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Integrated Genomic Analysis Highlights the Impaired PI3K-Akt Signaling Pathway in Alzheimer's Disease

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Abstract: Alzheimer's disease (AD), a leading cause of dementia, remained incurable, despite many advances in our knowledge about AD pathogenesis, underlying mechanisms are poorly understood. Transcriptome analysis showed efficiency in exploring these mechanisms; however, data are generated at a higher pace than interpreted and are almost inconsistent. Therefore we performed this meta-analysis to extract new knowledge from existing data and find the mechanisms involved in AD. Five temporal cortex transcriptomics datasets from 187 AD patients and 167 healthy controls were analyzed. Our analysis showed that the PI3K-Akt signaling pathway is significantly impaired in AD brains and was common among all datasets. Moreover, miRs targeting genes involved in the PI3K-Akt signaling pathway were identified. In conclusion, our results highlight the impaired PI3K-Akt signaling pathway in AD and suggested related miRs as the potential targets for early treatment and diagnosis of AD.

Keywords: Alzheimer's disease; meta-analysis; transcriptome; PI3K/Akt signaling pathway

1. Introduction

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disease that is characterized by the presence of senile plaques (SPs) and neurofibrillary tangles (NFTs) [1,2]. AD is the leading cause of dementia, accounting for about 60-70% of dementia cases, currently, around 50 million people are living with dementia globally, which is estimated to reach 82 million in 2030 and 152 in 2050 unless preventive strategies are found [3,4]. Two well-established risk factors for developing AD are Apolipoprotein E (ApoE) polymorphism and aging [5]. Despite many advances in our knowledge about AD pathogenesis, the underlying mechanisms are poorly understood and recent disappointing clinical outcomes of targeting two well-established AD pathological hallmarks, including NFTs and SPs have challenged our narrow understanding of AD pathogenesis [6,7].

The development of high-throughput technologies enabled us to investigate the changes at molecular levels corresponding to disease development and progression, however, huge amount of data are generated through these technologies at a higher pace than they are interpreted [8,9]. Microarray analysis as one of these high-throughput technologies has gained a great deal of attention among researchers from different fields, including AD. A growing number of research studies have been performed to elucidate the gene expression alteration in AD brains [10,11].

While microarray analysis proved to be an efficient tool to explore the AD-related changes at the gene expression level, however reproducibility of microarray analyses has always been questionable. Inconsistent results are commonly observed across different studies, furthermore considering the multifactorial nature of AD, different genetic backgrounds and lifestyles could affect these molecular changes across AD patients [12-14]. In this regard, a meta-analysis that combines the outcomes of several studies appeared as powerful tools to reduce the heterogeneity among published results and define the most reliable changes [15-19]. Currently, there are several meta-analyses on gene expression data from different brain regions of AD patients; including Li and colleagues meta-analysis in 2015 on six studies from the frontal cortex of AD patients [16], Moradifard and colleagues meta-analysis in 2018, which included data from six individual studies and further a sub-meta-analysis on the hippocampus and entorhinal cortex [20], and recently a meta-analysis on CA1 of the hippocampus DEGs by Hosseinian and colleagues in 2020 [12]. However, meta-analyses on other brain regions are not available. Therefore we herein performed a comprehensive meta-analysis on the temporal cortex microarray datasets from AD patients.

2. Materials and Methods

2.1. Search strategy and data collection.

A comprehensive search through Gene Expression Omnibus (GEO) was performed to find all eligible datasets from inception up to March 2021. “Alzheimer” and “temporal” were used as keywords and three filters including Homo sapiens, Series, and Expression profiling by array were employed. Differentially expressed genes (DEGs) between AD patients and healthy controls were obtained using the GEO2R tool and DEGs with adjusted P value < 0.05 were considered significant.

2.2. Integrated genomic analyses.

Herein for our meta-analysis, we used R package RobustRankAggreg. Unlike Rank Aggregation (RA) that detect the closest list to the input lists, RRA generates a relevant list of even irrelevant and incomplete input lists [21]. Robust DEGs for each brain region were considered significant if the Bonferroni-corrected p-value was < 0.05. Moreover, common genes between at least two datasets (common DEGs) were identified.

