Establishing Genomic/Transcriptomic Links Between Alzheimer's Disease and Type 2 Diabetes Mellitus by Meta-Analysis Approach

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Abstract: Meta-analysis methods exist for combining multiple microarray datasets. However, there are a wide range of issues associated with microarray meta-analysis and a limited ability to compare the performance of different meta-analysis methods. Using cDNA microarray technology (Partek Genomics Suite 6.6) and global pathway analysis with Ingenuity Pathway Analysis tool (IPA, Inc), we examined the transcript level in type 2 diabetes mellitus (T2DM) and Alzheimer's disease (AD) patients and controls. To understand the molecular link between T2DM and AD, we compared the gene expression pattern and pathway involved. Microarray analysis identified 235 differentially expressed genes between T2DM patients and controls; and 834 between AD and controls at two fold change and a false discovery rate of 0.05. Significantly changed expression of "myeloid leukemia cell differentiation protein 1; RAS guanyl releasing protein 1; S100 calcium-binding protein A8; prostaglandin- endoperoxide synthase 2; parvalbumin; endoplasmic reticulum aminopeptidase 1; phosphoglycerate kinase 1; Eukaryotic translation initiation factor 3 subunit F; Interleukin-1 beta; tubulin, beta 2A; glycine receptor alpha 1 and ribosomal protein S24" genes were highly associated with T2DM, whereas "neuronal differentiation 6; G-protein coupled receptor 83; phosphoserine phosphatase; bobby sox homolog or HMG box -containing protein 2; Glutathione S-transferase theta 1; alpha-2-glycoprotein 1 zinc-binding; Heat shock 70kDa protein 1B; transportin 1, Acidic leucine-rich nuclear phosphoprotein 32 family member B; Nuclear factor of activated T-cells 5; inositol 1,4,5-trisphosphate 3-kinase B; prenylcysteine oxidase 1 like" were found to be strongly related with AD. We also found a set of differentially expressed genes; "ARP2 actin-related protein 2; Cell division control protein 42; cytoplasmic polyadenylation element binding protein 4; Early growth response protein 1; ectonucleotide pyrophosphatase/phosphodiesterase 5; folate receptor 1; glutamate-ammonia ligase; hydroxy-3-methylglutaryl-Coenzyme A reductase; 3-hydroxy-3- methylglutaryl-CoA synthase; interleukin 1 receptor- like 1; leukemia inhibitory factor receptor; metastasis associated lung adenocarcinoma transcript 1; pyruvate dehydrogenase kinase, isozyme 4; phosphoserine phosphatase, parvalbumin, and tubulin, beta 2A" to be present in both dataset. Altered regulation of intracellular signaling pathways, including Ephrin receptor, liver X receptor/ retinoid X receptor; interleukin 6; insulin-like growth factor 1; interleukin 10 and 14-3-3-mediated signaling pathways were associated with T2DM as well as Alzheimer-type pathology. Our findings implicate diabetic disorders in the pathogenesis of AD, and provide a basis for future candidate studies based on specific pathways.

Keywords: Alzheimer's disease, canonical pathways, meta-analysis, microarray, transcriptomics, type 2 diabetes mellitus.

INTRODUCTION

Microarray-based expression profiling is a widely used, quick and inexpensive method to obtain information about the specific diseases. In recent years many researchers have embraced microarray technology and there has been an explosion in publicly available datasets. Examples of expression data repositories include Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/), ArrayExpress (http://www.ebi.ac.uk/microarray-as/ae/) and Stanford Microarray Database (http://genome-www5.stanford.edu/) as well as researchers' and institutions' websites. The use of these datasets is not exhausted, when used wisely they may reveal a depth of new information. Demand has increased to effectively utilise these datasets in current research as additional data for analysis and verification. Meta-analysis refers to an integrative data analysis method that traditionally is defined as a synthesis or at times review of results from datasets that are independent but related [1]. Meta-analysis has ranging benefits. Power can be added to an analysis, obtained by the increase in cohort size of the study. This aids the ability of the analysis to find effects that exist and is termed 'integration-driven discovery' [2]. Meta-analysis can also be important when studies have conflicting conclusions as they may estimate an average effect or highlight an important subtle variation [1, 3].

There are a number of issues associated with applying meta-analysis in gene expression studies. These include problems common to traditional meta-analysis such as overcoming different aims, design and populations of interest. There are also concerns specific to gene expression data including challenges with variable probes and probe

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sets, differing platforms being compared and laboratory effects. As different microarray platforms contain probes pertaining to different genes, platform comparisons become difficult when comparing these differing gene lists. Often the intersections of these lists are the only probes to be retained for further analysis. Moreover, when probes are mapped to their 'Entrez IDs' for cross platform comparisons, often multiple probes pertain to the same gene [4]. Due to reasons ranging from alternative splicing to probe location these probes may produce different expression results [5]. Ideal methods for aggregating these probe results in a meaningful and powerful way, is currently the topic of much discussion. Laboratory effects are important because array hybridisation is a sensitive procedure. Influences that may affect the array hybridisation include different experimental procedures and laboratory protocols [6, 7]. For more details of the difficulties associated with microarray meta-analysis please refer to Ramasamy *et al*. and other works [5, 8-12].

Genome-wide expression analysis with DNA microarrays has become a mainstay of genomics research. The challenge no longer lies in obtaining gene expression profiles, but rather in interpreting the results to gain deep insights into biological mechanisms. To get the better understanding of the disease mechanisms, the functional analysis of differential genes can be performed using a number of different methods [13]. Typically they rely on Gene Ontology and gene set enrichment analysis - based annotation of genes [14].

Diabetes is a disease that makes the body less able to convert sugar to energy. T2DM is by far the most common type and accounts for 90% of patients with diabetes [15] and is linked to lack of exercise and obesity. When diabetes is not controlled, too much sugar remains in the blood. Over time, this can damage organs, including the brain. AD is a progressive and fatal brain disorder that gradually destroys a person's cognitive memory and ability to learn, reason, make judgments, communicate and carry out daily activities. Scientists are finding more evidence that could link T2DM with AD, the most common form of dementia. However, primary molecular mechanisms underlying in risk of diabetic patients to develop AD later in life is largely unknown. In present study, transcriptomics and associated pathways analysis approach has been used to establish link between T2DM and AD.

MATERIALS AND METHODS

Patients and Samples

The study was performed on a series of T2DM and AD cDNA microarray dataset retrieved from public depository at NCBI's GEO database. We used an integrated bioinformatics approach to unify the data coming from different sources (blood or tissue) and platforms/arrays (GPL5188, GPL6244, GPL570, GPL96, GPL97, GPL8300 etc). Classification of the AD and T2DM samples to distinguish between affected and controls status were used as the sample information provided with the data series, we refer readers to the original manuscripts for more details regarding this status (Table **1**). Majority of dataset included in present meta-analysis study were small (~ 10 case + 10 controls), however few studies had large cohort size as well (GSE 5281: 74 AD + 87 Ctrl and GSE 18732: 45 T2DM + 47 Ctrl).

Gene Expression Analysis

Affymetrix .CEL files were imported to Partek Genomics Suite version 6.5 (Partek Inc., MO, USA). Prior to analysis, data series sharing common array chips were combined together to increase cohort size, while for different chips, we combined the differentially expressed genes after individual

Table 1. GEO Dataset Used for Meta-Analysis

GEO Data Series ID	Platform	Description	Source	References
GSE26972	GLP 5188, [HuEx-1_0-st]	3AD + 3 Ctrl*	Entorihnal cortex, Brain	[18]
GSE 38642	GPL 6244, [HuGene-1_0-st]	9 T2DM + 54 Ctrl	Pancreas, Islets	[23]
GSE 5281	GPL570, HG-U133_Plus_2	74 AD + 87 Ctrl	Different parts of Brain	[76]
GSE 16759	GPL570, HG-U133_Plus_2	4 AD + 4 Ctrl	Parietal lobe	[19]
GSE 28146	GPL570, HG-U133_Plus_2	7 Incipient + 8 Moderate + 7 Severe AD + 8 Ctrl	Brain	[20]
GSE 15932	GPL570, HG-U133_Plus_2	8 T2DM + 8 Ctrl	Peripheral Blood	[77]
GSE 23343	GPL570, HG-U133_Plus_2	10 T2DM + 7 Ctrl	Liver Tissue	[24]
GSE 25462	GPL570, HG-U133_Plus_2	10 T2DM + 40 Ctrl	Muscles Tissue	[25]
GSE 9006	GPL96, Human array U133A	12 T2DM + 24 ctrl	Peripheral Blood	[29]
GSE15623	GPL96, Human array U133A	9 T2DM + 5 Ctrl	Liver tissue, 4 Obese Cases	[28]
GSE 12643	GPL 8300, [HG_U95Av2]	10 T2DM +10 Ctrl	Myotubes	[30]
GSE 18732	GPL1392, Human Genome U133 Plus 2.0	45 T2DM + 47 Ctrl	Skeletal muscle	[26]
GSE 20966	GPL 1352, [U133_X3P]	10 T2DM + 10 Ctrl	Beta cells of Pancreas	[27]

