While all the genes are located in the true threshold region of the volcano plot, NGFR and PSEN1 are the most significant in terms of gene expression. This shows that the significant genes involved in neuronal apoptosis relate to the development of AD not just through the increase of neuronal cell death, but also play a role in the development of established biomarkers of AD.

Conclusions

This paper has revealed that genes involved in the regulation of apoptosis are upregulated in individuals with AD. From the eight genes included in the data on DisGeNet from the biological pathway of NRIF signals cell death from the nucleus, which were: APH1A, NCSTN, UBC, PSEN1, APH1B, UBB, NGFR, and PSENEN. It was found that all eight genes were differentially expressed in individuals with AD, and were upregulated, thus successfully testing the hypothesis. The most significant of these genes apparent by their FDR and log fold change were NGFR and PSEN1. As NGFR is related to mediated apoptosis and PSEN1 is a key component in the production of β -amyloid, this suggests that genes involved in neuronal cell death could be a potential biomarker for AD and a potential target for future therapeutics. Given the findings of this analysis, further research is needed to build an understanding of these significant genes and their specific role in neuronal apoptosis in order to further our understanding of the role it plays in the development and progression of AD.