2.3. Enriched pathways.

For pathway analysis, we submitted Robust DEGs list in The Database for Annotation, Visualisation, and Integrated Discovery (DAVID) and selected KEGG-pathways with Benjamini-corrected p-value less than 0.05, furthermore, pathways for common DEGs were also identified [22-24]. Finally, we found the pathways that were significant in both robust DEGs and common DEGs.

2.4. Targeting miRs.

To find miRs targeting genes involved in PI3K-Akt signaling pathway, GeneSet2miRNA (GS2M) was used in www.bioprofiling.de, which employed 11 prediction programs. ENTREZ GENE IDs for both Robust and common genes were submitted in GS2M

and miRs with Mont Carlo-corrected p-value less than 0.05 were considered statistically significant [25-27].

3. Results and Discussion

3.1. Search results and differentially expressed genes.

Our search through GEO yielded 24 datasets, of those 5 datasets including GSE118553, GSE5281, GSE36980, GSE122063, GSE132903 containing 187 AD patients and 167 healthy controls were included in our study. The basic characteristics of included studies are summarized in Table 1. DEGs between the control and AD group were identified using the GEO2R tool, where P-values less than 0.05 were considered significant.

Table 1. Basic characteristics of included studies.

Datasets	Country	Number of AD/CTR	Age (yrs.) AD/CTR	Postmortem interval (hours) AD/CTR	Reference
GSE118553	UK	52/27	82.9 ± 8.7/70.6 ± 15.9	39.9 ± 21.3/37.1 ± 20.7	[28]
GSE5281	USA	33/14	79.9 ± 6.9/79.8 ± 9.1	2.5/ 2.5	[29,30]
GSE36980	Japan	26/62	83.0 ± 5.7/83.0 ± 5.7	-	[31,32]
GSE122063	USA	12/11	80.9 ± 7.4/78.6 ± 8.5	8.0 ± 4.0/ 9.0 ± 3.0	[33]
GSE132903	USA	97/98	85.02 ± 6.75/84.98 ± 6.90	-	[10]

3.2. Results of integrated genomic analyses.

RRA analyses for identification of robust DEGs between datasets yielded a set of up and down-regulated genes that top five up and down-regulated robust genes are given in Table 2, furthermore, common genes between included studies were obtained using Venn diagram (Figure 1).

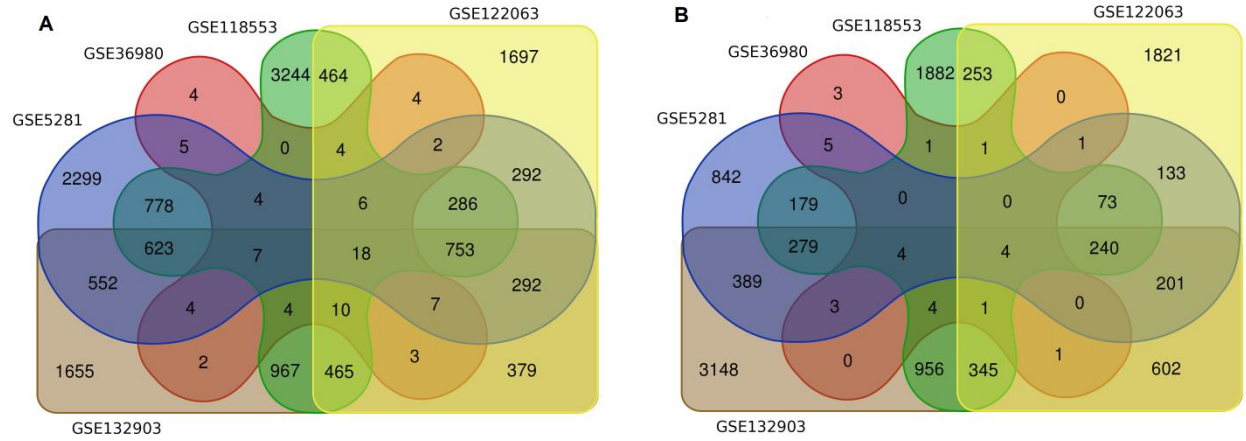

Figure 1. Common A) down-regulated and B) up-regulated genes between included studies, developed by Van der Peer Lab [34].

Table 2. Top five up and down-regulated robust genes.