Ctrl* = Control.

analysis of each chip type. The data was normalized using RMA normalization. Principal component analysis (PCA) was performed on all probes to visualize high dimensional data. PCA was used to assess quality control as well as overall variance in gene expression between the disease states. Analysis of Variance (ANOVA) was applied on the complete data set and differentially expressed gene list was then generated using False Discovery Rate (Benjamini Hochberg) of 0.05 with 2 fold change cut off. Disease and tissue type were two factors in ANOVA model and equal variance were assumed. Unsupervised two-dimensional average linkage hierarchical clustering was performed using Spearman's correlation as a similarity matrix. Diabetes is originated from pancreas (insulin production) whereas brain (plaque formation) is mainly affected in AD. Despite knowing the fact that that gene expression varies in different tissues; still we used different tissues to identify the common genes involved in both AD and T2DM. To correlate the transcriptomics of AD and T2DM, initially we searched for dataset of patients who first had T2DM and later developed AD but we could not find any of such study. Additionally Affymetrix also keeps upgrading expression chips according to recent available genomic information and chips are changed at probe level. Thus it is not appropriate to combine raw CEL file and the software (Partek genome suite 6.5) used in present study has also limitation of using different platforms data simultaneously. We, therefore, identified the differentially expressed gene for each platform separately and combined the list at the end.

Functional and Pathway Analysis

To define biological networks, interaction and functional analysis among the differentially regulated genes in breast cancer, pathway analyses were performed using IPA software (Ingenuity Systems, Redwood City, CA). A combined differentially expressed genes for AD and T2DM and their corresponding probesets ID, Gene symbol, Entrez gene ID as clone identifier, p-value and fold change values were uploaded separately into the IPA tool for core analysis revealing associated genetic network, canonical pathway, and biofunctions. The significance of the connection between the expression data and the canonical pathway were calculated by ratio and/or Fisher's exact. Significant genes passing the test criterion (e.g., p-value for ANOVA, t-test, correlation analysis, or possibly fold change) were functionally categorized by gene ontology.

RESULTS

Microarray provides an unbiased approach for identifying genome-wide changes in genes whose regulation is altered under pathological conditions [16, 17]. Many gene expression profiling studies of AD have been performed on RNA isolated from brain-tissue (18-22]. However pancreatic tissue, skeletal muscle tissue or peripheral blood samples has been taken by different group for diabetes to identify transcriptomic changes [23-30].

The main focus of this study was to determine the transcriptomic profiles of T2DM and AD and establish link between them. To identify genes that are involved in T2DM development and later increases the risk of AD in diabetic patient; we retrieved hundred of T2DM and AD specimens with paired control from GEO database, and analyzed the transcriptomic profiles of nearly 28,000 annotated genes. We performed PCA scatter plot for visualizing the high dimensional array data. In the scatter plot, each point represents a chip. We applied PCA for identifying outliers and major effects in the data. The results of PCA of the transcriptomic data showed that the samples from the same tissue type clustered tightly together. Clear differences were also observed between these diabetic and normal tissues revealing distinct expression profiles for the different tissue types (Fig. **1**). PCA mapping showed that 34.12% of the overall variance in the microarray dataset is depicted by the first three principal components.

Identification of Differentially Expressed Genes

In an effort to determine how diabetes affects hypothalamic function or increase the risk of AD, we did meta-analysis of retrieved microarray expression data to identify the differentially expressed genes for AD and T2DM. Comparison of the genome-wide expression of T2DM revealed 235 differentially expressed genes, 179 genes of which were upregulated and 56 genes were downregulated (2 fold changes, $p < 0.05$). Similarly we found 834 differentially expressed genes, 321 up and 513 down regulated genes for AD. (Only top up and down regulated genes shown in Table **2**). Cluster analysis also showed that the T2DM specimen agglomerated in various subsets according to disease and specimen type (Fig. **2**). On comparing the differentially expressed genes (DEGs), we found few genes namely ARP2 actin-related protein 2 homolog (yeast), cell division cycle 42 (GTP binding protein, 25kDa), cytoplasmic polyadenylation element binding protein 4, Early growth response 1, ectonucleotide pyrophosphatase/ phosphodiesterase 5, folate receptor 1, glutamate-ammonia ligase, hemoglobin gamma A /// hemoglobin gamma G, 3-hydroxy-3-methyl-glutaryl-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA synthase 1, interleukin 1 receptor-like 1, leukemia inhibitory factor receptor alpha, metastasis associated lung adeno-carcinoma transcript 1, pyruvate dehydrogenase kinase, isozyme 4, phosphoserine phosphatase, parvalbumin, sorting nexin 10, and tubulin beta 2A class IIa to be present in both T2DM and AD transcription profile (Table **3**). Among the these genes, many shown concordance in their expression pattern, however, ARP2 actin-related protein 2 homolog, Early growth response 1, folate receptor 1, glutamate-ammonia ligase, 3-hydroxy-3-methylglutaryl-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA synthase 1, parvalbumin and sorting nexin 10 genes were over expressed in T2DM and under expressed in AD. We also explored the literature to confirm the role of these genes in development of T2DM and AD using common pathway involved in later stages of life. ACTR2 - a major constituent of the ARP2/3 complex located at the cell surface and is essential to cell shape and motility through lamellipodial actin assembly and protrusion. ACTR2 (ARP2/3) is involved in signaling pathways like Ephrin receptor, IGF-1, CDC42, angiopoietin, axonal guidance, RhoA etc. We overlaid differentially expressed genes of T2DM and AD over Ephrin receptor signaling pathways to establish the molecular link between T2DM and AD (Fig. **3**).

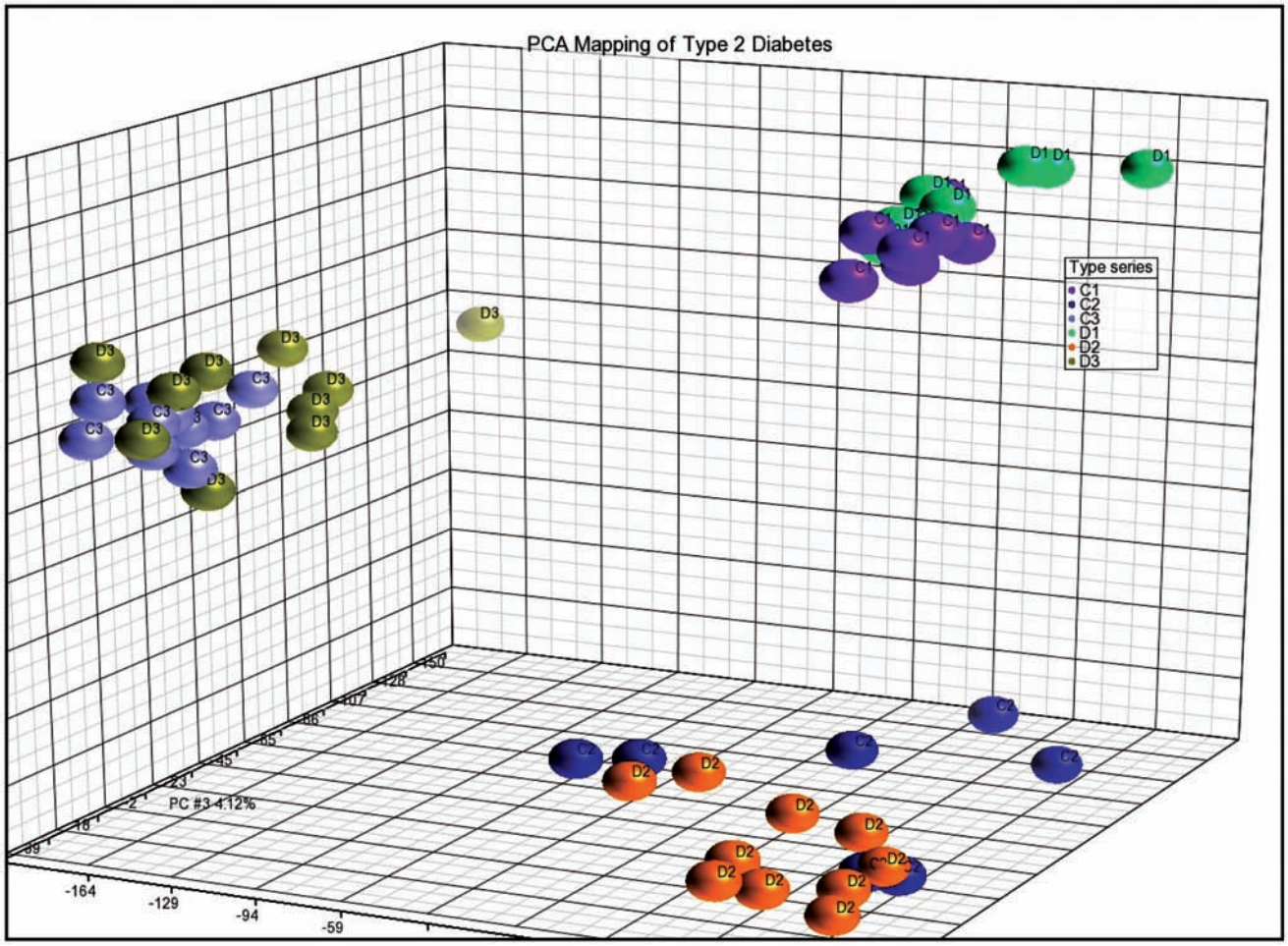

Fig. (1). Principal component analysis of transcriptomic data set of T2DM. Description: The top three principal components are plotted on the *X*-, *Y*-, and *Z*-axes, respectively. Overall variation between diabetes and normal, where each spot represents an individual array, can be seen by the clustering within each datasets (GSE 15932, D1 &C1; GSE23343, D2 & C2; and GSE25462, D3 & C3) and the separation between the diabetes and control.