Gene Symbol	Full name	Score
Robust up-regulated genes		
APLNR	Apelin Receptor	4.251491e-09
ITPKB	Inositol-Trisphosphate 3-Kinase B	3.801006e-06
AEBP1	AE Binding Protein 1	1.391380e-05
SERPINA3	Serpin Family A Member 3	4.576840e-05
ANLN	Anillin Actin Binding Protein	1.279258e-04
Robust down-regulated genes		
VSNL1	Visinin Like 1	4.370754e-08

Gene Symbol	Full name	Score
CARTPT	CART Prepropeptide	2.274075e-07
NPTX2	Neuronal Pentraxin 2	2.885267e-07
VGF	VGF Nerve Growth Factor Inducible	6.008304e-07
SYT13	Synaptotagmin 13	7.091239e-7

3.3. Pathways enriched by differentially expressed genes.

DAVID was used to find pathways enriched by robust and common DEGs, the top three pathways based on a number of genes and P-Values are given in Table 3, interestingly the PI3K-Akt signaling pathway was a top pathway enriched by both robust genes and common DEGs. Both phosphoinositide 3-kinase (PI3K) and protein kinase B (PKB or Akt) are kinases.

Table 3. Three top pathways enriched by up and down-regulated robust and common genes.

Pathway	Number of genes	P-Value
Robust up-regulated genes		
PI3K-Akt signaling pathway	10	1.70e-2
HTLV-I infection	8	2.60e-2
Rap1 signaling pathway	7	3.30e-2
Robust down-regulated genes		
Neuroactive ligand-receptor interaction	13	4.80e-4
GABAergic synapse	12	2.20e-8
Retrograde endocannabinoid signaling	12	1.40e-7
Common up-regulated genes		
Pathways in cancer	140	8.80e-14
PI3K-Akt signaling pathway	114	4.40e-9
Focal adhesion	80	3.50e-10
Common down-regulated genes		
Metabolic pathways	444	4.60e-7
Huntington's disease	108	1.20e-13
Alzheimer's disease	102	7.00e-16

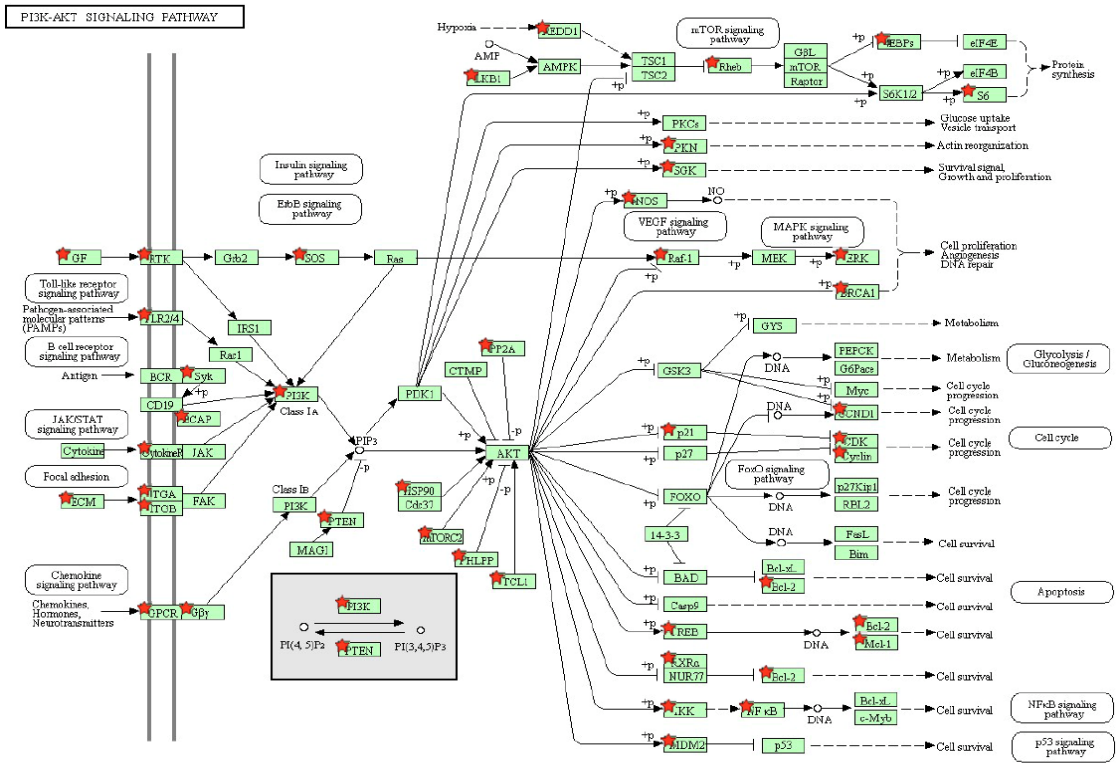

Figure 2. PI3K-Akt signaling pathway enriched by both robust and common genes that are marked by red stars reproduced with copyright permission from KEGG [24,35].

3.4. miRs targeting genes involved in the PI3K-Akt signaling pathway.

Results of miRs analyses for genes involved in PI3K-Akt signaling pathway using GS2M yielded 38 miRs, of those 7 miRs had the P-value less than 0.05 and showed to be statistically significant. The top three miRs based on a number of target genes and P-values are given in table 4; Including HSA-MIR-29B.4, HSA-MIR-29B.5 and HSA-MIR-29C.5 that target 21, 17 and 15 genes, respectively.

Table 4. Top three predicted miRs targeting genes involved in the PI3K-Akt signaling pathway

miR	P-value	Number of targeting genes
HSA-MIR-29B.4	7.57229967044858e-05	(21): CDK6, CDK2, COL1A2, COL4A1, COL4A4, COL4A5, COL6A3, COL11A1, ITGA6, ITGB1, LAMC1, LAMA2, MCL1, PDGFRB, PTEN, SGK1, COL5A3, GNG12, PDFGC, THBS2, ITGA11.
HSA-MIR-29B.5	1.28970003513928e-06	(17): CDK6, COL1A2, COL4A1, COL4A4, COL4A5, COL6A3, COL11A1, ITGA6, ITGB1, LAMC1, MCL1, PDGFRB, PTEN, SGK1, COL5A3, GNG12, PDFGC.
HSA-MIR-29C.5	0.000126067421820976	(15): CDK6, COL1A2, COL4A1, COL4A4, COL4A5, COL6A3, COL11A1, ITGB1, LAMC1, MCL1, PDGFRB, PTEN, SGK1, COL5A3, GNG12.

Herein, we have performed an integrated genomic analysis on microarray datasets from 187 AD patients and 167 healthy controls, our results showed significant up-regulation of genes involved in the PI3K-Akt signaling pathway in AD brains compared to healthy controls.

A PI3K-Akt signaling pathway is an essential modulator of insulin effects and its impairment is a key pathological event in type 2 diabetes (T2D), recently impaired PI3K-Akt signaling pathway has been reported in AD patients and has been proposed as a mechanism linking AD and T2D; however results are almost inconsistent, while most of the reports reported down-regulation of this pathway in AD patients [36,37], a few numbers of evidence indicated up-regulation of this pathway in AD and causing cognitive impairment [38,39], moreover, there is also some evidence indicating up-regulation of this pathway in an early stage of AD, possibly as a compensatory response which is then down-regulated upon AD progression [37,39,40]. PI3K and Akt are two kinases that are involved in different signaling pathways, including glycogen synthase kinase 3 beta (GSK-3β) inactivation; GSK-3β is responsible for tau protein phosphorylation and played a key role in AD pathogenesis, GSK-3β is usually inactivated through phosphorylation by Akt, which its activation is modulated by PI3K. Therefore, the mechanism for the role of the PI3K-Akt signaling pathway in AD pathogenesis is based on its deregulation leading to GSK-3β activation, which in turn increased tau phosphorylation [36]. However, the results of this study showed the up-regulation of the PI3K-Akt signaling pathway, which is in contrast to this mechanism, which is possibly a part of the compensatory response in an early stage of AD. Moreover, herein we have identified miRs targeting genes involved in the PI3K-Akt signaling pathway, which may be down-regulated in AD patients, specifically in early stages, although experimental studies are needed to confirm our results, these miRs may be potential therapeutic targets and biomarkers for AD treatment or early diagnosis.

4. Conclusions

In conclusion, in this study, we reached from thousands of differentially expressed genes in AD brain to tens of robust genes and tens of common genes between at least two

studies and showed the impaired PI3K-Akt signaling pathway in AD, subsequently, the miRs analysis showed the potential miRs targeting genes involved in PI3K-Akt signaling pathway with potential application as therapeutic and diagnostic biomarkers in AD.

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Conflicts of Interest

The authors declare no conflict of interest

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