Pathways and Networks Underlying T2DM and AD

To understand the mechanisms by which the genes alter a wide range of physiological processes, we examined molecular networks underlying T2DM and AD. Transcriptomic signatures of T2DM showed significant disruption in signaling pathways associating genes of the LXR/RXR Activation, IL-6 and IL-10 Signaling, Atherosclerosis Signaling, Granulocyte Adhesion and Diapedesis, Actin Cytoskeleton Signaling (Table **4**, Figs. **4**, **5**). Analysis by IPA shows a set of key genes from AD dataset that disrupt pathways such as 14-3-3-mediated Signaling, Huntington's Disease Signaling, NRF2-mediated Oxidative Stress Response, GABA Receptor Signaling, Remodeling of Epithelial Adherens Junctions, Germ Cell-Sertoli Cell Junction Signaling, Clathrin-mediated Endocytosis Signaling, and Rho GDP-dissociation inhibitor 1 (RhoGDI) Signaling (Fig. **6**). The pathway analysis revealed a strong correlation between the transcriptomic signature and the canonical pathways that have not been implicated in T2DM or AD before.

DISCUSSION

Gene microarray technology allows massively parallel analysis of most genes expressed in a tissue/blood, and therefore is an important new research tool that potentially can provide the investigative power needed to address the complexity of diabetes and neurodegenerative processes. Meta-analysis offers a way to enhance the robustness of microarray technology. The 'dataset cross-validation' meta-analysis approach observed within this study encapsulates a very real problem with microarrays; gene lists selected from one platform or study has a limited ability to be integrated. This indicates that the added power through meta-analysis produces more robust and reliable results, eventuating in a gene list that is not platform dependent but truly indicative of the disease.

Diabetes mellitus is associated with a variety of neurologic and cerebral complications, leading to increased risk of AD [31, 32]. Both type 1 (insulin-dependent) and type 2 (insulin-resistant) diabetes are associated with hyperglycemia; alterations in carbohydrate, lipid, and protein

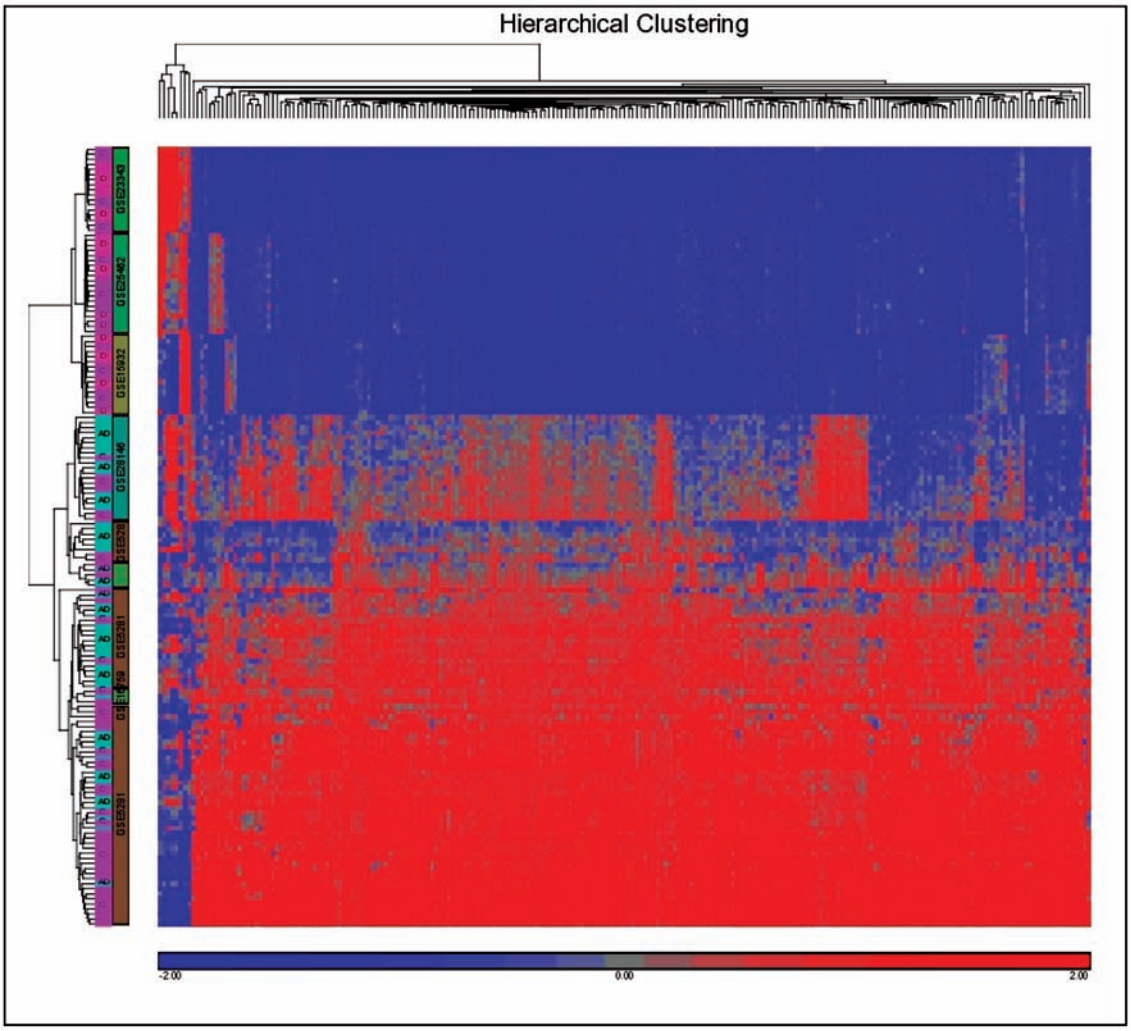

Fig. (2). Heat map of DEGs using Hierarchical clustering. Dendrogram shows the change in expression levels of genes in T2DM (GSE23343, GSE25462, and GSE15932) and Alzheimer's disease (GSE5281, GSE28146, and GSE16759) compared to their normal controls. The cluster color represents the normalized expression level of a given gene in a particular tissue type or histopathological condition given below and is colored according to the color bar at the bottom. Red denotes upregulation and blue denotes downregulation according the color scale. Each column is single gene and each row is a single experiment from each subject.

metabolism; and a variety of complications affecting tissues of the body. These complications extend to the central nervous system, where they range from acute alterations in mental status due to poor metabolic control to greater rates of decline in cognitive function with age [31, 33], higher prevalence of depression [34], and an increased risk of AD [32]. In present study, we focused on linking T2DM and AD at transcriptomics level and found 16 genes to be common in both. We have not checked the possibility of random chance of finding these genes in two completely unrelated datasets. We also showed that transcription profile of T2DM and AD are quite different from each other. However, gene set enrichment analysis and ingenuity pathway analysis of indicated many genes and pathways common in both T2DM and AD. Discussing the role of each and every identified genes and pathways are beyond the scope of present study, however identified genes and pathways are listed in tables. Here we show the link of T2DM and AD with following selected pathways.

14-3-3-Mediated Signaling Pathway

14-3-3 proteins were the first signaling molecules to be identified as discrete phosphoserine/threonine binding modules [35]. They are a family of conserved adaptor and scaffolding proteins expressed in all eukaryotic cells. There are seven known mammalian 14-3-3 isoforms, (β, ε, γ, η, σ, τ and ζ) having ability to bind a multitude of functionally diverse signaling proteins, including kinases, phosphatases, and transmembrane receptors. This plethora of interacting proteins allows 14-3-3 to play important roles in a wide range of vital regulatory processes, such as mitogenic signal transduction, neuronal development, apoptosis, cell cycle regulation, metabolic control, host-pathogen interactions, and pathogenesis [36, 37].

14-3-3 proteins were originally discovered as a family of proteins that are highly expressed in the brain. Through interactions with a multitude of binding partners, 14-3-3 proteins impact many aspects of brain function including

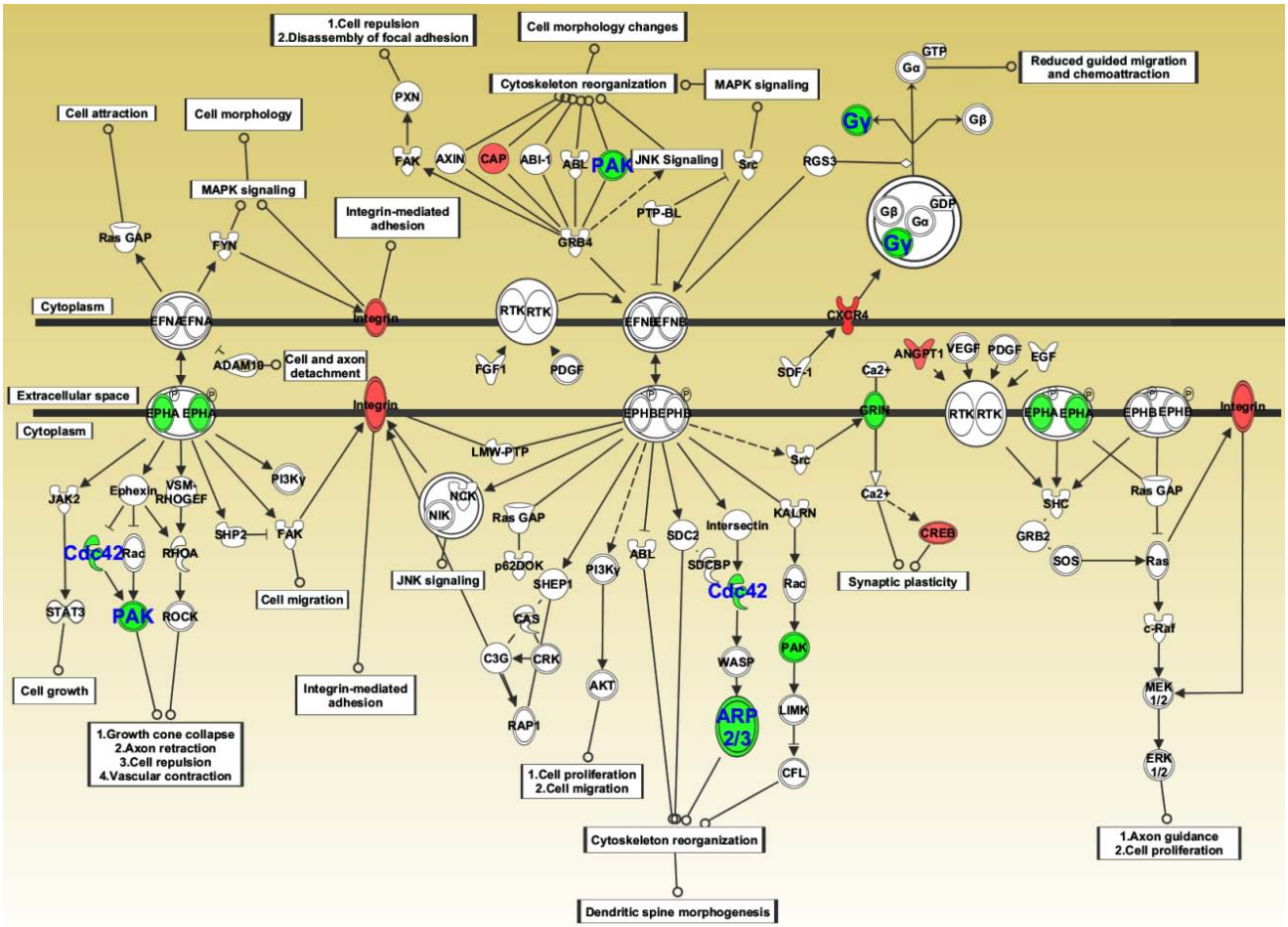

Fig. (3). Actin-related protein 2 homolog (ACTR2), a major constituent of the ARP2/3 complex is part of Ephrin receptor signalling pathway that is found to be significantly involved in both T2DM and AD. Red denotes up-regulated and Green denotes down-regulated overlaid gene transcripts. White denotes genes are not significant in our datasets. PAK, Cdc42, Gγ subunit of GPCR CXCR4, and ARP2/3 shown in blue, are common proteins involved in are T2DM and AD.

neural signaling, neuronal development and neuroprotection. Although much remains to be learned and understood, 14-3-3 proteins have been implicated in a variety of neurological disorders based on evidence from both clinical and laboratory studies [38]. Fountoulakis *et al*., did proteomic profiling of human brain by 2D electrophoresis and identified two isoforms, 14-3-3 γ and ε and found these two multifunctional proteins in several brain regions of aged patients with AD in comparison with control brains [39]. 14-3-3 proteins are also found in the neurofibrillary tangles in patients with AD [40]. Sluchanko *et al*. proposed that 14-3-3 should be considered an important participant of the complex process of tau aggregation and as a potential therapeutic target in treating AD. 14-3-3 interacts with nonphosphorylated tau and promotes its interaction and phosphorylation by a number of protein kinases. 14-3-3 induces aggregation of nonphosphorylated tau but does not affect aggregation of tau phosphorylated at specific sites [41]. Due to its acidic pI and high concentration in neurons, 14-3-3 can compete with tubulin for interaction with tau. Binding to phosphorylated tau, 14-3-3 might inhibit its dephosphorylation by protein phosphatases and by this means indirectly affect interaction of tau with microtubules and tau aggregation. It might also promote sequestration of dangerous small tau oligomers and stabilize tau aggregates. Its increased expression suggests a role in tuning microglia activation which reportedly follows the deposition of amyloid β fibrils and is generally considered a triggering factor in the early steps of the onset of AD [42].

Apart from AD, 14-3-3s are implicated in disease areas like obesity and diabetes too. 14-3-3 proteins mediate core cellular metabolism pathways, such as insulin signaling, autophagy, AMPK signaling, TOR signaling, and apoptosis, by regulating the activity of their key modulators [43]. 14-3-3β isoform regulates the activity of Akt, which mediates insulin signaling [44] and that of glucoseresponsive transcription factor, ChREBP (carbohydrate response element-binding protein), which plays a critical role in the glucose-mediated induction of gene products involved in hepatic glycolysis and lipogenesis [45]. Kim *et al*., did comprehensive bioinformatic analysis and suggested that regulating 14-3-3 function may be a therapeutic target for impaired metabolic disorders as well as hepatic insulin resistance (steatosis) and T2DM [46]. Lim *et al*., characterized the abundance and subcellular location of all

Table 2. Differentially Expressed Significant Genes in AD and T2DM Using Cut Off p value <0.05 and Fold Change 2. A Total 834 Genes in AD and 236 Genes in T2DM Passed the Criteria (Shown Only Top 10 Genes from Each Category)

Gene Symbol	Gene Name	Fold Change: Up/Down Regulated	p-Value
Type 2 Diabetes Mellitus			
PTGS2	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	5.4112	6.22E-08
S100P	S100 calcium binding protein P	3.96809	4.26E-17
PVALB	Parvalbumin	3.55777	0.000572
ERAP1	Endoplasmic reticulum aminopeptidase 1	3.39638	1.66E-21
CMTM6	CKLF-like MARVEL transmembrane domain containing 6	3.386	2.19E-18
PGK1	Phosphoglycerate kinase 1	3.38061	3.07E-21
CALML6	Calmodulin-like 6	3.24126	3.43E-06
EIF3F	Eukaryotic translation initiation factor 3, subunit F	3.13215	4.03E-15
IL1B	Interleukin 1, beta	3.04556	1.51E-06
PEG10	Paternally expressed 10	3.04163	0.000246
HBD	Hemoglobin, delta	-6.46233	3.59E-13
HBG1 /// HBG2	Hemoglobin, gamma A /// hemoglobin, gamma G	-3.89145	3.17E-16
OCLN	Occludin	-3.22359	1.20E-18
GLRA1	Glycine receptor, alpha 1	-2.92004	1.29E-05
CLEC2D	C-type lectin domain family 2, member D	-2.81207	2.68E-21
ALAS2	Aminolevulinate, delta-, synthase 2	-2.75868	1.89E-11
RPS24	Ribosomal protein S24	-2.5307	1.22E-23
EIF5A	Eukaryotic translation initiation factor 5A	-2.52499	0.005283
TUBB2A	Tubulin, beta 2A	-2.50715	2.39E-20
THEMIS	Thymocyte selection associated	-2.47733	6.96E-25
Alzheimer's Disease			
PSPH	Phosphoserine phosphatase	14.591	2.49E-06
HSPA1B	Heat shock 70kDa protein 1B	6.78252	0.014069
GSTT1	Glutathione S-transferase theta 1	5.34884	0.001869
AZGP1	Alpha-2-glycoprotein 1, zinc-binding	4.69948	0.000255
MAFF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	4.58778	5.29E-09
HSPA1B	Heat shock 70kDa protein 1B	4.57247	0.030391
DNAJB1	DnaJ (Hsp40) homolog, subfamily B, member 1	4.55671	0.043635
ANXA1	Annexin A1	4.45278	0.030145
SERPINH1	Serpin peptidase inhibitor, clade H (heat shock protein	4.34771	0.020741
DNAJB6	DnaJ (Hsp40) homolog, subfamily B, member 6	4.15108	0.016794
NEUROD6	Neuronal differentiation 6	-4.74083	0.019019
GPR83	G protein-coupled receptor 83	-4.22193	0.005070
CALB1	Calbindin 1, 28kDa	-4.13505	0.022166
MT1G	Metallothionein 1G	-3.87492	0.021796
WIF1	WNT inhibitory factor 1	-3.64782	0.001591
HLA-DRB6	Major histocompatibility complex, class II, DR beta 6 (pseudo)	-3.60383	0.046826
HTR2A	5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled //	-3.55887	0.042213
HSP90AB1	Heat shock protein 90kDa alpha (cytosolic), class B member 1	-3.51449	0.028148
VSNL1	Visinin-like 1	-3.46504	0.000217
NRN1	Neuritin 1	-3.46185	0.012741
SST	Somatostatin	-3.42891	2.14E-05

Table 3. Role of Differentially Expressed Genes Presents Both in T2DM and AD Dataset

Gene Symbol: Gene Name	Fold Change [Ref: T2DM]	Fold Change [Ref: AD]	Genes/Pathways Involved in T2DM and AD
ACTR2: ARP2 actin-related protein 2 homolog (yeast)	20.55391 [78]	-2.46845 [79]	ACTR2 - a major constituent of the ARP2/3 complex located at the cell surface and is essential to cell shape and motility through lamellipodial actin assembly and protrusion. Signaling pathways: Ephrin receptor, CDC42, IGF-1, angiopoietin, axonal guidance, RhoA
CDC42: cell division cycle 42 (GTP binding protein, 25kDa)	-2.03892 [80]	-2.37825 [81, 82]	MAP-kinase pathways; ERK, JNK and p38 activated by PAK, p21-activated kinase, family members. actin polymerization pathways; SCAR and WASP; and myosin activation filament assembly *via* PAK1/MLCK/myosin
CPEB4: cytoplasmic polyadenylation element binding protein 4	2.31332 X	2.43414 [83]	NMDA-induced signaling, regulate protein synthesis and synaptic plasticity in hippocampus highest levels in brain, kidney, heart, and fetal liver
EGR1: Early growth response 1	2.75034 [84, 85]	-2.35064 [86]	Signal transduction pathways involving the mitogen-activated protein kinases (MAPKs) cell proliferation, brain plasticity and learning, apoptosis.
ENPP5: ctonucleotide pyrophosphatase/ phosphodiesterase 5 (putative)	-2.1055 [87]	-2.13982 [88, 89]	Pantothenate and CoA Biosynthesis, Purine metabolism, Starch and Sucrose Metabolism. It may play a role in neuronal cell communication
FOLR1: folate receptor 1 (adult)	2.87604 [90]	-2.2601 X	a secreted protein that either anchors to membranes *via* a glycosyl-phosphatidylinositol linkage or exists in a soluble form and binds folic acid and its reduced derivatives, and transport 5-methyltetrahydrofolate into cells. Mutations associated with neurodegeneration due to cerebral folate transport deficiency.
GLUL: glutamate-ammonia ligase	2.0343 [91]	-2.4458 [92]	catalyzes the synthesis of glutamine and GABA from glutamate and ammonia. Glutamine and GABA are main source of energy and is involved in cell proliferation, inhibition of apoptosis, and cell signaling.
HBG1 /// HBG2: hemoglobin, gamma A and G	-3.89145 X	-2.55381 X	No Significant pathway found to be associated with T2DM or AD. Heme, oxygen, protein and metal ion binding. oxygen transporter activity
HMGCR: 3-hydroxy-3-methylglutaryl-CoA reductase	2.22623 [93]	-2.18519 [94, 95]	HMGCR is the rate-limiting enzyme for cholesterol synthesis and is regulated by sterols and non-sterol metabolites derived from mevalonate. It is suppressed by cholesterol derived from the internalization and degradation of low density lipoprotein (LDL) *via* the LDL receptor. Interaction between *HMGCR* and ABCA1 cholesterol-related genes modulates *Alzheimer's* disease risk.
HMGCS1: 3-hydroxy-3-methylglutaryl-CoA synthase 1	2.10163 [96]	-2.64968 X	HMGCS1 catalyzes the formation of HMG-CoA from acetyl-CoA and acetoacetyl-CoA. HMG-CoA levels seem to affect glucose-induced [Ca2+] signalling and insulin secretion in rat beta-cells, but no direct relation between HMGCS and diabetes was found until now. Inhibitors of HMG-CoA reductase are clinically used to prevent hyperlipidemia in type 2 diabetic patients.
IL1RL1: interleukin 1 receptor-like 1	2.33809 [97]	3.85103 [98]	IL1RL1 is a membrane receptor for IL-6, and IL-33, involved in TH2 inflammatory responses, eosinophilia and cell growth its stimulation recruits MYD88, IRAK1, IRAK4, and TRAF6, followed by phosphorylation of MAPK3/ERK1 and/or MAPK1/ERK2, MAPK14, and MAPK8
LIFR: leukemia inhibitory factor receptor alpha	2.26948 [99]	4.10647 [100, 101]	LIFR is a polyfunctional cytokine involved in Cytokine-cytokine receptor interaction and Jak-STAT signaling pathway that affects the differentiation, survival, and proliferation of a wide variety of cells
MALAT1: metastasis associated lung adenocarcinoma transcript 1	2.54809 X	2.24481 X	No Significant pathway found to be associated with T2DM or AD. Predictive marker for metastasis development in lung cancer
PDK4: pyruvate dehydrogenase kinase, isozyme 4	2.53208 [102, 103]	2.39544 [104-106]	PDK4 is overexpressed in skeletal muscle in T2DM, resulting in impaired glucose utilization. It also play role in AD through ERK signaling pathway
PSPH: phosphoserine phosphatase	2.10526 [107]	14.591 X	PSPH catalyzes the last step in serine biosynthesis. It is highly induced in proliferative normal keratinocytes
PVALB: parvalbumin	3.55777 [108, 109]	-2.35959 [110, 111]	Involved in calcium signaling Localised in fast-contracting muscles, where its levels are highest, and in the brain and some endocrine tissues
SNX10: sorting nexin 10	2.2198 X	-2.01543 X	No Significant pathway found to be associated with T2DM or AD. SNX10 has role in regulating endosome homeostasis, protein sorting, intracellular trafficking, osteoclast formation and resorption activity
TUBB2A: tubulin, beta 2A class	-2.50715 [112]	-2.30051 [113]	Apoptotic pathways; Posttranslational folding; Microtubule-based processes

[Ref:T2DM] and [Ref:AD] = Reference showing association of genes/pathways involved in T2DM and AD respectively.
X= No report showing disease association in literature for genes identified in our dataset.

Table 4. Canonical Pathways Predicted by Ingenuity Pathway Analysis. The Table Shows the Significantly Overrepresented Canonical Pathways Across the Whole Dataset of Differentially Expressed Genes

Ingenuity Canonical Pathways	-log (p-Value)	Molecules
Significant Canonical Pathways for DEGs of T2DM Dataset		
LXR/RXR Activation	4.61E00	IL33, IL1RL1, IL1B, S100A8, IL1R1, PTGS2, IL6, HMGC, APOD
IL-6 Signaling	3.92E00	IL33, IL8, IL1RL1, IL1B, IL1R1, IL6, MCL1, ATM
IL-10 Signaling	3.64E00	IL33, CCR1, IL1RL1, IL1B, IL1R1, IL6
Atherosclerosis Signaling	3.06E00	IL33, IL8, IL1B, S100A8, IL6, ALOX5, APOD
Granulocyte Adhesion and Diapedesis	2.91E00	IL33, IL8, CXCL9, IL1RL1, FPR2, MMP10, IL1B, IL1R1
Actin Cytoskeleton Signaling	2.82E00	MYH4, ACTR2, PAK2, CDC42, SSH2, FGF7, GNG12, ACTA1, ATM
Paxillin Signaling	2.77E00	ITGA2B, PAK2, CDC42, PTPN12, ACTA1, ATM
EIF2 Signaling	2.76E00	RPS24, RPL27A, RPL37A, EIF3F, PDPK1, RPS27L, RPS11, ATM
Hepatic Cholestasis	2.76E00	IL33, IL8, IL1RL1, IL1B, IL1R1, IL6, PRKCB
Graft-versus-Host Disease Signaling	2.63E00	IL33, TRD@, IL1B, IL6
Clathrin-mediated Endocytosis Signaling	2.63E00	ACTR2, RAB7A, S100A8, CDC42, FGF7, ACTA1, ATM, APOD
mTOR Signaling	2.61E00	RPS24, RHEB, EIF3F, PDPK1, RPS27L, RPS11, ATM, PRKCB
fMLP Signaling in Neutrophils	2.6E00	ACTR2, FPR2, CDC42, GNG12, ATM, PRKCB
B Cell Receptor Signaling	2.41E00	BCL10, EGR1, PDPK1, CDC42, PTEN, ATM, PRKCB
Significant Canonical Pathways for DEGs of Alzheimer's Disease Dataset		
14-3-3-mediated Signaling	6.47E00	MAP2K4, PIK3CA, TUBB3, YWHAG, PIK3C2A, YWHAH, TUBB4B, TUBB2A, YWHAZ, TUBA4A, TUBB, TUBA1B, PRKCG, FOS, PLCE1, TUBA3C/TUBA3D, YAP1, TUBA1C, SNCA
Huntington's Disease Signaling	6.44E00	MAP2K4, PIK3CA, VTI1A, HSPA1A/HSPA1B, PACSIN1, HSPA5, AP2A2, EP300, NSF, CDK5, CPLX2, VAMP3, DNAJB1, GNG4, GRIN2B, PIK3C2A, GNG3, STX1A, RPH3A, SNAP25, PRKCG, TAF9B, HSPA8, DNM1, ATP5B,
NRF2-mediated Oxidative Stress Response	6.4E00	MAP2K4, MGST1, PIK3CA, FTL, PIK3C2A, PRDX1, DNAJB4, HSPB8, ACTG1, MAFF, EP300, PRKCG, CUL3, GSTT1, FOS, STIP1, DNAJC1, SQSTM1, DNAJB6, DNAJB1, TXN, FKBP
GABA Receptor Signaling	5.64E00	DNM1, NSF, SLC32A1, AP2M1, GABRG2, GABRA4, GAD1, GABRD, GABRA1, AP2A2, GA
Remodeling of Epithelial Adherens Junctions	5.53E00	ACTR2, TUBB3, NME1, RAB5A, TUBB4B, TUBB2A, TUBA4A, TUBB, ACTG1, TUBA1B, DNM1, TUBA3C/TUBA3D, TUBA1C
Germ Cell-Sertoli Cell Junction Signaling	5.15E00	RND2, MAP2K4, PIK3CA, TUBB3, PIK3C2A, TUBB4B, TUBB2A, ITGA2, TUBA4A, CDC42, TUBB, TUBA1B, ACTG1, TGFBR2, PAK1, RHOQ, PAK3, SORBS1, TUBA3C/TUBA3D
Clathrin-mediated Endocytosis Signaling	4.58E00	ACTR2, AP2M1, PIK3CA, PIK3C2A, RAB5A, NUMB, ITGB8, SH3GL2, CDC42, AP2A2, ACTG1, DNM1, HSPA8, SYNJ1, AMPH, AAK1, RAB11A, CSNK2B, CTTN, ITGB5, RBP4
RhoGDI Signaling	4.44E00	RND2, ACTR2, ITGA2, WASF1, GNG3, CDC42, ACTG1, EP300, PAK1, CDH9, ARHGEF10, RHOQ, PAK3, EZR, CD44, CDH8, GNG2, GNG4, CDH13, PI4KA
Protein Ubiquitination Pathway	4.15E00	PSMB3, PSMA3, DNAJB4, HSPA1A/HSPA1B, UBE2N, HSPB8, HSPA5, PSMB6, UCHL1, PAN2, HSP90AB1, USP47, DNAJC1, DNAJB1, PSMB4, HSPD1, UBE3A, SKP1/SKP1P2, HSPA8, PSMB7, PSMB2, ANAPC5, DNAJB6, PS
Virus Entry *via* Endocytic Pathways	4.08E00	AP2M1, PIK3CA, PIK3C2A, ITGA2, ITGB8, CDC42, ACTG1, AP2A2, PRKCG, FOLR1, DNM1, CAV1, ITGB5
Sertoli Cell-Sertoli Cell Junction Signaling	3.88E00	MAP2K4, TUBB3, TJP2, TUBB4B, TGFBR3, TUBB2A, ITGA2, TUBA4A, CSDA, SYMPK, TUBB, CDC42, ACTG1, TUBA1B, SORBS1, TUBA3C/TUBA3D, TUBA1C, GUCY1B3
Gap Junction Signaling	3.62E00	PIK3CA, TUBB3, PIK3C2A, TUBB4B, TUBB2A, TUBA4A, TUBB, TUBA1B, ACTG1, PRKCG, PLCE1, CAV1, TUBA3C/TUBA3D, TUBA1C, GUCY1B3, PRKAR1A, HTR2A
Tec Kinase Signaling	3.59E00	RND2, MAP2K4, PIK3CA, PIK3C2A, TNFRSF10B, ITGA2, GNG3, ACTG1, PRKCG, STAT4, TLR4, FOS, PAK1, RHOQ, PAK3, GNG2

(Table 4) contd…..

Ingenuity Canonical Pathways	-log (p-Value)	Molecules
Epithelial Adherens Junction Signaling	3.44E00	ACTR2, TUBB3, TUBB4B, TGFBR3, TUBB2A, TUBA4A, WASF1, CDC42, TUBB, ACTG1, TUBA1B, TGFBR2, NOTCH2, SORBS1, TUBA3C/TUBA3D, TUBA1C
Aldosterone Signaling in Epithelial Cells	3.21E00	PIK3CA, PIK3C2A, DNAJB4, HSPA1A/HSPA1B, HSPB8, HSPD1, HSPA5, PRKCG, HSPA8, PLCE1, HSP90AB1, DNAJC1, DNAJB1, DNAJB6, PI4KA, HSPB1
eNOS Signaling	3.12E00	LPAR4, PIK3CA, CAMK4, PIK3C2A, HSPA1A/HSPA1B, FLT1, HSPA5, HSPA8, CCNA2, CALM1, CAV1, GUCY1B3, PRKAR1A
Axonal Guidance Signaling	3.01E00	KLC1, PIK3CA, TUBB, CDC42, PAK1, NFAT5, PLCE1, CDK5, SEMA3D, DCC, TUBA3C/TUBA3D, UNC5D, ADAM23, TUBA1C, GNG4, EPHA7, ACTR2, TUBB3, PIK3C2A, ADAMTS1, CXCR4, TUBB4B, TUBB2A, ITGA2, TUBA4A, GNG3, TUBA1B, PRKCG, PAK3, GNG2, PR

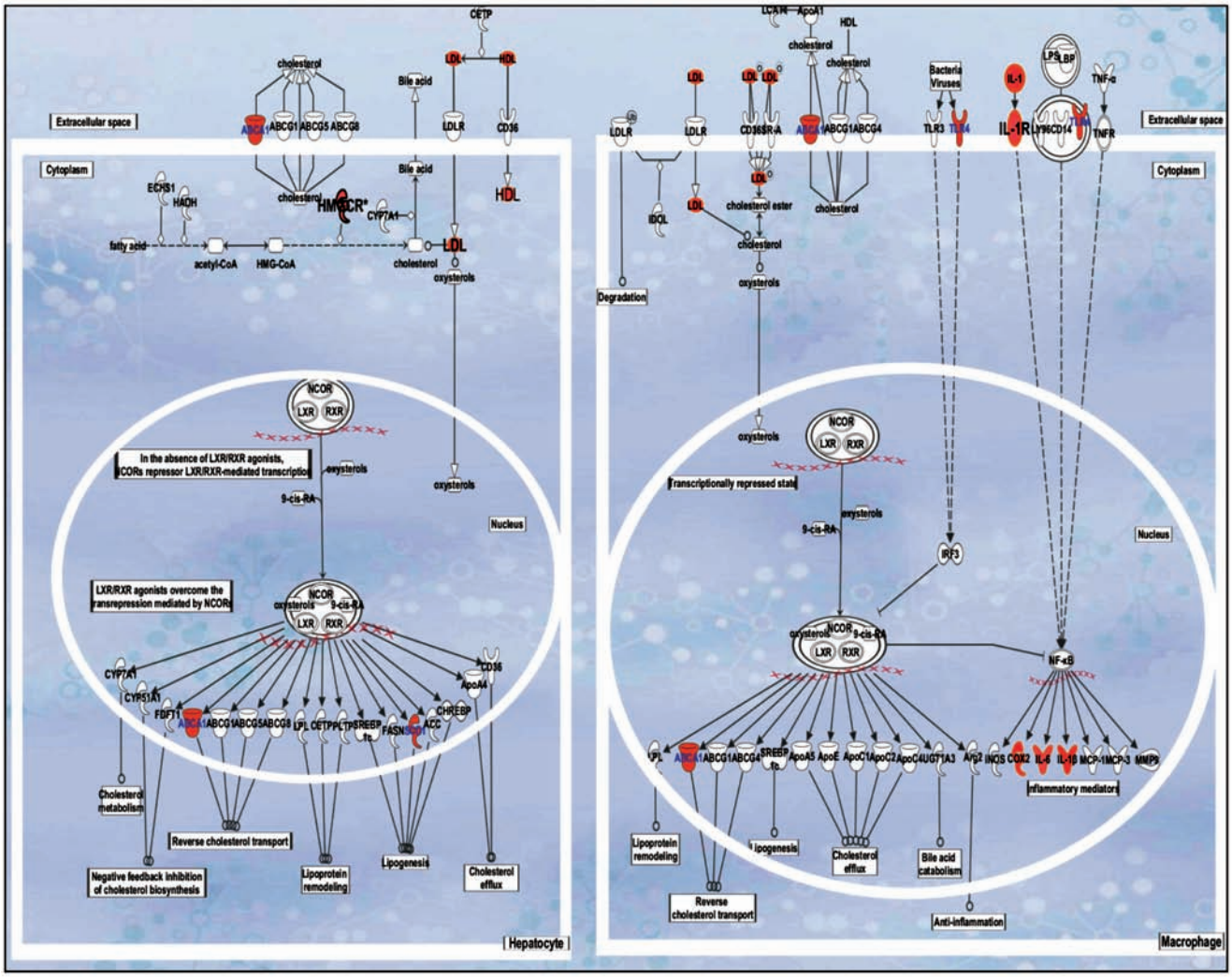

Fig. (4). LXR RXR activation signalling overlaid with significant genes of T2DM and AD dataset. Red denotes up-regulated and green denotes down-regulated whereas white denotes genes not significant in our datasets. HMGCR*, IL-1R, LDL and HDL were present in both T2DM and AD; IL-1, IL-6 and Cox2 were present in T2DM dataset only whereas ABCA1, SCD1, and TLR4 were AD associated genes.

seven 14-3-3 isoforms in mouse and human pancreatic beta cells and their study revealed specific regulation, localisation and anti-apoptotic roles among the isoforms. 14-3-3ζ specifically has role in preventing BAD-BAX mitochondrial localisation and protecting beta cells from multiple stresses, implicating that 14-3-3 proteins are pro-survival signaling hubs [47]. Another study done by using transgenic mice with cardiac-specific expression of a dominant-negative 14-3-3 protein mutant (DN 14-3-3), suggests that depletion of 14-3-3 protein induces cardiac oxidative stress, inflammation and remodeling after experimental diabetes induction mediated through p38 MAPK, MAPKAPK-2 and NF-κB signaling [48].

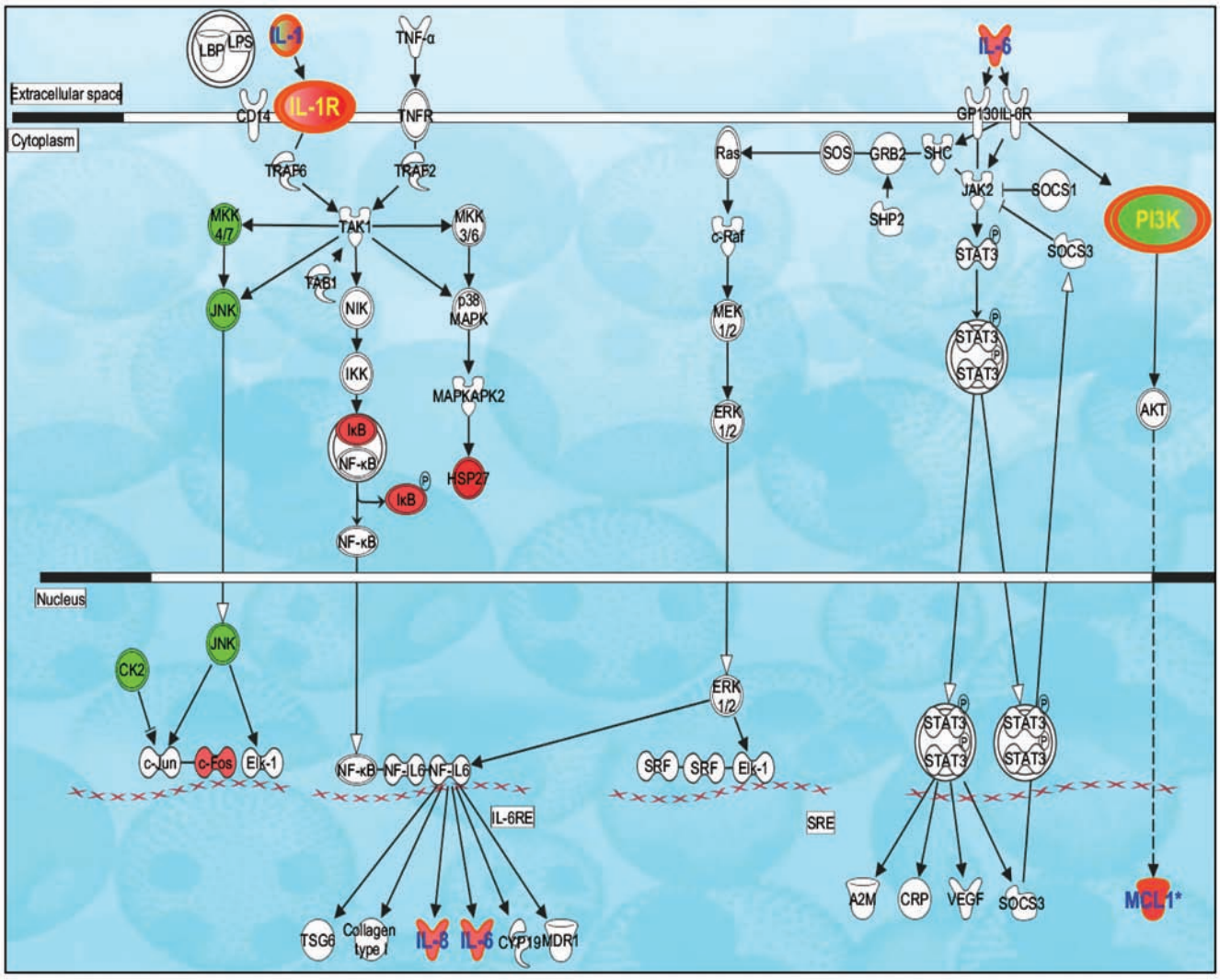

Fig. (5). IL-6 signalling pathway overlaid with significant genes of T2DM and AD dataset. Red denotes up-regulated and Green denotes down-regulated whereas white denotes genes not significant in our datasets. PI3K and IL-1R genes were present in both T2DM and AD; IL-1, IL-6, IL-8, MCL1* were present in T2DM dataset only whereas remaining were AD associated genes.

LXR/RXR (Liver X Receptor/Retinoid X Receptor) Activation Pathway

Liver X receptors (LXRs) are a member of the nuclear receptor family of transcription factors that are activated by oxysterol ligands and form functional heterodimers with the retinoid X receptors (RXRs). The RXRs are nuclear receptors that mediate the biological effects of retinoids by their involvement in retinoic acid-mediated gene activation. RXRα is the dimerization partner for the type II nuclear receptors that includes the LXR. LXR/RXR is involved in the regulation of lipid metabolism, inflammation, cholesterol to bile acid catabolism and glucose metabolism [49]. Activation of LXR decreases blood glucose levels in a number of diabetic animal models [50]. LXRβ activation in pancreatic β -cells increases insulin secretion and insulin biosynthesis [51]. The increase in insulin secretion is mediated *via* modulation of glucose/lipid metabolism [51, 52].

Two major targets of LXR and RXR (retinoic acid receptors) regulation are ABCA1 and SCD (Stearoyl-CoA desaturase), and the expression of these genes individually decreases the deposition of Aβ. LXRα and LXRβ double knockout mice develop neurodegenerative changes in brain tissue [53, 54]. LXR agonists are effective for treatment of murine models of atherosclerosis, diabetes, anti-inflammation, and Alzheimer's disease. T0901317, a LXR agonist decreases Aβ production in AD mouse model [55]. GW3965, a synthetic LXR agonist, improves glucose tolerance in a murine model of diet-induced obesity and insulin resistance by regulating genes involved in glucose metabolism in liver and adipose tissue [56]. However, treatment with rexinoids raises triglyceride levels (*via* transactivation of SREBP-1c by LXR/RXR heterodimers), suppresses the thyroid hormone axis, and induces hepatomegaly, thereby, restraining their use as therapeutic agents for the treatment of T2DM and insulin resistance [57].

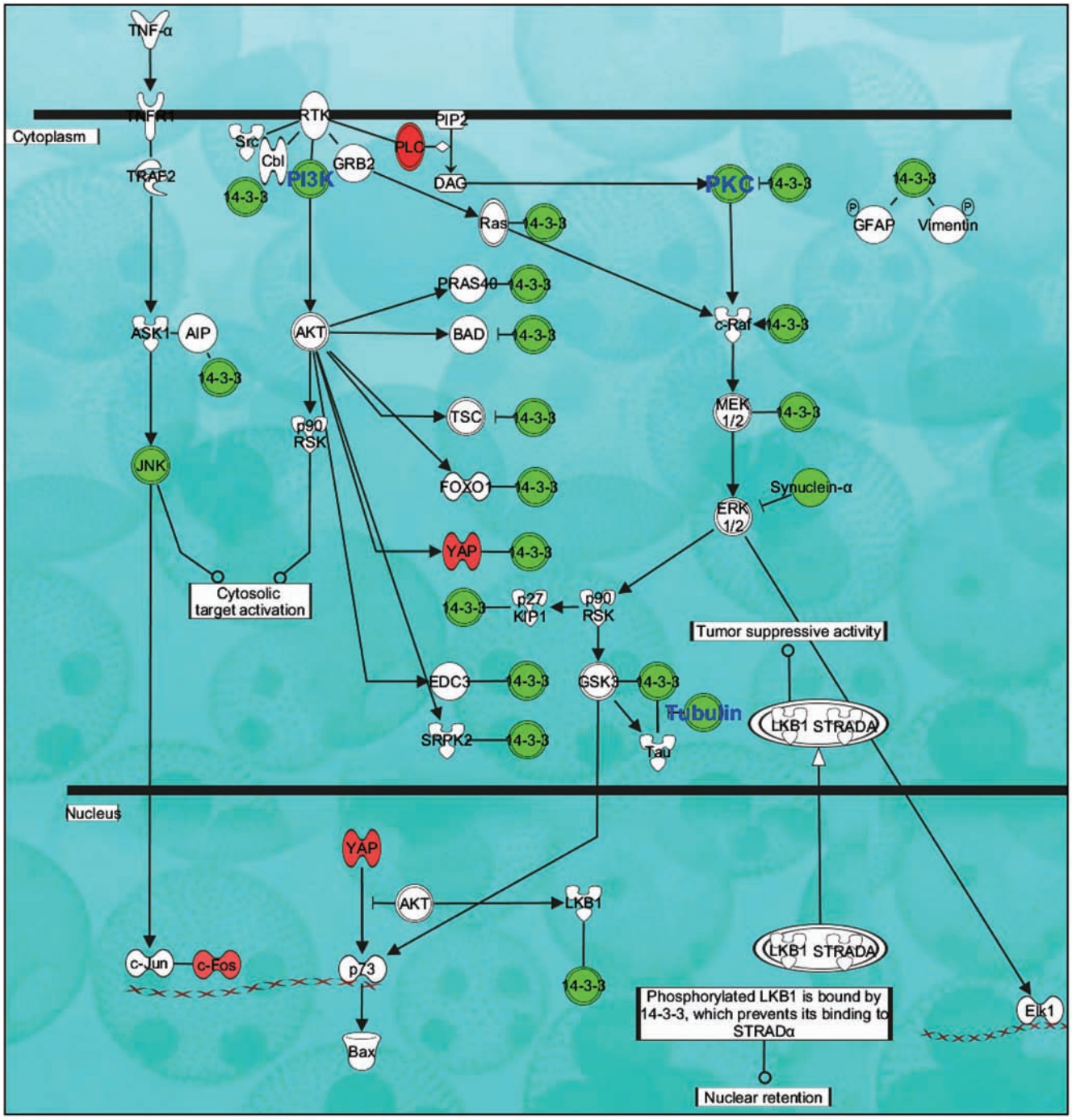

Fig. (6). 14-3-3-mediated signaling pathway overlaid with significant genes of T2DM and AD dataset. Red denotes up-regulated and Green denotes down-regulated whereas white denotes genes not significant in our datasets. PI3K, PKC and Tubulin are present in T2DM only whereas remaining are AD associated genes.

Ephrin Receptor Signaling Pathway

Ephrin receptors (Ephs) are a group of receptors that are activated in response to binding ephrin. Ephs form the largest known subfamily of receptor tyrosine kinases (RTKs). Both Ephs and their corresponding ephrin ligands are membrane-bound proteins that require direct cell-cell interactions for Eph receptor activation. Eph/ephrin signaling has been implicated in the regulation of a host of processes critical to embryonic development, angiogenesis [58], stem cell differentiation and cancer [59]. Unlike most other RTKs, Ephs have a unique bi-directional signaling capacity to initiate an intercellular signal in both the receptor-bearing cell ("forward" signaling) and the opposing ephrin-bearing cell ("reverse" signaling) following cell-cell contact [60].

EphA-ephrin-A-mediated beta cell communication is also bidirectional: EphA forward signaling inhibits insulin secretion, whereas ephrin-A reverse signaling stimulates insulin secretion. EphA forward signaling is downregulated

in response to glucose, indicating that, under basal conditions, beta cells use EphA forward signaling to suppress insulin secretion but under stimulatory conditions, they shift to ephrin-A reverse signaling to enhance insulin secretion. This explains how beta cell communication in pancreatic islets conversely affects basal and glucose-stimulated insulin secretion to improve glucose homeostasis [61]. Kaplan *et al.*, examined the role of the EphA2 receptor and ephrin-A1 ligand in human corneal epithelial cell migration by IHC analysis of healthy and diabetic corneal cells [62]. They found that EphA2 attenuates corneal epithelial cell migration when stimulated by ephrin-A1 ligand in a manner that involves the suppression of Akt. Elevated levels of ephrin-A1 may contribute to diabetic keratopathies by persistently engaging EphA2 and prohibiting Akt-dependent corneal epithelial repair processes.

Ephrin Bs are essential components of the Reelin receptor/signaling pathway control neuronal migration during the development of the nervous system. Loss of function of secreted glycoprotein Reelin in humans results in the severe developmental disorder lissencephaly and has been associated with other neurological disorders such as epilepsy, schizophrenia and Alzheimer's disease [63]. Eph receptors are a probable target for novel therapeutic strategies in AD. Reduced EphA4 and EphB2 receptor levels have been reported in postmortem hippocampal tissue from patients with incipient stage of AD [64]. Amyloid-β oligomers cause cognitive deficits in AD by impairing neuronal NMDA-type glutamate receptors, whose function is regulated by the receptor tyrosine kinase EphB2. EphB2 is part of the NMDA signaling pathway, depletion of EphB2 is critical in amyloid-β-induced neuronal dysfunction and restoring its expression rescues cognitive function in animal model of AD [65]. Recently, it has also been reported that γ-secretase-mediated EphA4 signaling pathway is involved in synaptic pathogenesis of AD [66]. EphA4 is a substrate of γ-secretase, and the γ -secretase-cleaved EphA4 intracellular domain (EICD) is known to enhance the formation of dendritic spines *via* activation of the Rac signaling pathway [67].

Interleukin 6 Signaling Pathway

IL-6 is a cytokine regulating acute-phase responses and lymphocyte stimulatory factors. The central role of IL-6 in inflammation makes it an important target for the management of infectious and inflammatory diseases. IL-6 responses are transmitted through Glycoprotein 130 (GP130) of JAK/STAT pathway, which serves as the universal signal-transducing receptor subunit for all IL-6-related cytokines. In addition, IL-6 also activates the extracellular signal-regulated kinases (ERK1/2) of the mitogen activated protein kinase (MAPK) pathway [68]. The upstream activators of ERK1/2 include RAS and the src homology-2 containing proteins GRB2 and SHC. The SHC protein is activated by JAK2 and thus serves as a link between the IL-6 activated JAK/STAT and RAS-MAPK pathways. The phosphorylation of MAPKs in response to IL-6 activated RAS results in the activation of nuclear factor IL-6 (NF-IL6), which in turn stimulates the transcription of the IL-6 gene [69].

Pro-inflammatory cytokines are important mediators of β-cell demise in type 1 as well as T2DM, where a state of chronic inflammation may persist [70]. Expression of IL-13 and IL-6 are reported to be altered in β -cells during enteroviral infection of islet cells, thereby influencing development of diabetes in humans. Interleukin-6 (IL-6) has been recently reported to have dual differential kind of role in modulating insulin sensitivity, with evidence as both an enhancer and inhibitor of insulin action [71]. Elevated brain levels of IL-6, secreted mainly from activated local astrocytes, contribute to pathological events including neuroinflammation and neurodegeneration [72]. Thus, inhibition of pathological IL-6 expression provides a rationale strategy for targeting the onset or further progression of neurological disorders including AD, multiple sclerosis, Parkinson's disease [73] and traumatic brain injury [74]. IL-6 is a crucial player in neuroinflammation owing to its influence on the three vital branches of this process: astrogliosis, microgliosis and blood–brain barrier integrity [75].

CONCLUSION

Microarray is most widely used tool for gene expression profiling of diseases. However, it brings several major bioinformatics and resource problems that frequently hinder the optimal application of this technology. Meta analysis of transcriptomics data is an important approach to address many of these concerns and to investigate the complexity and link of T2DM and AD. We identified many common genes and pathways that support the strong link between T2DM and AD and possibility of T2DM to act as risk factor for AD. Present study highlight that these genes and signaling pathways in diabetes are candidates for further investigation, both for understanding AD pathogenesis, and as potential targets for developing new treatment strategies which are specifically designed for diabetes and AD.

LIST OF ABBREVIATIONS

AD	=	Alzheimer's Disease
T2DM	=	Type II Diabetes Mellitus
IPA	=	Ingenuity Pathway Analysis
GEO	=	Gene Expression Omnibus
PCA	=	Principal component analysis
ANOVA	=	Analysis of Variance
DEGs	=	differentially expressed genes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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